Poster Abstracts Accepted for Presentation

July 26–30, 2015
Atlanta, Georgia

SCIENTIFIC POSTER SESSION SCHEDULE

Posters of the accepted abstracts can be viewed in the Exhibit Hall of the Georgia World Congress Center, on Tuesday, July 28 and Wednesday, July 29. All posters will be posted from 9:30am until 5:00pm. Presenting authors will be in attendance from 12:30pm until 1:30pm. Please refer to the onsite Abstracts Title Guide for a complete schedule of posters.

Below are the topics and their scheduled times.

TUESDAY, JULY 28, POSTER SESSIONS

9:30am – 5:00pm

Clinical Studies/Outcomes	A-001 – A-065 & A-341	S2
Endocrinology/Hormones	A-066 – A-126	S24
Factors Affecting Test Results	A-127 – A-176	S45
Hematology/Coagulation	A-177 – A-204	S64
Immunology	A-205 – A-241	S74
Mass Spectrometry Applications	A-242 – A-306	S87
Nutrition/Trace Metals/Vitamins	A-307 – A-331	S110
Animal Clinical Chemistry	A-332 – A-340	S119
Cancer/Tumor Markers	A-342 – A-385	S122

WEDNESDAY, JULY 29, POSTER SESSIONS

9:30am – 5:00pm

Cardiac Markers	B-001 – B-040	S136
Electrolytes/Blood Gas/Metabolites	B-041 – B-057	S150
Infectious Disease	B-058 – B-115	S156
Lipids/Lipoproteins	B-116 – B-138	S176
Management	B-139 – B-174	S184
Molecular Pathology/Probes	B-175 – B-207	S197
Pediatric/Fetal Clinical Chemistry	B-208 – B-224	S208
Point-of-Care Testing	B-225 – B-281	S215
Proteins/Enzymes	B-282 – B-297	S235
TDM/Toxicology/DAU	B-298 – B-344	S241
Technology/Design Development	B-345 – B-371	S258
Automation/Computer Applications	B-372 – B-390	S268

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Keyword Index S286

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Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Clinical Studies/Outcomes

A-001

Association between laboratory test turnaround time and emergency department length of stay: a retrospective US electronic health database analysis

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Background:

Rapid and accurate diagnosis is critical to providing timely and appropriate care in the emergency department (ED). Longer lengths of stay (LOS) in the ED correlate with higher inpatient service admission rates and additional inpatient LOS [1-2] 'Treat and release' patients (i.e. patients treated in the ED and subsequently discharged rather than admitted to inpatient services) represent a large proportion of ED visits in the US. In spite of the importance of laboratory test results in guiding patient management, there is currently a lack of studies examining the association between laboratory test turnaround time (TAT) and ED LOS. The objective of the present study was to examine the relationship between laboratory test TAT and ED LOS via retrospective analysis of a 'treat and release' ED population from a large US electronic health record (EHR) database (Cerner Health Facts®).

Methods:

ED visits from 2012 were included in the analysis if the patient was ≥18 years old, ≥1 laboratory test was ordered during the visit, ED LOS was <7 h, and the patient was discharged to home or the care of their family/caregiver. Laboratory test TAT for each patient was defined as the overall TAT (time between first test order and last returned result) for all tests ordered within 30 min of the first test ordered. LOS was defined as the time elapsed between ED admission and discharge. The relationship between TAT and LOS was examined via linear regression modeling, with and without adjustment for confounders, including patient and hospital characteristics. For regression analyses, the strength of the relationship between the TAT and LOS was assessed based on the statistical significance of the slope coefficient (p-value of <0.05 denoted statistical significance).

Results:

In total, 463,712 patient visits in the database met the defined inclusion criteria. After adjustment for confounders, regression modeling revealed a positive, statistically significant relationship between laboratory test TAT and ED LOS, such that a 10 min decrease in laboratory test TAT was associated with a 6.7 min reduction in ED LOS ($p<0.0001$). Examination of mean and median ED LOS revealed a similar relationship, and a 30 min decrease in laboratory test TAT from 61-75 min to 31-45 min resulted in a 19 min decrease in median ED LOS (from 226 to 207 min).

Conclusion:

The results of this analysis reveal a statistically significant association between laboratory test TAT and ED LOS, and suggest that laboratory test TAT is a key factor to consider during any efforts to improve ED efficiency. These results highlight the importance of developing and measuring shared TAT metrics between the ED and laboratories to help reduce LOS, as well as the potential benefits of processes aimed at improving laboratory efficiency. In order to more fully understand the implications of lab TAT and LOS reductions in different hospitals, future studies investigating the impact of lab TAT on factors such as wait time and ED throughput are warranted.

1. Carrier E et al. JAMA Intern Med 2014; 174:1843-6

2. Liew D et al. Med J Aust 2003; 179:524-6

A-002

Simple laboratory test results and mortality following coronary catheterization in Calgary, Alberta

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Background:

Simple laboratory tests such as the complete blood count and electrolytes are frequently measured in the acute care setting as they provide clinical information about immediate and short-term risk. They may also provide prognostic information on hospitalization and death following invasive procedures and discharge, although they are not frequently used for this purpose. Our objective was to examine the relationship between pre-procedural laboratory test results and mortality in patients undergoing coronary catheterization in Calgary, Alberta, Canada.

Methods:

Complete blood count, sodium, potassium, chloride and creatinine along with provincial healthcare number and test verification date were extracted from the laboratory information system of Calgary Laboratory Services (November 2009-June 2013) and merged to patient demographic (age, sex, smoking status) and outcome data from the Alberta Provincial Project for Outcome Assessment (APPROACH) , a province-wide cardiac catheterization registry. We included only data for the last lab panel run prior to catheterization. Logistic regression models using backwards elimination with 60-day post-catheterization mortality as the outcome were used to generate a parsimonious model containing only significant demographic and lab data predictors. All continuous variables were coded into quintiles. In the final model, we used interaction terms to test whether time between laboratory testing and catheterization had any impact on our results.

Results:

The final data set included 6275 patients and 301 deaths within 60 days of catheterization. After backwards elimination, the final model (odds ratios evaluated per 1 quintile increase) contained age (OR = 1.24, p=0.02), sex (OR = 1.75 for men vs women, p=0.04), sodium (OR = 0.75, $p < 0.01$), white blood cell count (OR = 1.30, p=0.02), mean corpuscular hemoglobin concentration (OR = 0.63, $p<0.01$), and red blood cell count (OR = 0.77, $p<0.01$). The c-statistic, an overall measure of model classification power, for these variables was 0.79. We found no clinically relevant differences in lab data-mortality relationships according to time between measurement and catheterization.

Conclusions:

In addition to age and sex, laboratory tests including sodium, white blood cell count, red blood cell count, and red cell hemoglobin concentration were predictive of 60-day mortality in patients undergoing coronary catheterization. These simple variables, many of which are automatically collected in lab information systems with minimal error, should be considered in risk assessments of coronary disease patients.

A-003

Clinical validation of the ReEBOV™ Antigen Rapid Test Kit for the point of care detection of Ebola Virus Disease

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Background: Ebola virus (EBOV) causes severe and often fatal viral hemorrhagic fever (Ebola Virus Disease; EVD). The 2014-2015 outbreak of EVD in West Africa is the deadliest of its kind, and has resulted in at least 9,556 deaths prompting an international emergency response. During the course of this outbreak, FDA and WHO approved qRT-PCR as a molecular diagnostic to detect EVD clinically; however with this technique a result is not available for 24hr or more and requires significant laboratory infrastructure and power. It became evident that there was a need for an easy-to-use, point-of-care test that can be performed in any clinical facility or field laboratory to aid in the rapid triage of suspect EVD cases. To assist in this effort

the Viral Hemorrhagic Fever Consortium (VHFC.org) led by Tulane University and Corgenix Inc. accelerated the development timeline of the ReEBOV™ Antigen Rapid Test Kit. This dipstick-format lateral flow immunoassay incorporates recombinant EBOV VP40 antigen and VP40-specific polyclonal antibodies to detect EBOV antigenemia in whole blood or plasma samples. Methods: Two clinical studies were performed with Tulane IRB and Sierra Leone Ethics Committee approval. A Corgenix sponsored banked plasma study (n=176) was conducted at Kenema Government Hospital (KGH), Kenema, Sierra Leone. Reference qRT-PCR utilized EBOV Zaire GP primers and probes (Trombley et al., 2010). The WHO conducted an independent clinical trial in field laboratories at Hastings and Prince of Wales, Sierra Leone that included venous whole blood (n=152) and banked plasma (n=140). WHO reference qRT-PCR was the RealStar® Filovirus Screen RT-PCR Kit 1.0 (Altona Diagnostics GmbH). Results: Analysis of the banked plasma sample study conducted at KGH did not include the estimated cut-off for qRT-PCR cycles (Ct). As such, the RDT Negative Percent Agreement (NPA) with qRT-PCR was 96.7% (58/60; CI 88.6% - 99.1%) and Positive Percent Agreement (PPA) was 62.1% (72/116; CI 53.0 - 70.4%). The qRT-PCR cycle range within the PPA included a Ct range of 24.7 ±4.4 for true positives and Ct range of 37.0 ±3.4 for false negatives which corresponded to the estimated Ct cut-off. The combined results of the WHO clinical study generated NPA 84.6% (165/195; CI 78.8 - 89.4%) and PPA of 91.8% (89/97; CI 84.4 - 96.4%). True positive range for qRT-PCR cycles was whole blood Ct 20.2 ±3.0 and plasma Ct 20.2 ±4.8. Conclusion: These clinical validation studies have demonstrated the ReEBOV™ Antigen Rapid Test is capable of detecting Ebola VP40 with sufficient accuracy as an aid to the diagnosis of acute EVD. Based in part on these findings, the ReEBOV™ Antigen Rapid Test Kit was granted FDA Emergency Use Authorization and the WHO listed the test as eligible for procurement in February 2015. The intended use is for the presumptive detection of Zaire EBOV in individuals with signs and symptoms of EBOV infection in conjunction with epidemiological risk factors. This Point of Care RDT represents a breakthrough in the detection of EVD for this and future Ebola virus outbreaks. This work is dedicated to our friends and colleagues who lost their lives during this tragic outbreak.

A-004

Estimation of Age and Gender- Specific Reference Intervals forTurkish Children Based on Hospital Patient Data

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Clinical interpretation of laboratory test results ise heavily dependent on the availability of reference intervals. For some parameters, pediatric test reference values vary from those of adults and/or among children of different ages based on their development and growth. Therefore, it is crucial to obtain age-matched laboratory test reference values. As the acquisition of samples from healthy children has become extremely difficult in recent years, clinical reference ranges calculated from routine laboratory data of patients using a statistical method have been used. Serum alkaline phosphatase (ALP) levels show great variation infants, children and adolescents.The aim of this study was to establish reference values of serum ALP in infants,children and adolescents up to18 years using hospital data.

Nineteen thousand two hundred thirty-one test results belonging to infants,children and adolescents stored in laboratory information system of Marmara University Pendik E&R Hospital between June 2014 and January 2015 were included in this study. ALP measurements were done according to IFCC method (AU 5800, Beckman Coulter, USA). Data were analyzed in accordance with CLSI C28-A3 guidelines on defining, establishing, and verifying reference intervals in the clinical laboratory. Outliers were removed after visual inspection and the Dixon test. The data is partitioned according to age and gender based on the partitions identified by CALIPER. Additionaly, we used the 90% confidence intervals as change by CALIPER. This subset of analyte values was used to check Gaussian distributionand establish lower and upper reference interval limits and the mean values.

These results suggest that calculating pediatric reference intervals from hospital-based data may be useful.The database will also be global benefit while establishing reference intervals .The data showed complex patterns in the concentrations of ALP during child growth and development,as well as sex differences.

ALP (U/L) Female Reference Intervals	Age	0-14 days n:182	15 days-<1year n:137	1-<10 years n:10856	10-<13 years n:3039	13-<15 years n:730	15-<17 years n:883	17-<19 years n:797
	Lower Limit	73	55	89	98	59	49	44
	Upper Limit	228	377	362	418	275	154	192
	Mean	153	195	206	222	138	87	88
ALP (U/L) Male Reference Intervals	Age	0-14 days n:182	15 days-<1year n:137	1-<10 years n:10856	10-<13 years n:3039	13-<15 years n:867	15-<17 years n:1069	17-<19 years n:671
	Lower Limit	73	55	89	98	90	64	58
	Upper Limit	228	377	362	418	432	339	213
	Mean	153	195	206	222	240	162	114

A-005

Status of vitamin D deficiency in Nepalese renal transplant recipients

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Background:

Vitamin D, apart from being an important part of the "calcium-vitamin D-parathyroid hormone" endocrine axis, has diverse range of "non-calcemic" biological actions such as induction of cell differentiation, inhibition of cell growth and immunomodulation. A high prevalence of vitamin D deficiency has been observed in renal transplant recipients (RTRs) worldwide. However, the exact prevalence of vitamin D deficiency is unknown in RTRs in Nepal. This study aimed to determine the prevalence of hypovitaminosis D in Nepalese RTRs and interrelations between serum 25-hydroxyvitamin D [25(OH)D] and other biochemical parameters.

Methods:

A total of 80 adult RTRs visiting the university hospital were enrolled in this cross sectional, observational study. The research protocol was approved by Institutional Review Board. Those requiring dialyses, with prior parathyroidectomy and receiving any vitamin D compounds after transplant were excluded. All the recipients had their serum analyzed for different laboratory parameters. Serum 25(OH)D and serum iPTH were measured using fully automated Enhanced Chemiluminiscent Immunoanalyzer, Vitros ECi™ analyzer (Ortho Clinical Diagnostics, Rochester, NY). The RTR population was categorized into recent transplant recipients (≤ 1 year) and long term recipients (> 1 year). The vitamin D status was defined as per NKF/KDOQI guidelines (vitamin D sufficiency: >30 ng/ml; insufficiency: 16-30 ng/ml; deficiency: ≤ 15 ng/ml). SPSS ver. 20.0 was used to analyze the data. Appropriate statistical tests were applied to compare variables between groups and establish correlation. P <0.05 was considered to be statistically significant

Results:

The mean age of the recipients was 38.11 ± 11.47 years, with 68 males and 12 females. Chronic glomerulonephritis was the leading cause of CKD and all the RTRs were under the "triple regimen" for immunosuppression: prednisolone, tacrolimus and mycophenolate mofetil. The two RTR population groups (recent and long term) didn't differ in demographic and biochemical characteristics. The median iPTH in the transplant population was 64.25 pg/ml (Range 17.5 - 405.8 pg/ml). 83.75 % of the transplant population had iPTH levels above the upper limit of the recommended range for their stage of CKD. The mean serum 25(OH)D was 24.62 ± 10.16 ng/ml, with 53.8 % categorized as vitamin D insufficient and 18.8 % as vitamin D deficient. The mean serum 25(OH)D value was not significantly different across other categories such as time post-transplant, gender and sunlight avoidance. There was a significant negative correlation between serum 25(OH)D and serum iPTH (Pearson's r = - 0.35, P = 0.001), but not so with the graft function

Conclusion:

There is a high prevalence of vitamin D insufficiency in RTR population in Nepal and it may exacerbate secondary hyperparathyroidism. The deficiency status is not corrected despite of nutritional improvement and normalization of GFR post transplantation. Therefore, routine evaluation and vitamin D supplementation coupled with sensible sun exposure could be an important strategy in improving the vitamin D status in RTRs to optimize the "non-calcemic" effects of vitamin D in this population. However, further prospective interventional studies are required to reinforce this idea.

A-006

Serum Procalcitonin as a biochemical marker for surgical site infection

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Background :

Surgical site infection (SSI) is a diagnostic challenge particularly in surgeries requiring application of prosthetic implants. Patients present with sero-sanginous wound discharge at the time of first post-operative wound dressing, which may turn-out to be precursor for frank SSI. Wound debridement if done early in such cases can be very fruitful in controlling the infection. The decision to wash and debride the wound early is a difficult one to make in the absence of frank signs of infection, and therefore warrants a marker to predict SSI. We thus aimed to study the role of procalcitonin in predicting SSI in patients undergoing clean elective orthopaedic procedures.

Material & Method

This prospective study was performed at a tertiary care super-specialty hospital, in south of India, and included all patients undergoing a clean elective orthopedic surgical procedure during the two year study period from September 2012 to June 2014. All immunocompromised patients and any patient with a known foci of infection pre-operatively or post-operatively were excluded. Serum samples for estimation of procalcitonin levels were drawn in the immediate preoperative period (day -0) and then on day 1 and day 3 following surgery. Procalcitonin levels were estimated by commercially available ELISA Kits (Ray Biotec®). The surgical wound was inspected on the 4th and 8th post- operative day under complete sterile conditions by the senior operating surgeon. The presence of wound discharge or any other local signs of infection at the operative site present any time during follow-up was noted. If any discharge was seen a swab from the wound was immediately sent for Gram stain and culture examination. Presence of any organism immediate wound lavage and debridement of the surgical wound was performed in the OR. All the patients were followed up for 3 months post- operatively.

Results

A total of 249 patients were recruited for the study out of which 39 patients were excluded because they could not be followed up for 3 months. 110 patients were excluded because other foci of infection could not be conclusively ruled out in these patients. This left us with 116 patients for final analyses.116 patients were divided into three groups - no wound complication (87), culture negative wound discharge (19), and culture positive wound discharge (10). The serum procalcitonin levels were increased (p value<0.001) in patients having postoperative wound discharge. On comparing the levels in patients with culture positive v/s culture negative wound discharge there was a statistically significant increase in the levels of procalcitonin on post-op day 3 (p value <0.001). ROC analysis revealed that serum rocalcitonin level of 70.23 pg/ml or greater on post-op day 3 could predict the presence of surgical site infection (Sensitivity, 88.9 %; specificity, 98.1% Area Under the Curve =0.996 , p<0.001). positive predictive value=81.18% ,Negative Predictive value= 99.04 %,accuracy = 97.4 %).

Conclusion

Estimation of serum procalcitonin levels in the post-operative period can guide the surgeon to perform an early wound lavage and debridement in such cases.

A-007

Serum Biomarkers and Obstructive Sleep Apnea

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Background: Obstructive sleep apnea (OSA) is a common disorder, characterized by repetitive episodes of complete (apnea) or partial (hypopnea) obstructions of the upper airway during sleep, with decreasing oxygen saturation and sleep fragmentation. More than 22 million American adults have OSA. Diagnosis is currently based upon overnight polysomnography, and patients are often not referred for this definitive testing. Up to 90% of individuals with OSA remain undiagnosed. OSA prevalence is increasing and may soon become the most common chronic disease in industrialized countries. Untreated OSA can lead to serious health consequences, including increased mortality. Patients with OSA have an elevated risk of coronary artery disease, cardiac arrhythmia, myocardial infarction, heart failure, stroke, diabetes, obesity, metabolic syndrome, memory decline, and work-related or driving accidents.

Objective: Given the significant health issues associated with untreated OSA, early diagnosis of this treatable disorder is critical. There is a large unmet need for biomarkers to identify individuals with possible OSA. Here we present data that demonstrates an association between OSA and metabolic and endocrine biomarkers.

Methods: A multicenter prospective trial was conducted enrolling consecutive symptomatic patients with suspected OSA. All subjects underwent a diagnostic sleep study (polysomnography). A non-symptomatic control group was also obtained from a separate Healthy Controls study. Eleven biomarkers were tested: HbA1c, CRP, IL-6, uric acid, EPO, cortisol, hGH, prolactin, testosterone, DHEA (Beckman Coulter UniCel DxC 600i Synchron® Access® Clinical Systems), and IGF-1.

Results: A total of 128 subjects have been enrolled in this ongoing study. Of these, 26 were diagnosed with moderate to severe OSA. OSA is more prevalent in males, and a Receiver Operating Characteristic (ROC) curve analysis of results from male subjects (n=70) was performed. Areas Under the Curve (AUCs) for diagnosis of moderate/severe OSA were >0.70 for HbA1c and CRP (p<0.001). AUCs were >0.60 for uric acid, IL-6, and EPO. AUCs were >0.60 for gender-specific markers (prolactin, testosterone, DHEA). AUCs were >0.50 for hGH, IGF-1, and cortisol. Many of the moderate/severe OSA subjects were pre-diabetic (A1c ≥ 5.7%), with high cardiovascular risk (CRP > 0.3). Individual biomarkers performed better or worse in specific clinical subgroups, e.g. A1c achieved significant group separation in obese subjects (p<0.05), as did CRP in non-obese subjects (p<0.01).

Conclusion: Our results identified promising biomarkers that may be useful in the diagnosis of patients with OSA.

A-008

Serum Neutrophil Gelatinase Associated Lipocalin (NGAL) can predict Chronic Kidney Disease stage and risk category proposed by KDIGO 2012, in a primary care population.

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Background: Chronic Kidney disease (CKD) affects more than 10% of the population in many countries worldwide, and is an important cause of death and loss of disability-adjusted life-years. On the other hand, awareness of health care providers and individuals is low. CKD is is defined by a reduction in glomerular filtration rate (GFR) and increased urinary albumin excertion. GFR can be accessed or calculated by various methods. NGAL is a biomarker of acute kidney injury, buth there is also evidence that its levels may reflect the active renal damage that underlies chronic disease.

Methods: The aim of this study was to evaluate serum NGAL levels association with various markers of renal impairment and its hability to predict CKD category and risk by the proposed Kidney Disease: Improving Global Outcomes (KDIGO) classification. We conducted a cross-sectional study of 334 outpatients assisted by the Family Medicine Program of Niterói, Brasil, aged over 44 years-old, from August 2011 to August 2012. Demographic and epidemiological data were obtained, serum creatinine, serum cystatin C, and urine microalbumin were tested. NGAL serum levels were determined by sandwich ELISA (Bioporto Diagnostics) from -80°C preserved samples, until first thawed. KDIGO G category was calculated according to creatinine and cystatine C formulas. Urinary albumin-creatinine rate (ACR) was also calculated, in order to determine KDIGO risk category.

Results: Two hundred ninety-six (88.6%) outpatients (182 women) were identified as G1 and G2 KDIGO categories, and 38 (11.3%) at G3a and higher categories (25 women). This findings, added to ACR results, led to the following risk category distribution: 73.6% low, 17% moderate, 5.3% high, 4% very high.

We observed statistically significant univariate correlations between NGAL and age (p=0.02), creatinine (p<0.000), cystatine C (p=0.02), microalbuminuria (p=0.002), and urinary albumin-creatinine rate (p=0.013), and GFR (p<0.000). However, none of those correlations were stronger than of creatinine (R2=0.28). When GFR was calculated by serum creatinine formula, a NGAL cut-off of 205.7 ng/ml predicted KDIGO category equal or higher than G3a, with a AUC_ROC 0.723, sensitivity of 63.2% and specificity of 74%. When GFR was calculated by cystatin C, a cut-off of 226.7 ng/ml showed a AUC-ROC 0.741, sensitivity of 63.3% and specificity of 78.5%. NGAL predicts moderate and/or higher CKD risk category with a cut-off of 226.7 ng/mL, a AUC-ROC 0.829, 70.4% sensitivity and 79.7% especificity.

Discussion: Serum creatinine, cystatin C and urinary albumin loss are commonly used to determine the level of impairment function and/or kidney injury. NGAL, a member of lipocalin superfamily, is highly expressed and released from renal tubular cells after various sources of renal injury. NGAL levels are associated with the severity of CKD, reflecting the phenomena of active and continuous kidney injury underlying the chronic condition. This study observed an association between serum NGAL

and other markers of renal impairment, also NGAL levels were able to predict the presence of moderately decreased renal function or worse. NGAL showed good specificity to predict a poor condition prognosis, determined by KDIGO 2012 higher risk categories.

A-009

Detoxifying activity of serum albumin demonstrated diagnostic utility in patients with kidney transplant dysfunction and pregnant women with preeclampcia

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Background: Serum albumin (SA) is an important component of the detoxifying system of the organism. In the circulatory system, SA is the main carrier protein whose detoxifying activity is the binding of hydrophobic toxins and their transport to hepatocytes. The detoxifying activity of SA can be significantly reduced when the SA molecules are overloaded with excessively high level of toxins. This significant reduction in the SA activity leads to an inadequate overall elimination of toxins from the organism. High overall toxin levels cause further deactivating of the SA transport molecules themselves. Such conditions finally result in the escalating intoxication of the patient. We investigated the clinical relevance of the novel, in vitro laboratory test for the detoxifying activity (DTE) of serum albumin, based on Electron Paramagnetic Resonance (EPR) spectroscopy of SA labeled with 16-doxyl stearic acid. Recent clinical study of post-surgery patients has already confirmed the clinical utility of the EPR test for prediction and diagnosis of septic intoxication.

Objective: To evaluate the clinical relevance of the EPR test of SA for patients who have a risk for developing intoxications of a non-infective origin.

Methods:

We used EPR analyzer AXM-09 (Albutran) and the set of reagents "ATA-test-T" to measure detoxifying activity of albumin in blood samples.

A) The observation study included 92 (45 males and 47 females) kidney transplant recipients. 54 of them had the kidney transplant dysfunction such as acute resection (30), ischemia/reperfusion injury (15), nephrotoxicity of calcineurin inhibitors (4) and combination of pathologies (5). The control group included 38 patients, which were observed in early (19/38) and late (19/38) postoperative periods.

B) The observation study included 189 pregnant women, including 97 with uncomplicated pregnancy (15-40 weeks) and 92 who suffer from preeclampcia (17-39 weeks).

Results:

A) In the patients with kidney transplant dysfunction, there the median value of DTE was found as 55% (34%; 81%) versus 123% (77%; 157%) for the patients of the control group. The diagnostic sensitivity and specificity of the test were 73% and 78%, respectively, for diagnosis of kidney transplant recipients with transplant dysfunction. The cut-off value of DTE was 80%. The ROC-analysis showed AUC of 0.86.

B) In the pregnant women with severe preeclampcia, there the mean value of DTE was 57% for second and 42% for third trimesters of pregnancy, versus 82% and 56%, respectively, for women with uncomplicated pregnancy. The diagnostic sensitivity and specificity of the test for were 64% and 92% (cut-off of DTE was 52%, AUC=0.79) for diagnosis of severe preeclampcia in second trimester, and 59% and 82%, respectively, in third trimester (cut-off 42%, AUC=0.75).

Conclusion: The in vitro EPR test of the detoxifying activity of serum albumin is a sensitive and noninvasive method, which clearly has a demonstrated diagnostic utility, for patients with kidney transplant dysfunction as well as for pregnant women with severe preeclampcia. Our results suggest that the detoxifying activity of serum albumin would be applicable as the marker for prediction and diagnosis of escalating intoxications of various origins.

A-010

Validation of anti-phospholipase A2 receptor antibody testing by ELISA and immunofluorescence

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Background: Recently, antibodies against phospholipase A2 receptor (PLA2R) in the kidney were determined to be a major target antigen among patients with idiopathic membranous nephropathy (iMN). In order to clinically interpret anti-PLA2R results, reference ranges need to be established in healthy individuals as well as patients with proteinuric renal diseases, including secondary causes of membranous nephropathy (those associated with systemic lupus, malignancy, infection). Importantly, it is currently unknown if patients with other proteinuric renal diseases can develop anti-PLA2 R antibodies. The objective of this study was to measure anti-PLA2R antibodies in a cohort of normal individuals as well as a cohort with a diverse set of biopsy-proven renal diagnoses.

Methods: This study was reviewed and approved by the Mayo Clinic Institutional Review Board. Serum from Mayo Clinic, Rochester patients who had recently undergone diagnostic native kidney biopsy to diagnose renal disease was obtained within two weeks of biopsy and frozen at -80°C until tested. Patients receiving protocol kidney transplant biopsies were excluded. Serum was assayed for anti-PLA2R antibodies using IFA (positive/negative) and quantitative ELISA kits from Euroimmun US (Morris Plains, NJ) in the Renal Testing Laboratory. Additionally, sera from 120 adult and 20 pediatric patients were obtained from the Mayo Clinic Department of Laboratory Medicine and Pathology Quality Management Services Biorepository and analyzed by IFA and ELISA in order to verify reference ranges.

Results: A total of 202 consecutive biopsy patients with available waste serum were identified. Of these, 6 (3%) had a biopsy diagnosis of iMN. Of these, 2 (33%) had a positive IFA and 1 (7%) a positive ELISA (>20 RU/mL). IFA and ELISA were negative for the remaining patients with biopsy diagnoses of amyloidosis (n=11), acute tubular necrosis (n=15), diabetic nephropathy (n=10), focal segmental glomerular sclerosis (n=23), IgA nephropathy (n=25), interstitial nephritis (n=9), minimal change disease (n=10), monoclonal protein-related diseases (n=13), vasculitis (n=20), other immune complex mediated diseases (n=18) and other disorders (n=41). Among adult reference donors, IFA was negative in 114 (95%) and indeterminant in 6 (95%) due to high background staining. ELISA analysis was negative in all 120 (<14 RU/mL, lower 95th reference range ≤4.04 RU/mL). Among the pediatric samples (n=20) all had negative IFA and ELISA (≤4.04 RU/mL) results.

Conclusion: Both the IFA and ELISA assays were 100% specific for anti-PLA2R mediated disease in normal reference range population and among a cohort with diverse biopsy proven renal diseases. Due to a low number of iMN cases in our cohort, assay sensitivity could not be assessed, nor could the prevalence of PLA2R versus non PLA2R-mediated disease be estimated. Furthermore, many patients with iMN had already been treated and may have been in serological remission. The reference range results suggest the adult range can be used in children.

A-011

An Exploratory Factor Analysis of Biomarkers in Patients with the First Anterior ST-Segment Elevation Myocardial Infarction Treated by Primary Percutaneous Coronary Intervention

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Background: Despite advances in treatment, acute coronary syndromes, which consist mainly of ST-segment elevation myocardial infarction (STEMI) and unstable angina/non-STEMI, present an enormous medical, social, and economic burden worldwide. Primary percutaneous coronary intervention (pPCI) is a therapy of choice for the management of patients with acute STEMI. Despite the improvement in morbidity and mortality in these patients, groups at high risk of complications and adverse clinical events remain. Identification of patients at risk for major adverse cardiovascular events (MACE) might help selecting candidates for aggressive treatment which may improve outcome or early discharge after pPCI. The aim of this study was to explore the factor structure of a circulating biomarkers of potential predictive value, measured in patients with the first anterior ST-segment elevation myocardial infarction treated by primary percutaneous coronary intervention.

Methods: A total of 100 consecutive patients with the first anterior STEMI successfully treated by pPCI (< 20% of residual stenosis and TIMI flow 3) within 6 hours after the onset of the chest pain were included. Blood samples were obtained immediately before starting cardiac catheterization. Quantification of biomarkers (IL-6, IL-7, IL-8, IL-15, MCP-1, MIP-1α, ICAM, L-selectin, E-selectin, TNFRI, TNFRII, and MMP-9) concentration was performed by Randox, Ltd. (Crumlin, UK), by using a biochip array analyzer (Evidence Investigator®). Factor analysis was carried out using the principle components analysis with Varimax orthogonal rotation of examined biomarkers. Multivariable logistic regression analysis was performed for prediction of 30 day MACE (cardiac death, non-fatal reinfarction, and target vessel revascularization) by using the individual factor scores as covariates in addition to significant univariate predictors of outcome. Kaplan Meier survival curves were generated for tertiles of the remaining significant factor from the multivariable analysis and the log rank test was used to compare event rate over time for the respective tertiles.

Results: Factor analysis yielded 4 uncorrelated factors (1 [logsTNFR1, sTNFR2, MMP-9], 2 [sICAM-1, sE-selectin is L-selectin], 3 [log IL-6, log IL-8, log MCP-1], and 4 [MIP-1α, log IL7, log IL15]). These factors explained approximately 67.6% of the total variance. Multivariable analysis, including all significant univariate predictors of outcome (atrial fibrillation, left ventricular ejection fraction, smoking) revealed factor 2 and factor 3 as a potent independent predictors of 30 day MACE. Kaplan-Meier survival estimates for MACE were lower in patients with factor 2 or factor 3 score in the highest tertile compared with those in the mid and in the lower tertile (Log rank test χ^2 =9.44, P<0.01: Log rank test χ^2 =8.37, P=0.01, respectively).

Conclusion: Clustering of multiple biomarkers by exploratory factor analysis is feasible and may give useful information in exploring different biomarkers in prognosis of STEMI patients. However, larger, prospectively designed trials would be required to further explore the utility of exploratory factor analysis for this application.

A-012

A New Theory For Reference Intervals and Analyte Test Reporting Based on Clinical Risks Derived from Readily-Available EMR Data

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Background: Reference interval cut-points for general diagnostic screening are usually determined by a methodology unrelated to medical outcomes: the central 95% of test values for a "healthy" cohort are defined as being the "Reference Interval" and the other 5% are flagged as "Low" or "High" to guide the physician toward diagnosis.

Problems with this method include difficulty of identifying a healthy cohort, assembling the number of subjects required for statistical power, and the leap of faith required to flag those outside the central area (indefensible logically: the entire cohort is defined as healthy a priori). These problems arise from an antiquated methodology, established in an era long before access to electronic medical records (EMR).

Methods: We extracted in-hospital tests (first test after admission) for serum potassium, sodium, and chloride (K, Na, Cl), discharge dispositions and demographics for 375,747 adult patient visits from Sarasota Memorial Hospital (Florida) EMR during the years 1998-2014. Similar extracts were performed at a major academic medical center in the northeast and a regional hospital in the southwest for 2012-2014. For each analyte, we calculated an Outcome Risk function:

$OR(x) = (ONOwithin\Delta x) / (ONOwithout\Delta x)$

where $ONOwithin\Delta x$ = odds of Negative Outcome for test results within Δx;

$ONOwithout\Delta x$ = odds of Negative Outcome for those not within Δx;

x = mean value of test results within an interval Δx;

Negative Outcome = all-cause in-hospital mortality.

The final OR(x) for each analyte was determined using JMP software to generate logistic regressions, adjusted for confounding variables.

Results: We found risk of mortality to be below average within these analyte intervals:

K = 3.4 to 4.4 mEq/L; Na = 136 to 144 mEq/L; Cl = 100 to 109 mEq/L.

Further, we provide evidence-based risk estimates (mortality odds ratios) for values outside of these cut-points. Identical cut-points were found with other Negative Outcomes, e.g.,1-year post-discharge mortality; and when using data from other medical centers. Our high K cut-point is much lower than the current standards (which vary from 5.1 to 5.4), but is in excellent agreement with recent clinical studies of AMI patients.

Conclusions We have sought a replacement methodology for reference intervals from perspective of modern clinical chemistry, and propose a novel method to associate risk of patient outcomes with analyte test values. Gathering tests and outcomes from whole populations via hospitals' EMR's, we avoid problems of defining a "healthy" population, relying instead upon the analysis of big data to determine clinically-safe reference interval cut-points. This allows reference interval cut-points to be generated by calculation of outcome risk functions and enables readily-available EMR data to be utilized in situ, associating actual patient outcomes with analyte values by each lab. We suggest replacing the old population-distribution method with this risk-function method for more meaningful guidelines from the lab to physicians.

A-013

Reduction of False-positive Bilirubin Results on the CLINITEK Novus Automated Urine Chemistry Analyzer

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Background: Urinary bilirubin is rarely an incidental finding. Patients with urinary bilirubin have either a transient or permanent interruption in the biliary tree and subsequent bile excretion, ultimately leading to the presence of urinary bilirubin. When detectable levels of bilirubin occur, an underlying pathology is possible and indicates the need for further clinical investigation. Unfortunately, even the most established methods can generate a high percentage of false-positive bilirubin results, often due to interfering substances and/or abnormal urine color. The CLINITEK Novus® Automated Urine Chemistry Analyzer employs a unique algorithm to decrease false-positive bilirubin results associated with these influences on the bilirubin reagent pad. Utilizing camera-based detection technology, this algorithm allows for the improved detection of true bilirubin, as confirmed by Icotest® reflex testing. We assessed false-positive CLINITEK Novus analyzer bilirubin results versus incidence on the CLINITEK Atlas® analyzer along with correlation of bilirubin results.

Methods: A clinical investigation was conducted to assess false-positive incidence using 1033 clinical samples ran on one CLINITEK Novus system and one CLINITEK Atlas system at three clinical sites. These 1033 samples consisted of 534 positive bilirubin results on the CLINITEK Atlas system from a total clinical population of 2773 specimens and a random assortment of 498 negative bilirubin results from the same clinical population. Reflex testing was conducted using one reagent lot of Ictotest tablets to identify false-positive results. Results were compiled in frequency tables. Positive agreement, negative agreement, exact agreement were calculated.

Results: All 499 of the negative specimens obtained from the CLINITEK Atlas system remained negative by the reference Ictotest assay. 496 of these samples were also negative by the CLINITEK Novus analyzer for a concordance of 99.4% (95% CI: 98.3-100%).

When the 534 positive samples from the CLINITEK Atlas system were tested with Ictotest, 417 of these samples were confirmed as positive and 117 were reported as negative. This is a false-positive rate of 21.9% on the CLINITEK Atlas analyzer, with a 95% CI for this false-positive rate between 18.5 and 25.7%. Of the 117 CLINITEK Atlas system-positive/Icotest-negative samples, 94 of them (80.3%; 95% CI: 72.0-87.1) were also negative by the CLINITEK Novus system. Of the 417 CLINITEK Atlas system and Ictotest-positive samples, 407 of these were also positive by the CLINITEK Novus analyzer, corresponding to a concordance of 97.1% (95% CI: 95.0-98.5%).

In terms of clinical sensitivity and specificity, the CLINITEK Atlas system demonstrated 100% sensitivity and 67% specificity. The CLINITEK Novus analyzer demonstrated 97% sensitivity and 95% specificity with the same population of clinical samples.

Conclusions: The CLINITEK Novus analyzer showed improved accuracy of bilirubin results compared to reference Icotest assay. Greater accuracy (fewer false positives, balanced sensitivity and specificity) in routine bilirubin urinalysis with the CLINITEK Novus analyzer served to decrease the time and costs associated with potentially unnecessary follow-up bilirubin testing.

A-014

Improving the diagnostic yield among catheter and non-catheter associated UTI's

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Background

Urinary tract infections (UTIs) are one of the most common types of healthcare associated infections. Catheter-associated urinary tract infections (CAUTIs) account up to 75% of all UTIs. Long term complications of CAUTI include longer hospital stays, patient discomfort and increased mortality/morbidity secondary to disseminated infections both locally and systemically.

Methods

Using a Sysmex UF-1000i™ urine particle analyzer, we examined a case series of CAUTI and SUTI (symptomatic or non-catheter associated) patients to model the likelihood of a positive screen prior to the diagnosis of a UTI by culture. In the initial study, a retrospective analysis of reviewed urine samples was collected over three months. Data was reviewed from traditional urine culture and urinalysis using the

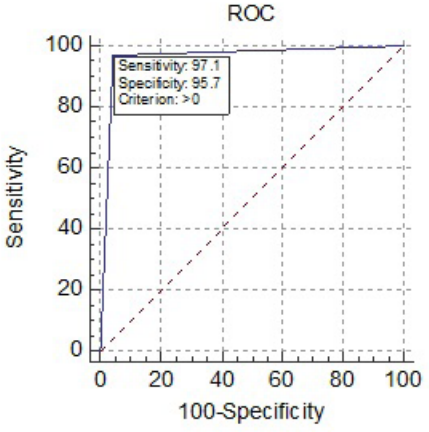
Sysmex UF-1000i™. Logistic regression was used to define what parameters where predictive of a positive culture: 1). Trace bacteria, 2). Trace yeast and 3). WBC greater than 15 k/μL. Using data mining, we identified 81 patients with either CAUTI (26) or SUTI (55) based on traditional urine culture. We then compared the likelihood that a patient in either group would have a positive screen.

Results

In the initial study, 4088 results were obtained. Screen performance revealed: a sensitivity of 98% (CI 97.4-98.4%), a specificity of 93.7% (CI 92.1-94.9%) and a positive predictive value (PPV) of 97.0% (CI 96.4%-97.6%). An ROC curve was obtained (see Figure 1). The positive LR is 15.5 (CI 12.57-19.12). Among the CAUTI patients, 100% had a positive screen on the UF-1000i™ and 90.9% of the SUTI patients had a positive screen.

Conclusions

The UF-1000i™ particle analyzer shows a high PPV and a high LR. Using a retrospective case series analysis, we confirm these values with a 93% positive screen among culture positive patients. This will allow clinicians to feel confident in accurately interpreting a screen result and treat patients before waiting 24-48 hours for culture results.



A-015

Diagnosis of Gestational Diabetes Mellitus by American Diabetes Association's and World Health Organization's Criteria and outcome in Pregnant Nigerian Women

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Background: Prevention of adverse pregnancy outcomes is an important goal in good obstetric practice. Gestational Diabetes Mellitus (GDM) is associated with adverse pregnancy outcomes. Several diagnostic criteria for GDM have been proposed and adopted in different countries. Recently, the American Diabetes Association (ADA) adopted the "International Association of Diabetes in Pregnancy Study Groups" (IADPSG) recommended diagnostic criteria for GDM. In Nigeria, there are no universally accepted diagnostic criteria for GDM. The ADA and World Health Organization's (WHO) diagnostic criteria are among the commonly used criteria. Clinical outcome data of diagnostic performance of these diagnostic criteria is lacking. The objective of this study was to compare the ADA diagnostic criteria for GDM with the WHO diagnostic criteria on the basis of predicting pregnancy outcome.

Method: This was a longitudinal study involving 130 pregnant women between 24-32 weeks of gestation. During a 75g-Oral Glucose Tolerance Test (OGTT), after at least 8hrs of overnight fast, venous blood samples were collected at 0hr (fasting), 1hr and 2hr for measurement of fasting, 1-hour and 2-hour glucose as well as fasting and 2-hour Insulin. Participants were classified as GDM and non GDM women based on the ADA and WHO diagnostic criteria. Five pregnancy outcomes were observed: pre-eclampsia, shoulder dystocia or birth injury, birth weight ≥ 4.0kg, clinical neonatal hypoglycaemia and birth asphyxia.

Results: Twenty-eight participants (21.5%) had GDM by ADA criteria (GDM_{ADA}) and 21 (16.2%) of women had GDM by WHO criteria (GDM_{WHO}). Only 15 (11.5%) met criteria for GDM by both criteria. Ten percent of Participants had GDM by ADA but not by WHO criteria whereas 4.6% had GDM by WHO but not by ADA criteria. The association of GDM with macrosomia was stronger in GDM_{WHO} women [OR=13.1, 95%CI(3.4-50.6)] compared to the GDM_{ADA} women [OR=5.3, 95%CI(1.5-18.9)]. GDM_{WHO} women were more likely to be associated with "one or more adverse pregnancy outcomes" compared to GDM_{ADA} women; OR=4.9, 95%CI (1.8-13.3) vs OR=3.5, 95%CI (1.4-8.8). Macrosomia or at least one adverse outcome, were more likely in patients with GDM who met the diagnostic criteria by both ADA and WHO criteria (P< 0.01). Conversely, women who met diagnostic criteria of GDM by one criterion but not the other criterion were not associated with macrosomia or at least one adverse pregnancy outcomes (P> 0.05).

Conclusion: Although ADA diagnostic criteria was more sensitive, diagnosis of GDM by WHO criteria was associated with higher adverse pregnancy outcomes in Nigerian women. A diagnosis of GDM that meets both WHO and ADA criteria provides stronger prediction for macrosomia or at least one adverse pregnancy outcome than a diagnosis that meets only WHO or ADA criteria. Therefore, a GDM diagnostic guideline that incorporates the strengths of both diagnostic criteria may be more useful in preventing adverse pregnancy outcome.

A-016

Intracranial gadolinium deposition quantified by ICP-MS following multiple contrast-enhanced MRI exams

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Background: Intravenous gadolinium-based contrast agents (GBCAs) are widely used in MR imaging, yet their safety and chemical stability remain topics of active investigation. In the current study, we sought to determine if repeated intravenous exposures to GBCAs are associated with neuronal tissue deposition.

Methods: In this single center study, we compared T1-weighted signal intensities from MRI exams and post-mortem neuronal tissue samples on 13 patients who underwent 4 or more gadolinium-enhanced brain MRIs between 2000-2014 (contrast exposed group) to 10 gadolinium-naïve patients (control group). All contrast-exposed patients had relatively normal renal function at the time of examination. Neuronal tissues from the dentate nuclei, pons, globus pallidus, and thalamus of these 23 deceased patients were harvested from our institutional biospecimen archive and subsequently analyzed by inductively coupled plasma mass-spectrometry (ICP-MS), transmission electron microscopy (TEM) and light microscopy to quantify, localize, and assess the effects of gadolinium deposition. Results were correlated with Pearson's test.

Results: Compared to neuronal tissues of control patients, all of whom demonstrated undetectable levels of gadolinium, neuronal tissues of GBCA-exposed patients contained between 0.2-58.8 μg gadolinium/g tissue, in a significant dose-dependent relationship that strongly correlated with signal intensity changes on precontrast T1 weighted MR images (ρ=0.38-0.95 for various tissues). Gadolinium deposition in the capillary endothelium and neuronal interstitium was only observed in the contrast-exposed group.

Conclusion: Intravenous gadolinium contrast material exposure is associated with neuronal tissue deposition in the setting of relatively normal renal function. Additional studies are needed to investigate the clinical significance of these findings.

A-017

Evaluation of the Clinical Application of Renal Function Parameters in Malignant Tumor Patients

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Abstract Objective: Following the use of chemotherapeutic agents and molecular targeted therapeutic drugs, renal function should be monitored in order to recognize renal impairment in patients with solid tumors or hematological malignancies. The glomerular filtration rate (GFR) is the most well-recognized measurement reflecting overall renal function but determining GFR using an exogenous marker is time-consuming and cumbersome. Estimates of glomerular filtration rate (eGFR) are introduced in order to circumvent the difficulty of measuring GFR. The present study is to explore the value of serum Creatinine(Cr), Cystain c(Cys C), Urea, β2 microglobulin (β2-MG), and Serum creatinine-cystatin C-based estimated glomerular filtration rate with an aim to diagnose renal dysfunction in malignant tumor patients.

Methods: A total of 725 malignant tumor patients who undertook therapies with specific antineoplastic agents (platinum-based chemotherapy, lapatinib, herceptin, etc) were recruited. Selection and staging criteria for patients were according to the 2012 CKD-EPI guideline: 1. One or more markers of kidney damage present

for >3 months. 2. Electrolyte and other abnormalities due to tubular disorders. 3. Abnormalities detected by histology. 4. GFR <60 ml/min/1.73 m2 (GFR categories G3a-G5). The levels of Serum Cr, Cys C, Urea, β2-MG, and Cr in the 24h urine samples of these patients were assayed with Beckman-Coulter AU5800 automatic analyzer. Creatinine clearance rate (CCr) was calculated using the Cockcroft-Gault equation, and the calculated GFR was called cGFR. The eGFR calculated based on 2012 CKD-EPI Cr equation was named mGFR. The eGFR calculated based on 2012 CKD-EPI creatinine-cystatin C equation was called uGFR. Data are presented as percentages, mean±SD. Differences between percentages were assessed by the $\chi 2$ test. Student's t test, analysis of variance test, and Pearson's correlation analysis were used for normally distributed variables. Deviation and average value of mGFR, uGFR and cGFR were regressed. We regarded P-values < 0.05 as significant. All statistical analyses were conducted using SPSS 19.0.

Results: Significant increases in Cys C and β2-MG were observed in each stage of renal function impairment in these patients as compared to the control cohort (P< 0.05). Serum Urea was significantly increased at the beginning of G2 stage (P < 0.05); however, serum Cr was only increased until G3 stage (P<0.05). The rate of abnormality of Cys C was higher at G1 to G3 stage in patients with renal function impairments compared with that of serum Cr in the same patients. A significant correlation was identified among the evaluations for mGFR, uGFR and cGFR (Γ=0.91 and 0.93). In addition, the concordance between uGFR and cGFR(-25.9~28.6) was higher than that between uGFR and mGFR(-20.3~61.0), while the accuracy between uGFR and cGFR (0.003) was better than that between uGFR and mGFR(0.225).

Conclusion: The detection of serum Cys C and β2-MG is favorable for the early diagnosis of renal function impairments in malignant tumor patients. A satisfactory applicable value was found in the clinical diagnosis of uGFR for renal impairment based on the outcome of joint serum CysC and Cr levels.

A-018

SERUM ADIPOKINES LEVELS IN PATIENTS WITH SARCOIDOSIS

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Introduction: Adipokines are different proteins synthesized by adipose tissue who have an influence on angiogenesis, hemostasis, lipid metabolism and immune system regulation. Sarcoidosis is a chronic inflammatory disease characterized by non-caseating granuloma which etiology is unknown yet. Adipokines may play an important role in the pathogenesis of sarcoidosis.

Aim: To determine the serum adipokines levels in patients with sarcoidosis and to determine a possible correlation with clinical and laboratory signs of disease.

Material and method: Forty-four biopsy proven sarcoidosis patients followed at a single center and age and sex matched 41 healthy volunteers were included in the study. Demographic, clinical, laboratory and radiological data were recorded and body mass index (BMI) was calculated in all patients. Routine laboratory tests (blood glucose, liver and kidney function test) were measured. Serum adiponectin and leptin levels were measured by ELISA method.

Results: Among 44sarcoidosis patients 13(29.5%) were male and 31(70.5%) were female. Twenty-one(47.7%) patients had erythema nodosum, three(6.8%) had uveitis, 40(90.9%) had arthralgia, 23(52.3%) had ankle arthritis, 15(34.1%) had enthesitis. Laboratory evaluation showed increased serum ACE level in 24(54.5%) patients, increased serum calcium level in 11 (25%) patients, increased serum D3 level in 5(11.4%) patients, increased ESR and CRP levels in 22(50%) and 23(52.3%) patients, respectively. Compared with the control group serum adiponectin levels were significantly higher in patients with sarcoidosis (p = 0.007). Serum adiponectin level were associated with arthralgia and ankle arthritis(p=0.007, p=0.006 respectively). Serum leptin levels were similar in sarcoidosis patients and controls (p = 0.327). There was no relationship between serum leptin level and disease features(p> 0.05).

Conclusion: In this study, high serum adiponectin level was detected in patients with sarcoidosis while serum leptin level were similar in the sarcoidosis and control group. Adiponectin, an anti-inflammatory protein may play an important role in the pathogenesis of sarcoidosis. Studies are needed to shed light on this topic.

A-019

Evaluation of early changes of cartilage biomarkers following arthroscopic meniscectomy in young Egyptian adults

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Abstract Background: The metabolic imbalance in the articular cartilage following meniscectomy includes an increase in cartilage degradation with an insufficient reparative or anabolic response resulting in structural, biochemical and mechanical changes that can progress from pre-clinical, to pre-radiographic, to radiographic damage of the joint.

Purpose: To evaluate combinations of imaging and biochemical biomarkers for cartilage breakdown, synthesis and quantity in the early period of post-arthroscopic meniscectomy.

Subjects and methods: Twenty young adults (three of them were females) who underwent unilateral arthroscopic partial meniscectomy were evaluated. The patients had a mean age of 32.5 years (range, 24-39), mean BMI of 28.5 kg/m2 (range, 24-34). Preoperative and six months postoperative US and MRI-based markers (cartilage thickness and volume, respectively) were quantified for medial and lateral tibio-femoral compartments for both knees. Preoperative, three and six months postoperative biochemical markers serum assays were measured; COMP and Col II (cartilage matrix breakdown) and PIICP (cartilage synthesis). These three markers were measured in an age, sex and BMI matched twenty healthy subjects for comparison.

Results: The meniscectomized knees had significantly lower total knee cartilage volume, P < 0.05 but non-significant mean thickness than the intact contralateral knees. Among the individual biochemical markers, PIICP had the highest significant diagnostic accuracy quantified as the area

under the receiver-operator characteristics curve (AUC) of 0.75 (95% confidence interval: 0.509- 0.912) higher than all others, P < 0.05 to distinguish subjects with progressive cartilage loss from non-progressors. Diagnostically, ratio of COMP and Col II to PIICP scored AUC of 0.90 (0.69- 0.98, higher than PIICP: P = 0.0001). For prediction of cartilage loss, none of the individual markers could be used.

Conclusion: Cartilage volume loss by MRI combined with changes in cartilage matrix turnover detected by molecular biomarkers may reflect the initial changes associated with cartilage degeneration that account for early OA.

A-020

Prevalence of Metabolic Syndrome among Ethnic Groups in China

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Background: To explore the prevalence of Metabolic Syndrome (MetS) among ethnic groups living in China.

Methods: A cross-sectional study was performed among 13263 subjects aged 35-98 years from five provinces in the North and South of China, including Han ethnic and six minorities:, Mongolians, Korean, Miao, Li, Tibetan and Tujia. The modified National Cholesterol Education Program Adult Treatment Panel III criteria were adopted to diagnose MetS.

Results: The crude prevalence of MetS ranged from 10.3% to30.3%. Koreans had the highest prevalence and the Miao had the lowest prevalence. After standardization of age using the data of the fifth national census conducted in 2000, the order of prevalence of MetS was Korean (24.1%), Mongolians (21.2%), Li (15.5%), Han (13.6%), Tibetan (12.7%), Miao (12.4%) and Tujia (10.1%). The prevalence of high blood pressure, hypertriglyceridemia and impaired fasting plasma glucose in Koreans was higher than in the other ethnic groups. Mongolians had the highest prevalence of central obesity; and the Tujia had lower high-density lipoprotein (HDL) cholesterol.

Consistent with previous research, logistic regression analyses showed that uric acid was significantly and positively correlated with MetS in the different ethnics.

Conclusions: The prevalence of Mets in Korean and Mongolians is higher than the other ethnics. Measures should be focused on these groups with high prevalence to promote disease prevention and control.

Keywords: Metabolic Syndrome, nationalities ethnic group, China

A-021

Investigation of role of food allergies in functional constipation etiology in childhood

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Introduction and Aim

Approximately 97% of cases with chronic constipation in childhood are associated with functional reasons. Some causes such as the delayed defecation, inadequate fluid and fiber intakes are important risk factors for functional constipation. In recent studies are shown that food allergy is also a risk factor for developing of functional constipation. However, the prospective studies in this subject are limited. Aim of this study is to determinate prevalence and effect of food allergies for functional constipation in children.

Methods

Functional constipation's diagnosis was performed according to Roma III criteria. Total 119 children with functional constipation were included in this study [61 female (51.3%), 58 male (48.7%)]. The mean age of these cases was 4.1 ± 2.6 (1-14) years. In each case, for the definitive and differential diagnosis, the results of hemogram, total IgE, specific IgE for the suspicious food allergens (cow's milk, egg-white, fish, wheat, peanut, soya bean, etc) and respiratory allergens (inhalant pollens, mites, animal hairs, etc), thyroid hormones, serum tissue transglutaminase-lgA, sweat test, and stool microbiological/parasitological tests were recorded. Also, the results of abdominal ultrasound and X-ray radiography were evaluated. The cases caused by organic diseases such as anatomic disorders, chronic illness, genetic syndromes, hypothyroidism, celiac disease and cystic fibrosis were excluded from the study. For the diagnosis of food allergy were used the elevated total IgE and specific IgE values and/or food elimination tests, and endoscopic methods.

Results

The significant IgE values (>100 IU/ml) were defined in 29 cases (24.4%). Food allergies were determined in 23 cases (19.3%), inhalant allergies were in 6 cases (5.0%). All cases diagnosed allergy had suffered from perianal disease (anal fissure and/or dermatitis). The most frequent food allergens were cow's milk and egg's white.

Conclusion

Food allergy has a high prevalence for functional constipation of childhood. Therefore, food allergy should be investigated in the cases with functional constipation.

A-022

Diagnosis of Infection Utilizing Accellix CD64

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Background:

Differentiating patients who are infected or not in the ICU can be very difficult. Present diagnostic tests remain inadequate. CD64 is constitutively expressed on the cell surface of PMNs and monocytes, but at low levels during the absence of infection. Upon invasion of a pathogen into the circulation, at a very early step of the immune host response, the expression level of CD64 on neutrophils increases dramatically. CD64 has a high specificity as its expression is not significantly elevated in malignancy of myeloid cells, any drug therapy (other than cytokines), clinical conditions with localized tissue damage, pregnancy and auto-immune disorders. The purpose of this study was to evaluate the Accellix CD64 instrument which provides results in 20 minutes in ICU patients with and without infections.

Methods:

The Accellix CD64 cartridge has all pre-analytical & analytical processing performed in the cartridge without further user intervention. This has been enabled by the development of a onetime use cartridge which includes onboard reagents and control material as well as an integrated flow cell. Infected (ICUi) and non-infected ICU patients (ICU Control-ICUc) and normal volunteers (C) had CD64 levels measured by the Accellix CD64

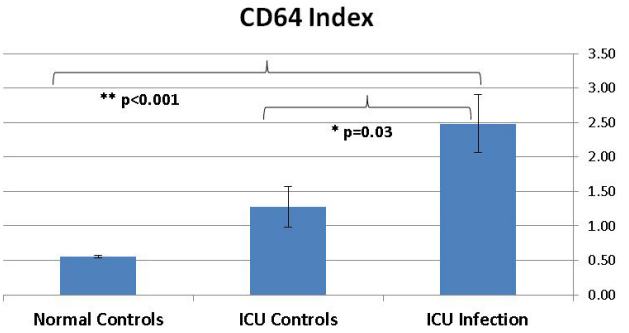
instrument. Measurements were calculated as 'CD64 index', i.e. the ratio

between the fluorescence of the PMN population and the fluorescence of control beads. ICU infection, ICU control and normal control patients' results can be seen in Figure 1.

Results:

Sixty patients were studied (ICUi-17, ICUc-13 and C-30). CD64 Index levels were higher (mean ± SEM) in ICU infection patients then ICU control and normal control patients (2.49 ± 0.42 vs. 1.28 ± 0.30 vs. 0.56 ± 0.02, p = 0.03 for ICU populations).

Conclusion: CD64 Index levels are higher in infected than non-infected ICU patients. Accellix CD64 is a promising instrument to differentiate infected from non-infected ICU patients in a timely manner.



A-023

Diagnosis and Prognosis of Sepsis in the Emergency Department - Usefulness of the Point-of Care Test PATHFAST Presepsin

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Background: Presepsin (sCD14-ST) serves as a mediator of the response to lipopolysaccharid from infectious agents. First evidence suggested that presepsin may be utilized as sepsis marker.

Objective: To examine the suitability of presepsin for diagnosis, prognosis and disease monitoring in patients suspicious of sepsis admitted to the emergency department (ED).

Methods: Presepsin was determined at presentation (baseline), after 8, 24 and 72 hours in 123 individuals admitted to the ED with signs of SIRS

(systemic inflammatory response syndrome) and/or infection. APACHE II score was established 24 and 72 hours after admission. 123 healthy individuals served as control group. Primary endpoint was death within 30 days. Presepsin was determined using the POC assay PATHFAST

Presepsin.

Results: Mean presepsin concentrations of the patient group at presentation and of the control group were 1945 ng/Ll and 130 ng/L, respectively (p<0.0001). Median presepsin levels at first presentation were 304, 544, 1944 and 2796 ng/L in differed highly significant in patients with SIRS (n=9), sepsis (n=74), severe sepsis (n=34) and septic shock (n=6), respectively. between comparable to the APACHE II score which has been established 24 hours after admission.

24 patients died during 30 days. The 30-day mortality was 19.5% ranging from 10 % to 32% between the 1st and the 4th quartile of presepsin concentration. ROC analysis revealed an AUC of 0.705. Non-survivors showed high presepsin values with

increasing tendency during follow-up while surviving patients exhibited significant lower values with decreasing tendency.

Conclusion: Presepsin demonstrated a strong relationship with disease severity and outcome. Presepsin provided reliable discrimination between SIRS and sepsis as well as prognosis and early prediction of 30-day mortality already at admission. Moreover, presepsin values showed close association to the course of the disease.

Course of presepsin during follow-up				
Presepsin Median (IQR), ng/L	Baseline	8 hours	24 hours	78 hours
Suvivors, n=114	590 (345-1396)	622 (367-1912)	574 (336-1610)	533 (324-1246)
Non-survivors, n=24	1763 (705-6616)	1859 (1001-5744)	1731 (809-4586)	2056 (811-5540
p-value	0.0046	0.0005	0.0033	0.0013

A-025

Effect of a hypnotic drugs on glycemic control in type 2 diabetes mellitus :Prediction of clinical improvement

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Objective:

Sleep disorder and/or increased stress are known to be worsening factors for glycemic control in patients with type 2 diabetes mellitus (DM). Some reports stated that better sleep improves insulin resistance, and that the combination of conventional antidiabetic and hypnotic drugs is sometimes administered to DM patients with a sleep disorder. This study was performed to evaluate whether the combination therapy with a hypnotic drugs improves HbA1c, and to establish a method of using clinical laboratory data to predict patients whose HbA1c will be improved.

Method:

We extracted 20,630 patients with type 2 DM who have been taking antidiabetic drugs and a hypnotic drug such as Zopiclone, Estazolam, Triazolam, Brotizolam, Zolpidem, and Diazepam, from a data warehouse of Kochi Medical School Hospital (from October 1981 to December 2014).

The starting day of this study was determined as period of 120 days without hypnotic drugs. HbA1c level at the starting day was determined as the pretreatment data. HbA1c level 30 to 120 days after the starting day was determined as the post-treatment data, and the difference of the HbA1c levels were compared. Cutoffs to identify HbA1c improvement were obtained by logistic regression and decision tree analyses by defining improvement and nonimprovement of HbA1c as the criterion variables, and the biochemical data and complete blood count data before the hypnotic therapy as explanatory variables.

Result:

The group receiving diazepam (n = 4,313) showed the greatest rate of HbA1c improvement (54.9%), followed by the group receiving zopiclone (n = 2,324; improvement rate, 48.4%). The other hypnotic groups also showed a similar rate. According to the logistic regression analysis (stepwise method), HbA1c, glucose (GLU), high-density lipoprotein cholesterol (HDLC), total cholesterol (T-CHO), and total protein (TP) were the important variables, with an area under the curve (AUC) of 0.68. The median of the selected group according to the prediction formula was -0.3 NGSP (%), whereas the median of the nonselected group was 0 NGSP (%). The Mann-Whitney U-test showed $P < 0.001$, suggesting a significant difference. According to the major cutoffs obtained by using the decision tree, patients showing HbA1c 6.3, T-CHO 156 and HDLC 29 or those showing HbA1c > 8.8; or those showing HbA1c > 7.0 and TP 6.1 tended to have improved HbA1c due to the hypnotic therapy.

Conclusion:

In type 2 DM patients receiving the combination therapy with a hypnotic drugs, HbA1c, T-CHO, HDLC and TP, identified those patients whose HbA1c will improve with the treatment. Moreover, diazepam had the greatest effect on glycemic control compared with other hypnotic drugs.

A-026

Novel Markers of Gestational Diabetes mellitus (GDM)

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Introduction: Gestational Diabetes Mellitus (GDM) is defined as any degree of glucose intolerance that occurs with pregnancy or is first discovered during pregnancy. The detection of GDM is important because of its associated maternal and fetal complications. At present, the standard protocol includes glucose challenge test (GCT) screening followed by a diagnostic 3-h oral glucose tolerance test (OGTT) at 24-28wks of gestation. These glucose-load methods have associated practical difficulties, poor patient compliance (prolonged fasting) and additional blood draws. Although glycosylated hemoglobin (HbA1c) has been a standard measure for monitoring diabetes, studies recommend limited utility of routine measurement of HbA1c for assessing glycemic control in pregnancy. Previous studies have proposed clinical utility of nontraditional shorter term glycemic biomarkers in gestational diabetes. Thus, the main objective of our study was to evaluate the efficacy of glycemia markers such as fructosamine (FRA), glycated albumin (GA), and 1, 5-anhydroglucitol (1, 5-AG) over the conventional 3-h OGTT in diagnosing GDM. Further, we wanted to establish diagnostic cut-off values for these markers.

Study Design: The current study was conducted at the Texas Children's Hospital laboratory using archived samples and was approved by Baylor IRB. A total of 124 samples were included in the study group categorized as group 1 (controls-normal screen; normal OGTT; n=34), group 2 (impaired GT-abnormal screen; normal OGTT; n=50) and group 3 (diagnosed GDM-abnormal screen; abnormal OGTT; n=40 according to the ADA criteria). Patient health information (PHI) was procured using the electronic medical records (EMR). All the three groups were analyzed for FRA, GA and 1,5-AG using the quantitative Enzyme linked immuno-sorbent assays (ELISA) using manufacturer instructions. The coefficient of variation (%CV) of these assays was <20%. Nonparametric statistical analysis was performed.

Results: Among the three proteins analyzed, fructosamine levels were not significantly different between the 3 groups by ANOVA ($p>0.05$) whereas glycated albumin levels were significantly different ($p <0.1$) in groups 1(controls) versus 2 (IGT) but not for groups 1 and 3 (GDM) respectively. 1, 5-AG levels were significantly different amongst all the groups [Controls - Median, Inter-Quartile Range 1.47U/L (1.21, 2.01); IGT- 1.01U/L (0.88, 1.15); GDM -1.16U/L (1.02, 1.26)] ($p <0.05$). Further, the cut-off values for each of these biomarkers were established using the receiver operator curves (ROC). Using 1.27U/L as the cut-off, 1, 5-AG represented 75% sensitivity and 65% specificity for diagnosing gestational diabetes.

Conclusion: Novel markers of GDM are urgently needed. These studies if confirmed with a larger sample size will provide an effective alternative to the diagnosis of GDM.

Keywords: Gestational diabetes mellitus, fructosamine, glycated albumin, 1, 5-anhydroglucitol, impaired glucose tolerance, biomarkers

A-027

Performance of Sofia Influenza A+B test compared to Luminex x-TAG respiratory viral panel assay in the context of institutional respiratory outbreak.

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Introduction: Influenza is an acute respiratory illness caused by Influenza A or B viruses that occur in outbreaks, mainly during the winter season. Rapid laboratory diagnosis of Influenza can help guide the clinical management of suspected patients effectively. Clinical sensitivities and specificities of the rapid influenza diagnostic tests varied considerably in the literature. Most of these studies are evaluated using previously frozen or stored specimens that had been previously tested positive. This study compares the performance of rapid Sofia Influenza A + B test to nucleic acid multiplex test x-TAG respiratory viral panel (RVP) assay in freshly collected nasal aspirates and measured simultaneously by both assays.

Methods: Retrospective data from 707 nasal aspirates (September 2014 to January 2015) collected from both adults and children tested simultaneously by both rapid Sofia Influenza A+B FIA immunofluorescence (Quidel, San Diego, CA) and qualitative nucleic acid multiplex RVP assay X-TAG Luminex technology (Luminex, Austin, TX) were analyzed.

Results: Concordance, analytical sensitivity and specificity were evaluated for both Influenza A, Sub types H1 and H3 and Influenza B. Prevalence for Influenza A by RVP is 16.3% and for Sub type H3 was 13.7%. Both non specific Influenza A and

subtype H3 were both positive in 13.8% of above population. None of the aspirates are positive for Influenza A Subtype H1. Only one out of 707 specimens was positive for Influenza B.

	Influenza A (Sofia- A vs. RVP-A)		Influenza A (Subtype H3) (Sofia-A vs. RVP-H3)	
	%	95% CI	%	95% CI
Sensitivity	56.7	46.1-64.9	65.0	54.6-74.4
Specificity	99.5	98.5-99.9	99.3	98.3-99.8
Positive Predictive Value	95.5	87.5-99.0	94.0	85.4-98.3
Negative Predictive Value	92.0	89.6-94.0	94.7	92.6-96.3
Agreement	92.4%		94.6%	

Conclusion: Sofia Influenza rapid test demonstrated good specificity and low sensitivity compared with a nucleic acid test for both Influenza A and Subtype H3. Sofia Influenza A + B test performed well in providing a rapid diagnosis, however confirmatory molecular testing is recommended for negative test results. Reevaluation of test performance should be periodically performed during outbreaks with the emergence and circulation of new influenza strains.

A-028

Cost-effectiveness Analysis in Prognosis of ST-Segment Elevation Myocardial Infarction: Choice of Optimal Laboratory Marker

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Background: The aim of this study was to explore discriminative abilities of several biomarkers of inflammation and hemodynamic stress as predictors for major adverse cardiovascular events (MACE) in patients with ST-segment elevation myocardial infarction (STEMI) treated by primary percutaneous coronary intervention (pPCI). Also, to assess their cost-effectiveness compared with the RISK-PCI score for the prediction of MACE during a 30-day follow-up after pPCI.

Methods: Using a decision model, we evaluated the costs, accuracy, and cost-effectiveness of each model. The RISK-PCI score was used as the baseline model. Other models were formed with the consecutive addition of selected markers: myeloperoxidase (MPO), high sensitive C-reactive protein, adiponectin, B-type natriuretic peptide (BNP), N-terminal-proBNP to the baseline model. A best-case model was formed from a combination of biomarkers to yield the best patient stratification algorithm. All models were assessed by their predictive probabilities using receiver operating characteristic curves. To accomplish our goals, we recruited 150 STEMI patients treated by pPCI. Composite 30-day major adverse cardiovascular events (MACE) was defined as cardiac death, non-fatal reinfarction, and target vessel revascularization. The analysis was performed from a third-party payer perspective.

Results: Only two strategies had outstanding discriminative abilities: the best-case model (RISK-PCI score+BNP+MPO) and RISK-PCI score plus BNP with area under the curve (AUC) values of 0.869 and 0.851, respectively. The cost-effectiveness ratio varied between 5199 € per AUC for the baseline model to RSD 9011€ per AUC for RISK-PCI score+NT-proBNP. After elimination of dominant strategies, the incremental cost-effectiveness ratio (ICER) for the remaining three strategies (baseline, RISK-PCI score plus BNP, and the best-case model) were calculated. For the RISK-PCI score plus BNP, the ICER (compared with the baseline model) was 18106 € per additional accuracy calculated for 100 analyses. The ICER for the best-case model (compared with the baseline model) was 84961€ per additional accuracy calculated for 100 analyses. Strategy involving hemodynamic stress biomarker BNP was more cost-effective than strategies involving inflammatory markers. Sensitivity analysis indicated that results were robust.

Conclusion: Our results support the feasibility of a multimarker approach for MACE prediction in STEMI patients treated by pPCI. The introduction of BNP in the clinical laboratory would be convenient and cost-effective.

A-029

Investigation of Red Blood Cells Alloantibodies after Transfusions in Southern Taiwan

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Background:

Development of red blood cell alloantibodies may lead to severe complications in patients receiving multiple blood transfusions and compromise the therapy.

Previous studies indicated that the rate of alloimmunization in chronically transfused patients was as high as 60 percent and specificity of alloantibodies varied for different regions, ethnics, and diseases.

Methods:

The study investigated the prevalence and type of unexpected red cell antibodies in a medical center with more than 1,200 beds. A total of 19,821 pre-transfusion records in 2012 were retrospectively reviewed for antibody screening tests at the facility in southern Taiwan where Han Chinese dominantly inhabited. Descriptive statistics were applied to determine the figures, based on the time interval between transfusion therapy and antibody detections.

Results:

The antibody screening test showed positive in 163 of the19,821 patients, indicating the overall alloimmunization rate of 0.82%. Further analysis revealed that 42.3 % (69/163) of cases with antibodies were found before arriving at the facility. The most common alloantibody identified was anti-Mi^a (47.9%), followed by anti-E (17.8%), anti-E+c (6.7%), anti-P1 (6.1%), anti-Le^a (5.5%), anti-M (4.9%), anti-Mi^a +E (2.5%), anti-Jk^a (1.8%), anti-S (1.2%) and the others (5.6%).

Additionally, 50.9 % (83/163) cases with alloantibodies were first identified after transfusion in this facility. Major antibodies detected after 7 days of transfusion included anti-Mi^a, anti-E, anti-P1 and anti-Jk^b.

Within one week, antibodies were found in 5 patients (6.0%), between 8-14 days in 11 patients (13.3%), between 15-30 days in 10 patients (12.0%), between 31-60 days in 6 patients (7.2%), between 1-12 months in 18 patients (21.8%) between 13-24 months in 10 patients (12.0%), and more than 2 years in 23 patients (27.7%). One specific case showed anti-S in18 months and anti-E in 21 months.

Furthermore, 45 cases (54.2 %, 45/83) of alloantibodies were developed after 2 units packed RBC administered and 33 cases (39.8 %, 33/83) were detected alloantibodies after more than 3 units packed RBC administered. Four cases (4.8%, 4/83) also developed alloantibodies after 12 units platelet concentrations administered, and 1 case (1.2%, 1/83) did them after 1 unit of Apheresis platelet was administered.

Conclusion:

In conclusion, the alloantibodies develop on the fifth day at the earliest posttransfusions. Platelet products containing small volume of red blood cells can stimulate formation of alloantibodies. Therefore, this study recommended that pre-transfusion testing be performed on blood samples collected within three days.

A-030

Potential role of biomarkers and cardiac imaging in scleroderma patients with subclinical myocardiopathy

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BACKGROUND

Scleroderma (Systemic sclerosis) (SSc), is a chronic autoimmune connective tissue disease which extensive fibrosis, vascular alterations and autoantibodies against various cellular antigens area among the principal features. SSc can cause pulmonary arterial hypertension (PAH) that is a major risk factor for death.

AIM

To evaluate in patients with SSc the relationship between different biomarkers and the clinical, immunological and biological profiles (inflammatory and profibrogenic) and parameters of the cardiac magnetic resonance imaging (MRI) and echocardiography.

METHODS

Twenty patients diagnosed with SSc were prospectively enrolled and compared to nine healthy controls. All underwent screening for cardiac complications evaluated by MRI and echocardiography. Systolic eccentricity index (EIsys) >1) and pulmonary-artery mean velocity (APVEL) <11.7 cm/s were defined as pathological. We measured

different serum biomarkers by multiplex technology for inflammation (IL-13,IL-6,TNFα), vasculopathy (VEGF), and fibrosis (endoglin, GDF-15, PDGFR and TGFβ) and cardiac biomarkers (NT-proBNP - Siemens Vista and hs-cTnT - Roche) by chemioluminiscence assays.

RESULTS

Mean age was 54 years and 80% were women. Two patients develop PAH. In multivariate analysis, TNF-α and GDF-15 where significantly higher in patients. We found a significant positive correlation between hs-cTnT in patients with arterial hypertension. Patients with SCL-70, anticentromere antibody and diffuse disease showed higher concentrations of PDGFR. One patient with PAH showed higher concentration for inflammatory and vascular biomarkers. We couldn't establish any correlation between biomarkers and right ventricular thickness. A positive correlation was found between hs-cTnT, endoglin and TGFβ2 and EIsys. Similar results were observed for NT-proBNP and APVEL.

CONCLUSIONS

PFGFR (biomarker for fibrosis) is increased in patients with autoantibodies.

Cardiac biomarkers (hs-cTnT and NT-proBNP) may be useful screening tools to identify subclinical cardiac disease in SSc patients which can develop PAH leading to right ventricular dysfunction, heart failure and death.

We couldn´t establish a relation between concentrations fibrotic biomarkers and ventricular thickness.

A-031

Evaluation of Soluble Urokinase Plasminogen Activator Receptor and Presepsin in Patients Presenting with Sepsis in the Emergency Department

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Background: Urokinase plasminogen activator receptor is expressed on the cell membrane of various cell types. Its soluble form (suPAR) is increased in critical ill patients especially with infectious diseases and sepsis. First evidence suggested that suPAR may serve as a prognostic marker. As presepsin has already shown convincing results in prognostication we compared suPAR and presepsin (PSEP) with procalcitonin (PCT) and the APACHE II score in patients presenting with sepsis in the emergency department (ED).

Methods: suPAR, PSEP, PCT were determined using commercial available assays (suPARnostic virogates, PATHFAST, BRAHMS Kryptor) in 69 patients with sepsis at admission to the ED. Primary endpoint was death within 30 days. The combined endpoint "major adverse event" (MAE) consisted of at least either the primary or at least one of the secondary endpoints intensive care, mechanical ventilation or dialysis.

Results: PSEP, PCT and APACHE II score differed significantly

between patients with sepsis, severe sepsis and septic shock (p-values: 0.0028, 0.01 and <0.0001, respectively) whereas the difference of suPAR was only slightly significant (p=0.0752). The 30-day mortality was 27.5%, ranging from 7.3% in sepsis to 44% in severe sepsis and 80% in septic shock. ROC analysis for discrimination between survivors and non-survivors revealed AUC values of 0.883, 0.727, 0.568 and 0.835 for PSEP, suPAR, PCT and APACHE II score, respectively. PSEP demonstrated a stronger relationship with 30-day MAE compared with suPAR and PCT: AUC-values: 0.753, 0.615, 0.610, respectively.

Conclusion: The prognostic accuracy of suPAR was superior to PCT but not to PSEP. Although suPAR provided reliable prognosis and prediction of 30-day mortality, the prognostic power of PSEP was superior to PCT and suPAR as well as to the APACHE score for outcome prediction (mortality and MAEs). PSEP was also superior in discrimination between sepsis, severe sepsis and septic shock.

Biomarker values in patients with or without MAEs and in survivors and non-survivors				
Medians (95% CI)	suPAR	Presepsin	PCT	APACHE II
No MAE, n=43	8.6(6.9-10.4)	782(556-988)	1.35(0.69-2.73)	14(12-19)
MAE, n=26	11.0(8.2-17.7)	1777(1081-3135)	2.17(1.08-15.9)	29(24-35)
p-value	0.1096	0.0003	0.1083	0.0001
Survivors, n=50	8.6(7.0-10.4)	804(615-992)	1.56(1.13-3.39)	18(13-23)
Non-survivors, n=19	13.2(8.5-18.9)	2124(1376-3563)	2.78(0.38-20.9)	29(24-36)
p-value	0.0643	0.0001	0.6917	0.0003
AUC	0.727	0.833	0.568	0.835

A-032

Molecular cytogenetic study of deletion on derivative chromosome 9 in a CML patient with BCR/ABL rearrangement

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Background: The BCR/ABL gene rearrangement is the causing factor in chronic myeloid leukemia (CML). In most cases, it is cytogenetically identified a translocation between chromosomes 9 and 22, resulting in the presence of the Philadelphia chromosome (Ph). Deletions around the breakpoints on the derivative chromosome 9 including ABL and BCR sequences in Ph+ CML patients are thought to have prognostic value. The present study reports a case of translocation involving chromosomes 9 and 22, with a possible deletion on derivative 9 identified by conventional cytogenetic and confirmed by fluorescence in situ hybridization (FISH).

Methods and results: In september 2014, sample of a 41 year old patient with lecocitoses, severe anemia and thrombocytosis was referred to our institution to perform cytogenetic and molecular testing.. Cytogenetic analysis showed the presence of the translocation between chromosomes 9 and 22 as well as a possible deletion of the derivative chromosome 9 in sixteen among the twenty examined metaphases. The karyotype was: 46,XX,t(9;22)(q34;q11.2)?del(9)(q22q13)[16]/46,XX[4]. FISH using the LSI BCR/ABL Dual-Color, Dual Fusion (D-FISH) probe showed one fusion, one green and one red signal (1F1G1R) confirming the presence of the BCR/ABL1 translocation and deletion on derivative chromosome 9. Multiplex qualitative reverse transcriptase-polymerase chain reaction detected the transcript BCR/ABL, isoform p210 (b3a2). Real-time quantitative polymerase chain reaction (RQ-PCR) was performed after the beginning of Gleevec therapy to quantify BCR/ABL1 transcript and evaluate the treatment response. The level of BCR/ABL1 expression was 0.54% (IS), indicating a good molecular response.

Conclusion: FISH can be used as an adjunct to the karyotyping to detect the deletion on derivative chromosome 9. Patients evaluated in this study were good molecular response to treatment, although some authors suggest inferior survival as well as poor response to therapy in CML patients carrying derivative 9 deletions.

A-033

The Economic Burden of Confirmatory Testing for Cardiac Troponin and Human Chorionic Gonadotropin: A Multi-Site Laboratory Database Analysis.

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Background: Erratic false positive human chorionic gonadotropin (hCG) and cardiac troponin (cTn) results have been reported on multiple analytical platforms. Many laboratories minimize the reporting of such results through confirmatory testing i.e. re-centrifugation and reanalysis of select patient samples. However, this practice delays clinical decision making and also increases healthcare costs. We analyzed retrospective data from Edmonton, Alberta to quantify the economic burden of such confirmatory testing to the laboratory.

Methods: hCG confirmatory testing practices are uniform across the city; any sample with 5 U/L < hCG < 100 U/L is re-centrifuged and reanalyzed. Four months of hCG data were extracted from three laboratories. In contrast, cTn confirmatory testing

varies across the city. One laboratory re-centrifuges and reanalyzes all first-time results >0.15 microgram/L; a second laboratory does so if cTn >0.10 microgram/L; a third laboratory does not regularly confirm results. Four months of cTn data were extracted only from the two laboratories that perform confirmatory testing. We analyzed the data for the total number of tests, number of first-time positive results, and number of false positive results. This information was combined with costing for test supplies and technician time to calculate the overall cost of confirmatory testing.

Results: The table summarizes the results of our analysis. The overall financial burden of hCG and cTn testing ranged from 8,154 USD per annum (small community hospital) to 11,816 USD per annum (large academic center).

Conclusions: To limit the risk of using a potentially false-positive hCG or cTn result to inform clinical decision-making, many laboratories have confirmatory testing policies in place. Our results indicate that such retesting is of considerable economic burden to any laboratory. Improvements in analytical precision of these assays as well as improvements to preanalytical sample quality could reduce or eliminate this burden.

	Patients Seen Annually	Total # Tests (doesn't include repeats)	First-Time Positives	Repeated First-Time Positives	False Positives	False Positive Rate (%)	Cost to Lab Per Annum (USD)
HCG							
Academic Center	700,000	2,342	108	99	44	44	2,417
Large Community Hospital	450,000	9,076	188	173	35	20	4,223
Small Community Hospital	11,900	826	71	63	0	0	1,538
cTn							
Academic Center	700,000	13,465	1180	385	26	7	9,399
Small Community Hospital	11,900	5,289	344	271	17	6	6,616

A-034

Plasma cytokines augment Procalcitonin levels in septic patients admitted to Oncological Intensive Care Unit

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Background: Sepsis biomarkers may be useful to rule out infection, serve as markers of disease severity including need for ICU admission, and in evaluating the patient's clinical course.

Objective:

Our objective was to evaluate the diagnostic and prognostic value of procalcitonin (PCT) and 5 select cytokines (TNF-α, IL-1β, IL-6, IL-8, interferon-gamma (IFN-γ) in cancer patients admitted to the ICU with suspected severe sepsis and septic shock.

Methods: We conducted an observational quality improvement study that evaluated 25 patients who were admitted to the ICU with suspected severe sepsis or septic shock over a 6-month period. One patient was

excluded due to specimen availability limitation.

Design: Prospective, observational study.

Setting: Adult oncology medical-surgical ICU at a tertiary cancer center.

Subjects: 24 patients (10 with suspected severe sepsis or septic shock and 14 control subjects).

Interventions and measurements: Serum PCT (VIDAS Brahms PCT, Biomerieux) and plasma TNF-α, IL-1β, IL-6, IL-8, and IFN-γ) (MSD electrochemiluminescence assay (Meso Scale Discovery, Gaithersburg, MD) levels were measured within 24h (Day 1) of ICU admission.

The samples were batched and the PCT and cytokine results were not available for clinical use. Receiver operating curves (ROC) for PCT and the 5 cytokines and their relationships to the Sequential Organ Failure Assessment (SOFA) scores on Day 1 and patient outcomes were analyzed.

Results: The mean SOFA score on Day 1 of the septic patients was 8.9 (range 2-14). The AUC for PCT, TNF-α, IL-1β, IL-6, IL-8, IFN-γ on Day 1 were 0.871, 0.806, 0.51, 0.889, 0.722, and 0.833, respectively. The highest AUC was demonstrated when IL-6 was coupled with PCT (0.9) and was lowest with IL-8 and PCT. 50% of the septic patients died. Nonsurvivors had higher levels of PCT, IL-6 and IL-8 on Day 1.

Conclusions:

Our preliminary findings suggest that plasma levels of IL-6 augment PCT in diagnosing severe sepsis/septic shock in cancer patients admitted to the ICU. A larger, more comprehensive study is needed to substantiate these findings.

A-035

Utility Of A.D.A. Levels In Body Fluids From Peri urban Locality-India

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BACKGROUND-Adenosine deaminase (ADA)is rapid sensitive inexpensive diagnostic biomarker in body fluids for diagnosis of tuberculosis.

AIM- To assess the specificity and sensitivity of ADA levels in body fluids for diagnosis of tuberculosis.

Material and Method- Adenosine deaminase (ADA) in 111 body fluids- 72 pleural fluids, 13 ascitic fluids, 26 cerebrospinal fluids and 3 serum samples was estimated by Gisti G Galanti method using kit manufactured by Tulip diagnostics.

Negative and positive were classified using cut of limits as per the kit reference.

These results were correlated with final diagnosis achieved by radiological, cytological, fluid protein level, ESR, and clinical correlation.

Results- Pleural fluids, out of 72, 62 were true positive with sensitivity 92 % specificity 100 %.

Limitation of the study _TB culture, molecular methods were not used.

CONCLUSION - ADA is rapid sensitive and inexpensive diagnostic biomarker for tuberculous pleural effusion.

A-036

The ALaRMS Score: Validation of Lab-based Mortality Prediction in a Swiss Population

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Background:

ALaRMS is an inpatient mortality predictive model (Acute Laboratory Risk of Mortality Score, published in 2013) based on additive scoring of routine laboratory measurements. This model was assumed to yield accurate mortality predictive information with an AUC of 87% in an U.S. population. In our study, we validated this model in a swiss tertiary hospital cross-sectional cohort.

Methods:

The laboratory data included the years 2012 and 2013 with a total number of 56101 cases. 54418 patients survived hospitalization. As the Inselspital uses troponin T testing and ICD-10 classification, the scoring had to be adapted accordingly. We computed ALaRMS scores for all cases, added the information about survival, and generated ROC curves and AUROCs with bootstrapped confidence intervals.

Results:

In our cohort, we found a slightly lower, but comparable AUC of 83.04% (95% CI 82.06 - 84.03) for the ALaRMS model alone.

Outlook:

In a subsequent step, we aim to optimizing the ALaRMS model by penalized logistic regression modeling to take full advantage of the quantitative nature of our laboratory data and to narrow down the number of laboratory tests needed for efficient and effective mortality prediction.

A-037

Obesity and Oxidative Stress

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Background:

Obesity is become one of the most common risk factor of metabolic syndrome. Obesity and its releated conditions are lead to oxidative stress and inflammation. The aim of this study was to investigate the relationship between inflammatory parameters and obesity.

Methods:

37 obese adolescents (19 boys and 18 girls, median age:11, age range 6-16) were randomly select from obese children who were received to Bezmialem Vakif University Pediatric Endocrinology Clinic in Turkey. The control group consisted of 37; age- and gender- matched healthy children. All children's weight and height measurements were performed by the same person who used same equipments. BMI was calculated that body weight (kg) divided by height in meters squared.

After these measurements, HOMA-IR, types of cholesterol, CRP, total antioxidant activity (TAS), total oxidant status (TOS), thiol, catalase and paroxanase (PON) were assayed in serum samples. The SPSS software (ver. 11.5 for Windows; SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results:

Differences between groups were evaluated by Mann Whitney U test. BMI (P<0,001), HOMA-IR (P<0,001), total cholesterol (P<0,001), HDL cholesterol (P<0,001), LDL cholesterol (P<0,001), triglyceride (P<0,001), CRP (P=0,034), TAS (P<0,001), TOS(P=0,006), thiol (P=0,001) are significantly higher in obese group. There were no difference in catalase(P=0,152) and PON (P=0,273) between two groups.

Conclusion:

This study suggests that obese children are exposed to more oxidative stress than normal-weight children. Their lipid profiles are more abnormal than control group. Therefore, obese children are more prone to cardiovascular disease than normal-weight children.

A-038

Aldosterone Status in Patients with Obstructive Sleep Apnea and Resistant Hypertension.

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Background: There is a strong association between Obstructive Sleep Apnea (OSA) and Resistant Hypertension (RH) and also between RH and hyperaldosteronism. Previous studies have shown controversial results about aldosterone and renin measurements related to OSA severity in patients with RH. The objective of this study was to evaluate the relationship between OSA and aldosterone status in patients with RH. **Methods**: We evaluated patients with RH and OSA (determined for polysonnography with apnea / hypopnea index - AIH). It was considered moderate apnea AIH > 15, and severe when AHI > 30. All patients were evaluated with 24-hour Ambulatory Blood Pressure Monitoring (ABPM); laboratory tests including plasma renin activity and serum aldosterone (in a first visit) and aldosterone measured in 24-hour urine (in a second visit). Patients were instructed to withdraw spironolactone four weeks before blood tests; 24-hour urine was obtained in use of all antihypertensive scheme including diuretics and spironolactone. Aldosterone was determined by Radioimmunoassay (Siemens, Los Angeles, CA); renin was determined by Chemiluminescence using Liaison Direct Renin kit (DiaSorin, Saluggia, Italy), the results were converted to renin activity and after used to calculate aldosterone-to-renin ratio (ARR). Statistical analysis was performed with SPSS 17.0; we compared severe and moderate OSA in a bivariate form; continuous variables were analysed with T test for normal distribution or Mann Whitney for abnormal; X^2 test was used for categorical variables. **Results:** 122 patients were evaluated, 48 (39%) men, the mean age was 62 ± 7 years; AHI average was 40 ± 20. Patients with AHI > 30 (severe OSA) had increased serum aldosterone (11.6 ± 7.4 ng/dL vs 8.5 ± 5.2 ng/dL, p = 0.042) and plasma renin activity (8.0 ± 11.2 ng/mL/h vs 2.9 ± 6.7 ng/mL/h, p=0.008) when compared to group with moderate OSA. ARR and Urinary aldosterone were similar in both groups (10.9 ±17.1 vs 14.3 ± 22.6, p= 0.42 and 8.9 ± 5.1 mcg/24h vs 7.7 ± 6.6 mcg/24h, p= 0.24, respectively). **Conclusions**: Severity of OSA was associated to higher serum aldosterone and higher plasma renin activity in patients with RH. There is no association with hyperaldosteronism based on ARR. Similarly, there was no statistical difference in Urinary Aldosterone levels based on severity of apnea, what probably was associated to use of antihypertensive drugs.

A-039

The Levels Of Vaspin And Chemerin Levels And Correlation Between Cardiologic Parametres In Patients Wıth Psorıasis Vulgaris

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BACKGROUND: Significant associations between psoriasis and obesity or being overweight have been observed. Adipokines are involved in the pathogenesis of psoriasis and they are biomarkers of obesity-related inflammation. Chemerin is a newly identified adipokine and high systemic chemerin level was found as an independent marker of the metabolic syndrome. Vaspin is the anti-inflammatory adipokine, is discussed as a new link between inflammation and obesity. This study investigated vaspin and chemerin levels in the serum of psoriatic patients and healthy controls. and an association between vaspin-chemerin levels with predictors of subclinical cardiovascular disease in patients with psoriasis.

METHODS: A total of 56 patients suffering from psoriasis and 32 age-¬matched controls were included in the study. Vaspin and chemerin serum levels were analysed by ELISA. All patients were evaluated by transthoracic Doppler echocardiography. The psoriasis area severity index (PASI) was calculated in all psoriatic patients.

RESULTS: The serum concentration of soluble chemerin was significantly higher in psoriatic patients compared to healthy controls (330.9±70.1 ng/mL, 300.8±59.6 ng/mL, respectively; p=0.04). There was no significant difference in the serum concentration of soluble vaspin between psoriatic patients and the control group (129.9 pg/mL, 126.4 pg/mL, respectively; p>0.05). The epicardial fat tissue (EFT) was significantly increased (0,33±0,13 cm vs. 0.25±0.12 cm; p=0.04) in patients with psoriasis compared with the controls. Flow mediated dilatation (FMD) was lower in psoriatic patients compared to the control group (6,66±2,44, 11,77±3,28, respectively; p=0,000). It was found the ratio of mitral peak velocity of early filling to early diastolic mitral annular velocity (E/E') for the psoriatic group to be higher than the control (5,59±1,98, 4,69±0,94, respectively; p=0,032). The ratio of early diastolic mitral annular velocity (E') to late diastolic mitral annular velocity (A') was lower in psoriatic group than the control (1,05±0,4 vs. 1,32±0,47, p=0,014). The myocardium performance index (MPI) was found lower in psoriatic patients than the control (0,54±0,06 vs. 0,60±0,09, p=0,009). There was no correlations between vaspin with FMD, EFT and other echocardiography parameters. Chemerin correlated with ratio of mitral peak velocity of early filling (E) to mitral peak velocity of late filling (A).

CONCLUSIONS: Patients with psoriasis have higher blood levels of chemerin. In psoriatic patients there was subclinical cardiac disfunction.There was a correlation between serum chemerin level and cardiac diastolic disfunction that was not previously shown. These findings suggest that chemerin may play a role in the pathogenesis of psoriasis and can be used as markers of the disease. Further studies, investigating the interplay between vaspin, chemerin, inflammation, and psoriasis are needed.

A-040

A prospective multi-site evaluation of the intra-menstrual cycle variability of anti-müllerian hormone (AMH) using an automated AMH immunoassay

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Background and Objective: Access AMH[1,2] is a biomarker that is being developed for evaluating response to controlled ovarian stimulation in women undergoing in vitro fertilization procedures. Published results for intra-menstrual cycle variability lack agreement. The purpose of this study is to determine whether or not AMH levels vary significantly across the normal menstrual cycle.

Methods: 24 apparently healthy women were prospectively enrolled from 2 sites with IRB-approved informed consent. Blood samples were collected 2 times per week throughout each complete menstrual cycle (21 to 35 days) starting with baseline (day

2 to 4). Eligibility criteria: ≥ 18 years to ≤ 45 years, both ovaries present, no polycystic ovary syndrome (PCOS), no history of ovarian surgery, no exposure to cytotoxic drugs or pelvic radiation therapy, no recent contraceptive use, and no other recent hormonal therapy. Serum samples were tested on the Beckman Coulter Access 2 immunoassay analyzer[3]. Age-adjusted mixed-effects models were constructed to estimate intraclass correlation (ICC) and within-subject variability across the menstrual cycle.

Results: 191 specimens were collected from 24 women (mean age 35 years; range 24 to 45 years). Older age was significantly associated with lower mean AMH values (p-value = 0.004). There was no evidence of a linear trend in AMH levels across cycle days (p-value = 0.409). AMH showed more variability for levels ≥ 3 ng/mL and less variability for levels < 3 ng/mL. The estimated ICC was 0.94 (95% confidence interval, 0.89-0.96), indicating that 6% of the overall variability in AMH was due to within-subject variability.

	N			Within Subject		Between Subject		
AMH Category	Subjects	Samples	Mean AMH (ng/mL)	SD	CV	SD	CV	ICC
All	24	191	3.9	0.76	19.4%	3.06	78.3%	0.94
≥3 ng/mL	12	96	6.6	1.06	15.9%	2.93	44.1%	0.89
<3ng/mL	12	95	1.1	0.22	19.3%	0.79	69.3%	0.93

Conclusion: No trend in AMH results was observed throughout a normal menstrual cycle. Fluctuations in AMH results during the menstrual cycle accounted for only 6% of the overall variability.

[1]Not available in the US; [2]Not intended as off-label promotion of any BCI product; [3]All trademarks are the property of their respective owners.

A-041

Unexpected karyotype: Down syndrome with a de novo (dup21q) with a mother carrier of a rob(14;21)

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Introduction: Down syndrome, the most frequent form of mental disability caused by a microscopically demonstrable chromosomal aberration, is characterized by a well defined and distinctive phenotypic features and natural history. Most individuals (95%) with trisomy 21 have 3 free copies of chromosome 21; in about 5% of patients, 1 copy is translocated to another acrocentric chromosome, most often chromosome 14 or 21. In 2 to 4% of cases with free trisomy 21 there is recognizable mosaicism for a trisomic and a normal cell line. Objective: Here we describe a patient with classical features of Down Syndrome with a (dup21q) and her mother carrier of a rob(14;21). Methods: The cytogenetic study was held after chromosome analysis of metaphases obtained from culture of peripheral blood lymphocytes PHA-stimulated, Giemsa Banding and karyotyping according the ISCN 2013. Results: The cytogenetic analysis demonstrated a 45,XX,rob(14;21)(q10;q10) (fig.1) and 46,XX,+21,rob(21;21)(q10;q10) (fig.2). Discussion: Most de novo t(14;21) trisomies originate in maternal germ cells . The mean maternal age is 29.2 years. In de novo t(21;21) Down Syndrome the situation is different , in most cases the t(21;21) is an isochromosome (dup21q) rather than the result of a Robertsonian translocation caused by a fusion between 2 heterologous chromatids. About half were of paternal and half of maternal origin. In de novo t(21;21) true Robertsonian trisomy 21 cases, the extra chromose 21 was maternal. In this case the expected karyotype would be the presence of the translocated chromosome rob(14;21) maternaly inherited but a de novo event : a (dup21q) happened leading to the same diagnosis of Down syndrome. We hypothetized that in this case the (dup21q) is an isochromosome probably of maternal origin as she is a carrier of a translocation with altered recombination patterns and nondisjunction enabling the formation an isochromosome.

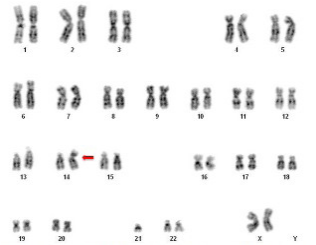

Figure 1: 45,XX,rob(21;21)(q10;q10) the mother karyotype showing the Robertsonian Translocation, involving the chromosomes 14 and 21 (red arrow)

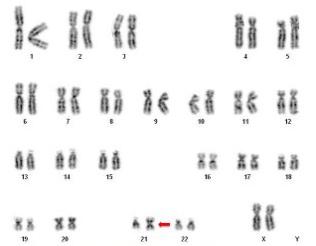

Figure 2: 46,XX,+21,rob(21;21)(q10;q10) The daughters karyotype showing the Robertsonian Translocation, involving the chromosomes 21 with trisomy of long arm of chromosome 21 (red arrow)

A-042

Double aneuploidy with a karyotype 48,XXY,+21

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Introduction: A chromosome abnormality reflects an atypical number of chromosomes or a structural abnormality in one or more chromosomes including autosomes and sex chromosomes. Double aneuploidy is the presence of two different chromosomal abnormalities in the same individual and it's a relatively rare condition. Trisomy 21, also named Down Syndrome (DS) is caused by the presence of an additional autosome, affecting 1 to 700 live births. Klinefelter Syndrome (KS) is a genetic condition characterized by an extra X chromosome resulting in a 47,XXY karyotype. This karyotype exists in roughly between 1 to 500-1000 live male births. Though trisomy 21 and numerical sex chromosomes anomalies are both common disorders, the co-occurrence of both is rare. A high percentage of double and multiple chromosomal aneuploidies were observed in spontaneous abortion and mostly involving acrocentric chromosomes. Only a few cases of double aneuploidy (DS+KS) have been reported in the literature since its first report by Ford et al in 1959. Objective: Here we present a case of postnatal diagnosis of double aneuploidy DS +KS. Methods: The cytogenetic study was held after chromosome analysis of metaphases obtained from culture of peripheral blood lymphocytes PHA-stimulated, Giemsa Banding and karyotyping according the ISCN 2013. Results: The cytogenetic analysis demonstrated a 48,XXY,+21 karyotype (Fig.1). Discussion: Double aneuploidy usually are associated with advanced maternal age, caused possibly by non-disjunction during meiosis, which may cause chromosomal changes sexual or autosomal in offspring. The patient with double aneuploidy can present characteristics of both chromosomal anomalies. The occurrence of double aneuploidy +21 and XXY is rare, published data are scarce, and yet, the incidence and risk of recurrence are difficult to determine. This case report may add to other cases in order to improve the study of the incidence and recurrence of this rare finding.

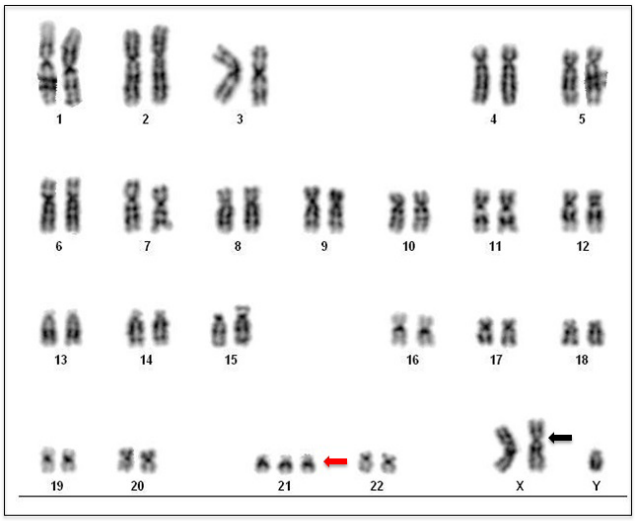

Figure 1: 48,XXY,+21 Karyotype showing the double aneuploidy , trisomy 21 (red arrow) and the extra X chromosome (black arrow)

A-043

Comparison of Performance: Siemens CLINITEK Novus Urine Analyzer and CLINITEK Advantus Urine Analyzer

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Background: The ability to offer a full set of solutions to accommodate different testing volumes or sites with high correlation of results between analyzers is imperative for all healthcare networks. As patients move between care environments (physician office to a specialized clinic, hospital emergency department, or inpatient setting), clinicians need consistent results to ensure that the test result information leads to the same clinical decision. This requirement for end-to-end solutions is not

new but has recently become more urgent in discussions between clinicians and laboratory management.

Comparable performance among urine analyzers, whether used in a centralized lab, a point-of-care testing site, or a backup situation, is essential in the clinical environment. This is particularly true in the instance where one instrument is unavailable and another must match its level of performance in terms of sensitivity, specificity, and reliability to ensure consistent test outcomes. The objective of this study is to compare the performance of three strip types on the CLINITEK Advantus® instrument to the CLINITEK Novus® PRO 12 cassette on the CLINITEK Novus instrument, using contrived urine samples.

Methods: The study analyzed the CLINITEK Novus urine analyzer using the CLINITEK Novus PRO 12 reagent cassette and the CLINITEK Advantus urine analyzer using MULTISTIX® 10SG, MULTISTIX 10LS, and CLINITEK® Microalbumin 9 reagent strips. Testing was conducted on two lots of each reagent with two instruments of each type. Clinical specimens were contrived to each of the CLINITEK Novus PRO 12 cassette block outputs for each analyte using a single instrument.

Results: Excellent performance was observed with albumin, bilirubin, ketone, protein, and nitrite tests across the entire reporting range. 99.0% of all results for all analytes fell within 1 target block output. A false-negative rate of 1.8% and a false-positive rate of 0.2% were observed when removing pH and specific gravity testing. 93% of pH results were within 0.5 pH units and 100% of specific gravity results were within 1 block when rounding CLINITEK Novus system quantitative results to CLINITEK Advantus system block outputs.

Conclusion: Providing an end-to-end solution for urine analyzers requires comparable performance of systems, whether used in a centralized lab, a point-of-care testing site, or a backup situation. This study demonstrates that the CLINITEK Novus analyzer and CLINITEK Advantus analyzer provide strong result agreement allowing for consistent clinical decision making.

A-044

High "Normal" Potassium Poses Mortality Risk for All Patients

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Background: Recent studies show increased mortality in acute myocardial infarction (AMI) patients with serum potassium levels of 4.5-5.0 mEq/L, which is within the reference interval used by most laboratories. These findings have created an unresolved controversy challenging established potassium repletion therapeutic targets. We hypothesize this higher risk is applicable generally, not just to AMI patients.

Methods: Retrospective cohort study of 375,747 hospital visits at Sarasota Memorial Hospital for years 1998-2014; and for years 2012-2014 at a major academic medical center in the northeast and a regional hospital in the southwest. Primary outcomes were in-hospital and one-year mortality. Models of mortality were generated and fit by logistic regression, yielding multivariate adjusted odds ratios for potassium-linked mortality.

Results: Utilizing logistic regression with adjustment for possible confounding factors, our analysis for all patients, independent of diagnosis, yields lowest mortality at potassium values from 3.5 to 4.5 mEq/L, with significantly higher risks beyond 4.5 mEq/L. For both the AMI cohort and the non-AMI cohort, in-hospital all-cause mortality odds ratios were above 1.8 ($p<0.001$) for potassium between 4.5 and 5.0 mEq/L (within the usual reference interval); and were above 3 ($p<0.001$) for potassium between 5.0 and 5.4 mEq/L (often considered within the reference interval). Adjusting for serum Creatinine levels > 2.0 mg/dL produced the same high "normal" mortality risks. Our findings hold for one-year post-discharge mortality, as well as in-hospital mortality. While the risk functions differ in detail between AMI and other patients, we find that both show minimum risk within the same cut-points, with substantial increased risk above 4.5 mEq/L.

Conclusions: Our analysis extends the AMI finding: all patients have an increased mortality risk for serum potassium levels above 4.5 mEq/L. The etiology of death associated with mild hyperkalemia remains unclear. Presence of renal insufficiency appears not to account for this increased mortality. Without prospective studies, our findings cannot establish safety or danger of potassium repletion therapeutic targets. Finally, we point out that standard reference intervals are not based on patient risk, but are defined as the central 95% of test results for a "healthy" cohort. Reference interval cut-points would be more meaningful with a risk-based methodology.

A-045

Assessment of Hyperkalaemia in the Medical Emergency Unit

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Objective: To assess the prevalence, severity and risk factors of Hyperkalaemia and its effect on mortality and length of hospital stay of patients requiring emergency medical care.

Methods: A retrospective analysis was carried out on the first potassium level on admission of all patients admitted through the Medical Emergency Unit (ward F1), from January 2013 to March 2014, of Tygerberg Hospital, a tertiary health center in Western Cape Province, South Africa. Using the computerised hospital database we retrieved information on the following: (1) Number of patients that presented with Hyperkalaemia on admission (2) Severity of Hyperkalaemia classified as mild, moderate and severe (3) Risk factors; age, sex and diagnosis on admission (4) Clinical impact; length of hospital stay and hospital mortality. Hyperkalaemia was considered at serum potassium >5.2 mmol/l while critical hyperkalaemia >6.0 mmol/l according to established cut-offs for the reference population.

Results: Over a period of 15 months One hundred and thirty nine thousand one hundred and fifty three (139,153) requests for serum potassium was received by the laboratory out of which thirteen thousand one hundred and sixty four (13164) accounting for ten percent (10%) of the total request was from the medical emergency unit. 1 out of every 5 of the serum potassium result from the medical emergency unit was abnormal. The overall prevalence of hyperkalaemia was 8.1% and 12.3% of this was within the critical range. Gender was not a significant risk factor (M:F, 1.1;1) while mean age of presentation was 52 ± 2.6 years. Diagnosis on admission was related to the Genitourinary (25.8%), Cardiovascular (15.3%), Pulmonary (14.2%), Endocrine (11.9%), Central Nervous system (7.3%), Sepsis (6.5%), Cancer (5.3%), Haematological (4.3%), Gastrointestinal system (4.3%), others (5.8%). In hospital mortality and length of hospital stay was higher ($p<0.05$) among patients with hyperkalaemia (38%, 9 days) than in patients with normal serum potassium (6%, 7 days).

Conclusions: Hyperkalaemia is a common problem among patients requiring emergency medical care. It results from various medical conditions and it is associated with high In-hospital mortality and longer length of hospital stay. It is therefore important to recognize this problem early and institute early treatment measures in order to improve the prognosis.

A-046

Laboratory Job Safety/Hazard Analysis

S. M. Ahmer, S. A. Baloch, N. Ali. *Aga Khan University & Hospital, Karachi, Pakistan*

INTRODUCTION: During an External Quality Audit in 2013, Administrator surveyor raised a query over handling of formaldehyde in Lab Patient Service Center and suggested the use of charcoal mask .It was a mammoth task for clinical lab which has a network of 209 Laboratory Patient Service Centers including 7 Integrated Medical Center, and 12 Satellite lab across Pakistan. Safety and employee health is considered prime responsibility of the management of clinical laboratory at The Aga Khan University Hospital. Total cost of the project calculated was PKR 2,210,000 ($22800) @ PKR 10,000 ($100) for each charcoal mask required for patient service center. A group comprising of Clinical Lab Management and Employee Health & Safety (EHS) was formed to evaluate the requirements of the project. During the evaluation EHS made a recommendation to check (STEL) first. A short-term exposure limit (STEL) is the acceptable average exposure over a short period of time, usually 15 minutes as long as the time weighted average is not exceeded.

Objectives: To check the Short Term Exposure Limit (STEL) of Formaldehyde. Based on the results obtained we will decide whether Charcoal mask is needed at our collection points if it exceeds two parts formaldehyde per million parts of air (2 ppm) as a 15-minute STEL.

Methods: The first step was to check the volume of formaldehyde 10 % in a specimen received for biopsy followed by addition of formaldehyde 10 %, if the quantity was insufficient. EntryRAE Gas detector was used to take readings in the initial 15 minutes every hour.

Results: We conducted studies in fifteen different collection points. In all cases no reading crossed the upper limit which was >2.0

Conclusion: All our readings remained well within the acceptable range; therefore there was no need of charcoal mask in our collection points.

References: A short-term exposure limit (STEL) is the acceptable average exposure over a short period of time, usually 15 minutes as long as the time weighted average is not exceeded. STEL is a term used in occupational health, industrial hygiene and toxicology. In United States STEL of a chemical has been defined as legal limit for exposure of an employee to a chemical substance. The Occupational Safety and Health Administration (U.S. OSHA) has set OSHA-STELs for chemicals. STEL assessments are usually done for 15 minutes and expressed in parts per million (ppm), or sometimes in milligrams per cubic meter (mg/m3).

Disclosure of Interest: None Declared

A-047

Association of Interleukin 18 -607A/C and -137C/G polymorphisms with oxidative stress in renal transplant recipients

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Background: IL-18 mediates a wide range of inflammatory and oxidative responses including renal injury, fibrosis and graft rejection. It has been reported that the promoter -607 and -137 polymorphisms of IL-18 influence the level of cytokine IL-18 expression. This prospective observational study investigated the association between oxidative stress with IL-18 607 and -137 polymorphisms in renal transplant recipients.

Patients and Methods: This study included 75 renal transplant recipients (28 female, 47 male; mean age: 38.28 ± 13.03) from living related donors. Blood samples were collected immediately before and after transplantation at day 7 and month 1. Serum IL-18, creatinine, cystatin C, CRP and oxidative stress markers (TOS, TAC) were measured. Oxidative Stress Index (OSI) was calculated. Polymorphisms of the promoter region of the IL-18 gene, IL18-607A/C and -137C/G were determined by analysis of "real-time PCR/Melting curve". GFR values were estimated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Formula. SPSS 20.0 software was used for statistical analysis.

Results: Serum creatinine, cystatin C, CRP, IL-18, TOS, OSI levels were significantly decreased after transplantation. Posttransplant levels of serum TAC and estimated GFR demonstrated consistent significantly increases. Serum IL-18 levels were significantly higher in patients with IL-18-137 GG genotype before transplantation. While pretransplant levels of serum IL-18 were found significantly higher in patients with IL-18-607 CC genotype, we also observed significantly higher estimated GFR (CKD-EPI) values in CA genotype (p=0.012).

Conclusion: We determined that oxidative stress was decreased significantly after transplantation. In this study, although we observed significantly differences in serum IL-18 levels and some GFR markers according to genotypes, the influence of polymorphisms on oxidative stress has not been clearly shown.

A-048

INCREASED CIRCULATING INTERLEUKIN-23 IN PATIENTS WITH SARCOIDOSIS

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Introduction: Sarcoidosis is Th1-mediated chronic granulomatous disease characterized by non-caseating granulomas formation. Interleukin-23(IL-23) is a protein encoded by the IL-23 gene in humans and is produced by dendritic cells and macrophages. IL-23 and IL-12 share a common p40 subunit and are important proinflammatory cytokines.This study aim to determine the serum IL-23 level in patients with sarcoidosis, and to determine a possible correlation with clinical and laboratory findings of the disease.

Methods: Forty-four biopsy proven sarcoidosis patients followed at a single center and 41 healthy volunteers were included in the study. Demographic, clinical, laboratory and radiological data of all patients were recorded. Serum samples from all the patients and the control group were taken and IL-6, IL-12, IL-17, IL-23 were measured by ELISA method.

Results: Among 44sarcoidosis patients 13(29.5%) were male and 31(70.5%) were female. Average patient age was 47.4 years, mean disease duration was 3.2year. Twenty-one(47.7%) patients had erythema nodosum, three(6.8%) had uveitis, 40(90.9%) had arthralgia, 23(52.3%) had ankle arthritis, 15(34.1%) had enthesitis. Laboratory evaluation showed increased serum ACE level in 24(54.5%) patients, increased serum calcium level in 11 (25%) patients, increased serum D3 level in 5(11.4%) patients, increased ESR and CRP levels in 22(50%) and 23(52.3%) patients, respectively. Compared with the control group higher serum IL-23 level were found in patients with sarcoidosis (p = 0.01). Serum IL-23 were associated with ankle arthritis (p=0.02). Serum IL-6, IL-12 and IL-17 levels were similar in sarcoidosis patients and control group (p=0.128, p=0.212, p=0.521 respectively).

Conclusion: In our study we found increased serum IL-23 in patients with sarcoidosis, while serum IL-6, IL-12 and IL-17 were detected as normal. Despite our results are somewhat contradictory to other studies in literature, sarcoidosis still should be discussed if is Th1/Th17 disease or not?

Multicenter studies are needed in this regard.

A-049

SERUM SALUSIN-ALPHA IS INCREASED IN PATIENTS WITH PSORIATIC ARTHRITIS

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Introduction: Psoriatic arthritis (PsA) is a chronic inflammatory disease characterized with skin lesions and joint involvement. Mechanisms of developing early atherosclerosis in PsA patients is not elucidated yet. Salusin- α and salusin- β are two new bioactive molecules. Salusin-β has been reported to have pro-atherosclerotic effects, but salusin-α has anti-atherosclerotic effects. Mechanisms of atherosclerosis developing in PsA patients may be associated with salusins.

Aim: The purpose of this study was to identify the serum salusin- α and salusin- β levels in patients with PsA and to determine the possible relationship with the disease findings.

Material and methods: Forty patients diagnosed with PsA according to CASPAR classification criteria and 40healthy volunteers were included in the study. Demographic, clinical, laboratory and radiological data were recorded in all patients. Disease activity indexes (PASI, BASDAI, BASFI, HAQ) were recorded. Serum salusin- α and salusin-β level were measured by ELISA method.

Results: Among 40PsA patients 13(32.5%)were males and 27(67.5%) were female, mean age was 48.5year and mean disease duration was 2.4year. Demographic and clinical assessment of patients were as follows: 20(50%) patients had family history, 18(45%) patients were smoker, 19(47.5%)patients had HLA-B27 positivity, 33(82.5%) had sacroiliitis, 36(90%)had enthesitis, 23(57.5%) had DIP joints and nail involvement , 26(65%)had wrist involvement, and 11(27.5%) had ankle involvement. 20 (50%) patients had elevated CRP level and 25(62.5%) patients had elevated ESR level. Compared with the control group PsA patients had elevated serum salusin- α level(p = 0.004). The relationship between serum salusin- α level and ankle arthritis and dactylitis were determined (p = 0.04, p = 0.03). Serum levels of salusin- β were similar in PsA patients and controls (p = 0.285). Serum salusin- β level were correlated with ankle arthritis (p = 0.02).

Conclusion: We found elevated serum salusin- α and normal serum salusin- β levels in PsA patients. Salusins may plays an important role in the pathogenesis of early atherosclerosis development in patients with PsA. In this regard, there is a need for multicenter prospective studies.

A-050

Evaluation Between Enzymatic and Jaffe Creatinine Assays for Estimation of Glomerular Filtration Rate by the CKD-EPI Equation in Renal Transplant Recipients

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BACKGROUND: The aim of this study was to compare the agreement between serum creatinine measured by modified Jaffe and enzymatic methods and their effect on eGFR as calculated by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation in renal transplant recipients.

METHODS: We studied 75 kidney transplant recipients (28 female, 47 male; mean age: 38 ± 13). Blood samples were collected after surgery at day 7 and month 1. Serum creatinine levels were analysed by modified traceable Jaffe method (Cobas 8000, Roche Diagnostic) and by an enzymatic method (CREA plus, Cobas 8000, Roche Diagnostic) in Cobas 8000 analyser. GFR was estimated by CKD-EPI equation. Data were expressed as mean ± standard errors. Bland-Altman analysis was performed to calculate limits of agreement.

RESULTS: Serum creatinine level measured by the enzymatic and Jaffe methods was similar at day 7 (1.22±0.42 vs 1.30±0.40 mg/dL, respectively, p=0.06) and at month 1 (1.23±0.34 vs 1.32±0.41 mg/dL, respectively, p=0.17). The correlation between the two methods was higher at day 7 after transplantation (r=0.94 vs 0.77). Comparison of eGFRs revealed a high correlation (r=0.91, r=0.93, p<0.05). The biases between Jaffe and enzymatic creatinine assay were 0.07 (95% CI= 0.05-0.1) at 7th day, 0.09 (95% CI= 0.04-0.14) at month 1 respectively. eGFR results based on Jaffe assay were found lower than eGFR based on enzymatic assay (bias: -4.59, 95% CI=-6.46 to -2.71 at day 7, bias: -3.59, 95% CI=-5.25 to -1.92 at month 1, respectively).

CONCLUSIONS: Accurate and precise measurements of serum creatinine are needed for a more reliable estimation of GFR as support for reliable clinical decision making. Although the enzymatic assay is not free of interference from various substances, may lead to less variability in serum creatinine measurements than Jaffe techniques in renal transplant patients.

A-051

Performance evaluation of the cobas u 701 urine analyzer

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Background: The cobas u 701 (Roche Diagnostics) system provides an automated solution for sediment microscopy using imaging analysis intended for the in vitro quantitative determination of erythrocytes (RBC) and leukocytes (WBC); the semi-quantitative determination of squamous (SEC) and non-squamous (NEC) epithelial cells, bacteria (BACT), and hyaline casts (HYA); and the qualitative determination of pathological casts, crystals, yeasts, mucus, and sperm in urine. This study evaluated the analytical performance of this system including experiments for precision, recovery of semi-quantitative range borders, carry-over and method comparison versus other automated systems (IRIS IQ 200), and conventional microscopy using Kova chamber technology.

Methods: This study tested 315 remnant, de-identified urine samples for method comparison. Each methodology was performed according to CLSI guidelines and manufacturer's package insert instructions. In addition, the operability was verified by routine simulation runs (including system stress runs) and completion of a detailed questionnaire. Precision was assessed by calculation of standard deviations (SD), or coefficient of variance (CV %) within acceptable agreement rates. Recovery of defined concentration ranges was assessed for HYA, BACT, SEC, NEC by diluting high positive samples and measuring in triplicate on cobas u 701 system and the predicate methods. The cobas u 701 system was tested for sample carryover using the Broughton model with subsequent measurements of high positive and negative samples.

Results: Repeatability and intermediate precision were all within the manufacturer's acceptance limits. SDs for RBCs and WBCs in low concentration samples were well within < 2 cells/µL and < 1 cell/µL limits respectively. Intermediate CVs were < 10% for the QC in the low pathological range (at about 220 and 450 cells/µL for RBC and WBC respectively). Repeatability experiments for semiquantitative and qualitative parameters confirmed reproducible recognition of negative and positive samples (distributed over 2 concentration ranges).

Results obtained for HYA, BACT, SEC, and NEC were within the expected concentration ranges.

No significant deviations for sample carryover were found for any parameter tested.

Method comparison of cobas u 701 system results versus conventional microscopy provided regression slopes of 1.04 and 0.98 for RBC and WBC respectively (specificity results of 81% - 97% and sensitivity results between 51 - 100%). The cobas u 701 system versus the Iris IQ 200 results for method comparison yielded a lower agreement for RBC (slope of 1.26, higher scattering) and sensitivity ranged from 32 - 97%. However, reclassification for all results on Iris IQ 200 improved the results in the statistical calculation.

User interface, availability of maintenance wizards, throughput, and imaging technology are beneficial as documented by the detailed questionnaires completed by each operator.

Conclusions: The analytical performance of the cobas u 701 system met our expectation for a new urinalysis system. The agreement with visual microscopy without requiring reclassification provides improvement for routine workflow. The imaging technology is a useful system for microscopic urine testing. The cobas u 701 urine analyzer standardizes the entire urine testing procedure for microscopy, reduces operator intervention and offers centralized result management.

Disclaimer: cobas u 701 is not cleared or approved for use in the USA

A-052

Associations of single nucleotide polymorphisms in precursor-microRNA (miR)-125a and the expression of mature miR-125a with the development and prognosis of autoimmune thyroid diseases.

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Background: It is important to search the biomarker to predict the development and prognosis of autoimmune thyroid diseases (AITDs) such as Hashimoto's disease (HD) and Graves' disease (GD). MicroRNA (miR) bind directly to the 3' untranslated region of specific target mRNAs to suppress the expression of proteins, promote the degradation of target mRNAs and regulate immune response. miR-125a is known to be a negative regulator of regulated upon activation normal T cell expressed and secreted (RANTES), interleukin (IL)-6 and transforming growth factor (TGF)-β; however, its association with AITDs remains unknown.

Methods: To clarify the association between AITDs and miR-125a, we genotyped the rs12976445 C/T, rs10404453 A/G and rs12975333 G/T polymorphisms in the MIR125A gene, which encodes miR-125a, using direct sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods in 155 patients with GD, 151 patients with HD and 118 healthy volunteers. Among GD patients, 60 GD patients had been treated with methimazole for at least five years and were still positive for anti-thyrotropin receptor antibody (TRAb) (intractable GD) and 45 GD patients had maintained an euthyroid state and were negative for TRAb for more than two years without medication (GD in remission) and 50 patients who could not be categorized to intractable GD or GD in remission groups at the time of analysis. All patients with GD had clinical histories of positive TRAb and thyrotoxicosis. Among HD patients, 59 HD patients had developed moderate to severe hypothyroidism before 50 years of age and been treated with thyroxine (severe HD) and 41 untreated, euthyroid HD patients were over 50 years of age (mild HD) and 51 patients who could not be categorized to severe HD or mild HD groups at the time of analysis . All patients with HD were positive for anti-thyroid microsomal antibody (McAb) and/or anti-thyroglobulin antibody (TgAb) and all patients with mild HD had a palpable diffuse goiter. All healthy volunteers were euthyroid and negative for thyroid specific autoantibodies (control subjects). All patients and control subjects are Japanese and unrelated.

Results: We determined that the CC genotype and C allele of the rs12976445 C/T polymorphism were significantly more frequent in patients with HD compared with control subjects (P<0·05) and in intractable GD compared with GD in remission (P<0·05). The expression of miR-125a was correlated negatively with age (P=0·0010) and down-regulated in patients with GD compared with control subjects (P=0.0249).

Conclusion:miR-125a expression in PBMCs and the rs12976445 C/T polymorphism were associated with AITD development and prognosis.

A-053

Severe acute pancreatitis and serum C-Reactive Protein

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Background: Severe acute pancreatitis (SAP) is associated to high mortality rates. Elevated serum C-Reactive Protein (CRP) levels in patients with acute pancreatitis have been proposed as a prognostic marker of the disease. The aim of this study was to assess the quantification of CRP in serum of patients with acute pancreatitis, through its monitoring during the first 72 hours and determine their relationship with SAP.

Methods: We studied patients with acute pancreatitis. Serum CRP levels were measured initial, 24, 48 and 72 hours after admission in the emergency room. Acute pancreatitis was established in patients with acute abdominal pain and serum amylase > 300 IU/L (reference values: < 100 IU/L). Serum CRP was determined by turbidimetric immunoassay in Dimension EXL (Siemens®) with reference values: < 0.5 mg/dl. Patients were classified into two groups according to the type of acute

pancreatitis: edematous acute pancreatitis (EAP) and SAP. Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC) using the software MEDCALC®.

Results: We studied 17 patients with ages between 17 and 86 years old (mean = 56.4), 7 women and 10 men. Twelve patients were EAP and 5 were SAP. No statistically significant differences were found between EAP and SAP according to the serum CRP levels initial and 24 hours ($p > 0.05$). The AUC of serum 48 hours CRP levels for diagnosis of SAP was 0.955 ($p < 0.0001$), optimal cut-off value was 3.3 mg/dl exhibiting 100% sensitivity and 81.8% specificity. The AUC of serum 72 hours CRP levels was 0.970 ($p < 0.0001$), optimal cut-off value was 3.3 mg/dl exhibiting 100% sensitivity and 90.9% specificity.

Conclusions: Serum CRP levels measured after 48 hours of evolution of the disease showed high diagnosis efficacy to predict whether an acute pancreatitis is edematous or severe.

A-054

Comparison of robust and traditional statistical methods for evaluation of blood glucose measurements in an external quality assessment program

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Objectives: External quality assessment (EQA) evaluates the performance of laboratories by means of interlaboratory comparisons, and is an important measure for accuracy and reliability of the laboratory results. Parametric statistics are the more commonly known approach in an external quality assessment program. However, due to the variability of laboratory performances, the distribution of data is often abnormal, including some outlying values. Robust statistical methods, which are based on weighted data and are resistant to the presence of outliers can be used alternatively for EQA evaluation.

Design & methods: In this study, we compared the performance scores obtained by using different statistical methods: Parametric statistics (arithmetic mean and standard deviation) and non-parametric robust statistics (median and robust standard deviation). A total of 1008 glucose results of three lots at different concentrations, submitted through the Kbudek-EQA reporting system, were collected and analyzed by Kbudek-EQA evaluating system and SPSS 17.0. Participants were evaluated in their peer groups.

The data distributions of three sets were tested by Kolmogorov-Smirnov Z test. The distribution of results was not Gaussian. "Consensus of Participants' Results" approach was used to determine the assigned value for a test material. z-scores were calculated using both standard deviation and robust standard deviation. The z-score values outside the ±3 standard deviation (SD) were evaluated as a procedure that needs investigation. These were considered unsatisfactory, while the values inside the ±2 SD limits were considered satisfactory, and z-scores outside the ±2 SD limit but inside the ±3 SD limits were considered questionable.

Results: We found the rate of the unsuccessful and questionable performance scores of the three lots to be 4.9%, 10.1%, 11.1% respectively, by using robust statistics and 2.1%, 6.8%, 7.9% by using parametric statistics. Two methods of evaluation of performance didn't show statistically very good agreement (McNemar's test, $P<0.05$ for all the three lots).

Conclusion: External quality assessment including extensive participants often result in abnormal distribution. Robust statistical methods are insensitive to slight deviation for a given probability model and can estimate the population parameters utilizing robust algorithm. We can conclude that robust statistical method may be more appropriate for EQA result analysis in selected cases.

A-055

Molecular Epidemiology Analysis of Klebsiella pneumoniae Carbapenemase-2 in Regional Hospital in Taiwan

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Background:Carbapenems are important last-line β-lactams antibiotics for treatment of multidrug resistant bacteria. *Klebsiella pneumoniae* carbapenemases (KPCs) are serine β-lactamases that have become a major cause of multidrug resistant nosocomial infection worldwide because they confer on the bacteria which resistant to most of β-lactams antibiotics including penicillins, cephalosporins, monobactams and carbapenems. *Enterobacteriaceae* (mainly in *Klebsiella pneumoniae*) with carbapenems resistance conferred by bla_{KPC} are rapid spread which may result in major global health problems. Consequently, the prevalence monitoring of KPC-producing *Klebsiella pneumoniae* becomes an inevitable task in the clinical workplace. In this study, we investigated the expression of bla_{KPC-2} by polymerase chain reaction (PCR) method and established genotype profile by pulse-filed gel electrophoresis (PFGE) in carbapenem-resistant *Klebsiella pneumoniae* (CRKP).

Methods:According to Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S21), the clinical specimens collected from August 2011 to November 2014 were tested for drug susceptibility to imipenem (IPM), meropenem (MEM), and ertapenem (ETP) using disk diffusion method. The production of carbapenemase was detected by the modified Hodge test (MHT), and confirmed bla_{KPC-2} gene expression by PCR. The genetic relationship among isolates was analyzed by PFGE using restriction enzyme *Xba*I.

Results:The 109 CRKPs clinical specimens were isolated and analyzed from En Chu Kong hospital of Taiwan. Of which, seventeen (15.6 %) isolates showed carbapenemase activity, and twelve (11 %) isolates contained KPC-2 gene. PFGE analysis showed two patterns for bla_{KPC-2} positive isolates. Five isolates were positive to MHT, but showed negative result by PCR. This is likely due to the presence of other carbapenemases.

Conclusion:This study reveals KPC-2-producing *Klebsiella pneumoniae* have become a significant concern in our hospital. Together, we have established PFGE method which helps differentiate infectious strains efficiently while controlling the outbreak of nosocomial infection.

A-056

The Association of Lead and Cataracts and Vision-Related Problems in Perimenopausal Women

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Background:

Around the world, women are subject to an earlier incidence of cataracts, have a higher risk for cataracts, and also have a higher risk for other vision-related problems than men. Previous research has indicated an association between endogenous lead stored in long bones and cataracts in men over 60 years of age; however, a similar study in women did not reveal an association.

Methods:

This case control study was conducted to investigate whether perimenopausal mobilization of endogenous lead serves as a possible causative factor for women's generalized vision issues and cataract incidence in particular. Secondary data were gathered from 1,416 women aged 40 to 55 years of age from the 2005-2006 and 2007-2008 National Health and Nutrition Examination Surveys. Variables of interest included vision-related issues, cataract surgeries, bone density data, blood lead levels, and markers of lead mobilization.

Results:

The results of the logistic regression analysis in the absence of confounders (OR = 1.50, 95% CI [1.08, 2.09]) indicated that endogenous lead is a possible causative factor for the low-level vision problems women experience during their perimenopausal years. Chi-square analysis of mobilized lead was also associated with cataract surgeries, $p < .05$.

Conclusion:

This appears to be the first report of a possible association between lead and cataracts in women and highlights the need for women to be studied in the context of their biology when their clinical results differ from those of men. These results should engender positive social change initiatives to minimize women's perimenopausal exposure to endogenous lead. Recommendations for further research include a case control study, which includes plasma lead levels, and a longitudinal study.

A-057

Evaluation of Performing Immunology Tests with the Lithium-heparin Tube

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Background:

Nowadays, integration of chemistry and immunoassay analyzers is the trend to reduce the cost in most medical laboratories. Therefore, the study aimed to assess the appropriateness of performing immunoassay tests with the Li-heparin tube.

The study analyzed 24 immunoassay items, including 12 qualitative and 12 quantitative items. Eight of the 24 items on the package inserts do not show whether the Li-heparin tube is applicable.

Methods:

Paired venous blood samples from OPD patients, 10 males and 10 females, were collected in the serum separating tubes (SST) and Li-heparin tubes simultaneously. After clotting in the SST tubes for 30 minutes, paired samples were centrifuged for 10 minutes at 2851 relative centrifugation force (RCF). Immunoassay items, 24 tests totally, were performed on ABBOTT ARCHITECT i2000 autoanalyzer, and data were compared based on two different tubes. Desirable bias of within subject biological variation (CVw%) were used as an indicator of error allowances.

Results:

Sensitivity and specificity of both tubes for 12 qualitative tests reached consistently. Statistically significant differences ($p<0.05$) between two tubes were shown on E2 and FSH, and the correlations were 0.995 and 0.993 respectively. The mean difference of both tubes were 7.9% for E2 and 17.3% for FSH; however, the error allowances defined from within subject biological variation were 18.1% for E2 and 8.7% for FSH. According to the error allowances from the within subject biological variation, this study showed the bias of both tubes on E2 were acceptable, but the bias for FSH did not meet the acceptable criteria.

Conclusion:

The study indicated that verifying the information from package inserts was a must. Among the 24 tests, FSH could not be analyzed with the Li-heparin tube, and it was not complied with the manufacture recommendations. The findings from this study were expected to be applied in the resources integration and auto barcode-labeling machine implementation in the modern clinical laboratory in the future.

A-058

Value of C3 ,C4 and combined detection of multiple biomarkers in differential diagnosis between benign and malignant ascites

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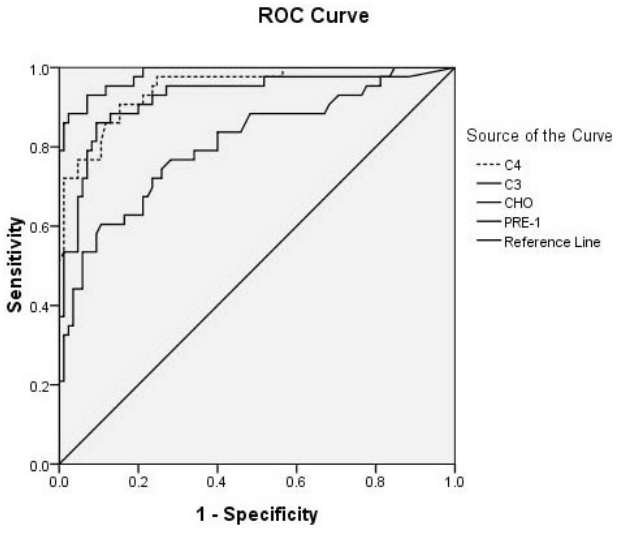
Background: Ascites are pathological accumulation of fluid in the peritoneal cavity, found in a wide variety of conditions. Metastasis of tumor cell into ascites accounts for over 10% of patients who present with ascites.The complement (C) system is a major component of innate immunity. It eliminates invading microorganisms, transformed cells and molecular aggregates from tissues and biological fluids. Some researchers have found that complement may elevate in some malignant tissues and fluid.

Objectives: To investigate the value of complement C3,C4 and combined detection of multiple biomarkers in differential diagnosis between benign and malignant ascites.

Methods: 128 Ascitical datas from Department of Clinical Laboratory,the First Hospital of Jilin University,from March to August in 2014, were analysised, including 85 benign and 43 malignant ascites. Immuno-scatter turbidmetry was used to measure the quantity of C3,C4,hs-CRP and FERR, electrochemiluminescence for AFP,CA125. CA199 and CEA, enzyme reaction rate method for CHO. Receiver operating curve and Logistic Regession were used to assess the value of the individual and multiple tumor markers to differentiate the malignant ascites from benign ascites.

Result: Compared with the benign ascites, CA125, CA199, CEA, C3, C4, CHO, hs-CRP and FERR in malignant ascites were significantly elevated($P<0.05$). The sensitivity of the individual marker was 55.8%,44.2%,60.5%,86%,90.7%,60.5%,79.1%,51.2%;while the speciality for 85.9%,96.5%,92.9%,90.6%,84.7%,89.4%,74.1%,76.5%;accuration for 75.8%,78.9%,82%,89.1%,88.3%,79.7%,75.8%,68% separately.

Conclusion: Complement C3 and C4 are elevated in maglinant ascites, providing the most valuable diagnosis for maglinant ascites. Combined with other biomarkers, complement could be a valuale tool to differentiate the maglinant ascites.The deposit of complement in ascites could help tumor cell more easily to eacape the surveillance of our immune-system. Targeted on relevant complements and receptors may provide a new therapy to malignant ascites.



A-059

The Comparison Of Low Flow Vs. High Flow Anestesia On Oxidative Stress During Pnoperitoneum Performed In Laparoscopic Cholecystectomy

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Pneumoperitoneum performed in laparoscopic surgery can be defined as a specific case/model of ischemia/reperfusion injury. We wanted to compare the effects of low gas flow anestesia to high gas flow anesthesia on oxidative stress and inflammation parameters in laparoscopic cholecystectomy in which oxidative stress is triggered by pneumoperitoneum.

55 patients aged between 18-70 years with an ASA status of I or II, scheduled to undergo elective laparoscopic cholecystectomy were enrolled in the study. The patients were randomized into two groups as high flow (HF) group and low flow (LF) group. Gas flow was 4L/dk (50% O_2, 50%air) in HF group (n=28) and 1L/dk (50% O_2, 50%air) in LF Group (n=27). ECG, NIBP, SpO_2, $etCO_2$, and BIS monitoring were applied to every subject and inhalational anesthetic concentration was set to maintain a BIS value between 40-60. All patients' O_2, inspiratory and expiratory concentrations of inhalation agent desflurane, BIS, blood pressure, pulse, peripheric oxygen saturation values were recorded during the operation. Venous blood samples were taken after the induction of anesthesia, during the termination of pneumoperitoneum and postoperative 24th hour for MDA, nitrotyrosine, CRP, cortisol, IL-6 analyses. Serum MDA levels were measured by HPLC (Ultimate3000,ThermoDionex, USA) with a flourescence detector. Within-run precision values were 1.8-5.5% and between-run precision values were 6.5-9% for 0.40-1.55 umol/L MDA, according to manufacturer's claim. The lower detection limit was 0.02 umol/L. Serum nitrotyrosine (SunRed, China) and IL-6 (Assaypro, USA) levels were measured by sandwich enzyme immunoassays. Cortisol levels were measured with a chemiluminescent immunoassay (DxI,BeckmanCoulter, USA). Serum CRP levels were measured immunoturbidometrically (Integra400, RocheDiagnostics, Germany).

MDA and nitrotyrosine levels were not significantly different when HF and LF groups are compared according to their sampling times; induction, during the termination of pneumoperitoneum and postoperative 24th hour. There were significant decreases in MDA levels between pneumoperitoneum termination vs. postoperative 24th hour and induction vs. postoperative 24th hour in HF and LF groups (47,97 umol/L vs 32,42 umol/L, P=0.000 and 47,97 umol/L vs 42,03 umol/L, P=0.000, respectively for HF group; 49,99 umol/L vs 35,37 umol/L, P=0.002 and 46,53 vs 35,37 umol/L, P=0.000, respectively for LF group).

The increase observed in nitrotyrosine levels between pneumoperitoneum termination vs. postoperative 24th hour and induction vs. postoperative 24th hour in HF and LF groups were not significant. When MDA and nitrotyrosine levels were compared according to the duration of operation (<60minutes and >90minutes), no significant

difference was observed. There was no significant difference of CRP, cortisol and IL-6 levels among Group HF and Group LF.

Ischemia/reperfusion injury occurring during pneumoperitoneum performed in laparoscopic operations exhibit a timewise decrease in MDA levels, which could be explained by activation of antioxidant systems. As for the possible protective effect of

LF anesthesia vs. HF anesthesia, no protective effect of LF anesthesia was observed with regards to oxidative stress and inflammatory markers in our group. Further research with greater number of subjects is required to confirm the changes observed which have not reached statistical significance.

A-060

MEASUREMENT OF PRO- AND ANTI-INFLAMMATORY CYTOKINES IN ACUTE STROKE. HOW USEFUL ARE FOR THE PREDICTION OF PATIENTS SUSCEPTIBLE TO IFECTIONS?

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BACKGROUND: Stroke affects the normally well-balanced interplay of two supersystems: the nervous and the immune system. Immunodepression after stroke increases the susceptibility to infection, the most relevant complication in stroke patients. Stroke-associated infections (SAI) have been reported in 21-65% of patients with stroke. The pathophysiology of SAI is not clearly understood yet. The incidence of SAI as well as the cytokine profiles were assessed in patients with acute stroke.

METHODS: In a prospective study 100 patients with acute stroke were enrolled. Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10) and tumor necrosis factor-a (TNF-a) were measured upon admission and at 24, 48, 72 hours thereafter, with a final measurement on day-7. Stroke severity was measured at the time of admission and during hospitalization with the Scandinavian Stroke Scale (SSS). Eighty healthy subjects served as controls. All cytokine levels were quantified in EDTA plasma samples employing a biochip array kit on the Evidence Investigator analyzer (Randox).

RESULTS: Mean age(SD) of the patients was 75.2(9.4) years. The mean time(SD) between the onset of neurological symptoms and hospital admission was 3.22(1.58) hours. SAI were diagnosed in 46 (46%) patients (urinary tract infections=13, respiratory tract infections=29, both=2, sepsis=2). Mean SSS(SD) was significantly lower in patients with SAI compared to those without SAI, upon admission 22.08(15.48) vs 40.68(13.65), p=0.0001 and during hospitalization 21.80(25.52) vs 51.18(13.01), p=0.0001.

Upon admission, we observed significant increases in patients with SAI compared to those without SAI (mean±SE), in IL-6 (24.27±8.32 vs 6.03±1.61, p=002), IL-8 (15.39±3.49 vs 3.54±0.50, p=0.001) and IL-10 (5.35±2.16 vs 1.51±0.39, p=0.015) whereas TNF-a/IL10 ratio was significantly decreased(2.94±0.98 vs1.63±0.18, p=0.043). The diagnostic accuracy of a single measurement of IL-6 [AUC=0.853 (95%CI 0.794-0.913), P<0.0001], IL-8 [AUC=0.822 (95%CI 0.754-0.890), P<;0.0001] and IL-10 [AUC=0.762 (95%CI 0.680-0.843), P<0.0001] upon hospital admission, for diagnosis of SAI was high. Logistic regression analysis where SSS-score, IL-6, IL-8, IL-10 and TNF-a/IL-10-ratio upon admission were inserted as variables, revealed IL-6 (OR=1.10, 95%CI 1.01-1.24), IL-8 (OR=1.24, 95%CI 1.12-1.31) and IL-10 (OR=1.08, 95%CI 1.04-1.15) as independent predictors of SAI.

CONCLUSION: Measurement of IL-6, IL-8 and IL-10 upon admission can reveal patients, candidates for subsequent SAI

A-061

Analysis of allergens in patients with allergic shin diseases in South China

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Objective: To investigate the levels and types of allergen-specific IgE antibodies in the serum of patients with allergic skin diseases and provide relevant basis for the clinical diagnosis,treatment and prevention of allergic skin diseases.

Methods: 822 patients of allergic skin diseases including 136 cases of atopic dermatitis, 172 cases of eczema and 514 cases of urticaria, who were treated in Peking University Shenzhen Hospital in 2014, were recruited. Western blot was used to detect the specific IgE antibodies in serum.

Results: The allergic skin diseases included atopic dermatitis, eczema and urticarial and their total positive rates of allergen-specific IgE allergen antibody in serum were 49.3%, 45.1%, 49.4%, respectively. The result was no significantly different. The positive rates of allergen-specific IgE were 75.0%, 80.8%, and 67.4% for the above-mentioned three allergic skin diseases in children respectively, and these rates were significantly higher than in the adult group (47.0%, 41.8%, 46.5%). In patients with three allergic skin diseases, the allergen species with a higher positive rate were the same, which were dust mites (23.5%, 20.3%, 22.0%), crab (17.6%, 15.7%, 22.2%) and marine fish combinations (14.0%, 15.1 %, 12.1%). In patients with three allergic skin diseases, the content of allergen-specific IgE antibody was mainly level 1 in density (48.0%, 49.4%, 43.0%). The majority of patients were positive for two or more allergens.

Conclusions: Allergic skin diseases in children are closely related to the contact of allergens. The house dust mites group, crab and marine fish group are the main allergens in South China.Patients with allergic skin diseases should avoid contacting the related allergens in order to reduce the occurrence of allergic diseases.

A-062

A Study Of sdLDL-C And Insulin Resistance In Apparently Healthy Obese Young Adults Of Southern Part Of Indian Subcontinent

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Background:

Indian subcontinent is a land of diversities with not just 49 ethnic groups but wide regional variation in diet from one geographical area to another that decides the health status of the population. Unlike earlier times developing countries too are facing the challenge of obesity and the modern epidemics have a common root cause tapering to obesity. Obesity during adolescent and young adulthood usually persists to adulthood in almost 70-80% cases and gives rise to early onset of type 2 diabetes mellitus, cardiovascular disorders and metabolic syndrome insulin resistance being the common link to all. Not many studies have been done in India on young adult health which is the group that can be targeted for early prevention of such modern epidemics.

Objective:

The present study was taken up to study the prevalence of insulin resistance in apparently healthy young adult obese population and study its correlation with different cardiovascular risk factors like lipid profile and sdLDL-C.

Material and methods:

In a randomized control study 106 apparently healthy young adults in the age group of 21-34 years were chosen from the community out of which 45 were obese and 61were age and gender matched non-obese controls. They were divided in obese and non-obese groups based on their BMI and cut-off BMI was 25Kg/m^2. Along with physical parameters fasting plasma glucose, lipid profile and routine biochemical parameters were assayed by standard kit methods and plasma insulin was measured by ELISA kit. Different lipid ratios, atherogenic index and insulin resistance were calculated. Atherogenic index was measured by the formula Log (Triglycerides/ HDL-C). Insulin resistance was measured by HOMA-IR model and QUICKI index. Small dense LDL-C (sdLDL-C) was quantified by modified Tsutomu –Hirano method.

Results:

In the obese group BMI, waist circumference (WC) and waist-hip ratio (WHR) elevated significantly (p=0.0001) and TG, VLDL-C and sdLDL-C as well as atherogenic index elevated significantly (p<0.001).Significant Hyperinsulinaemia (p<0.0001) was found in the obese group and 50% of obese cases had hyperinsulinaemia. Insulin resistance calculated by HOMA-IR and QUICKI index was statistically significant (p<0.0001) in obese. Linear regression analysis showed sdLDL-C (R^2=0.08, p=0.05 at 95% C.I.), hyperinsulinaemia (R^2=0.089, p=0.054 at 95% C.I.) and insulin resistance (R^2=0.099, p=0.03 at 95% C.I.) significantly dependent on WC and atherogenic index was significantly dependent on TG (R^2=0.0036, p=0.05 at 95% C.I.) rather than any other lipid factors. On ROC analysis either method of insulin resistance showed equal efficacy (AUC for HOMA-IR= 80.3% and QUICKI = 80.14%; C.I. 95%) and atherogenic index turned out to be a better predictor than sdLDL-C (AUC for Atherogenic index= 76.14% and QUICKI = 71.46%).

Conclusions:

Carbohydrate-rich diet increases TG and hence protein rich diet is advisable. For Indian subpopulation WC and WHR should also be evaluated along with BMI. Insulin resistance should be identified early and interventional measures should be taken in

terms of physical exercise and insulin receptor sensitizers for a short-term. sdLDL-C rises earlier than total cholesterol and hence a CVS risk predictor.

A-063

Prognostic Utility of AFP in Acute Liver Failure

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Objective

Higher alpha-fetoprotein (AFP) levels are indicative of liver regeneration but alone are not of minimal help in determining prognosis in patients with acute liver failure (ALF). The purpose of this study is to assess the utility of AFP concentration in combination with various severity of illness scores (SIS) [Sequential Organ Failure Assessment (SOFA) score, Model for End Stage Liver (MELD) score, and Acute Physiology and Chronic Health Evaluation II (APACHE II) score] to determine if AFP will improve the predictions made by the SIS score(s) alone in the setting of ALF.

Methodology

This was a retrospective study with 810 ALF patients from the US Acute Liver Failure Study Group (ALFSG) who have AFP values that were determined from case report forms (CRF) or from sera collected on study day 1.. The SOFA score, MELD score, and APACHE II score were calculated for each patient from available CRF data. We compared each severity of illness score to the severity score combined with the Log_{10} AFP value to predict outcomes (transplantation, death, non-spontaneous survival [death or transplant 21 days or less from study admission]) for ALF from all etiologies and for ALF due to acetaminophen (APAP) toxicity alone (n = 306) using logistic regression; the Hosmer-Lemeshow (HL) test was used to evaluate the fit of the model to the data using the criteria $p<0.15$ for good model fit. Predictions from logistic regression were used to determine the area under the curve calculated using receiver operating characteristic (ROC) analysis.

Validation

Separate logistic regression models for each outcome were calculated for each of the SIS scores with and without AFP, for both All ALF etiologies and APAP alone, and for AFP alone. When all were examined, 5 combinations of both the SIS alone and the SIS plus the log_{10}AFP were significant ($p<0.05$). These 5 pairs were examined using ROC analysis. The AUCs were calculated for 3 MELD scores, 1 SOFA score and 1 APACHEII score.

MELD/All Etiologies/Death: AUC for AFP = 0.590, MELD = 0.664, MELD + AFP = 0.689

MELD/All Etiologies/Non-SS: AUC for AFP = 0.563, MELD = 0.720, MELD + AFP = 0.727

MELD/APAP only/Non-SS: AUC for AFP = 0.587, MELD = 0.733, MELD + AFP = 0.748

SOFA/All Etiologies/Non-SS: AUC for AFP = 0.560, SOFA = 0.680, SOFA + AFP = 0.695

APAPCHEII/All Etiologies/Non-SS: AUC for AFP = 0.558, SOFA = 0.629, SOFA + AFP = 0.651

Conclusion

While AFP alone has been shown to be a limited prognostic indicator in ALF as seen with the low AUCs that were all less than 0.591, when it was added to severity of illness scores the prognostic ability of the SIS scores did show improvement, particularly with MELD, with improvement from 0.007 to 0.025. The AFP improved the AUCs for the MELD, SOFA and APACHEII scores' ability to predict non-SS for all etiologies by 0.07, 0.015, and 0.022, respectively.

A-064

Effects of Natural Opium on Atherosclerosis assessed by Carotid Ultrasound, hsCRP, Cholesterol, Glucose, BMI and Blood-Pressure studied on Opium-abusing and Non-abusing Indian Outpatients: A Cross Sectional Pilot Study

T. Mittal[1], A. Hazra[2], S. Tarafdar[1], N. Nebhinani[1], P. Khera[1], D. Har[3], V. Singh[1], P. Sharma[1], S. Mandal[1]. *[1]AIIMS Jodhpur, Jodhpur, Rajasthan, India, [2]Christian Medical College, Vellore, Tamil Nadu, India, [3]Independent, Bangalore, India*

Background:

In developing countries including India and the middle east Natural Opium is currently the second most abused substance after tobacco. Decades of confusion exist about whether opium protects or harms the cardiovascular-system. Recent large Iranian Cohort studies suggest that Opium independently caused increased mortality risk (especially cardiovascular) after adjusting for Smoking, Age and Sex.

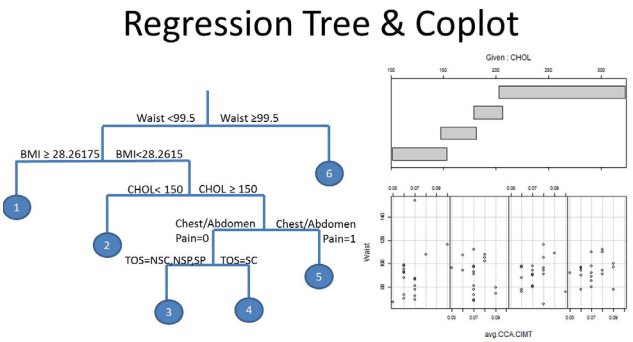
The population around Jodhpur harbors world's highest density of natural opium abusers. Most of the studies on effects of opium so far were done in Iran. We wanted to do a pilot study to see if the population around AIIMS Jodhpur has similar cardiometabolic associations with opium.

Methods:

A pilot cross sectional study is done on adult males out patients comparing Chronic Opium abusers with tobacco smoking and nonsmoking controls. We collected history of opium consumption, smoking, comorbidities and medications. We measured atherosclerotic predisposition directly by carotid intima-media thickness (CIMT), also hsCRP, Random Blood Sugar and total Cholesterol which could all be measured on nonfasting encounters. We also measured BP, BMI, and waist-circumference. Statistical Analysis done by "R" v3.1.1 and R-Studio: Average-Common-Carotid-CIMT used as the target variable and measure of atherosclerotic outcome; other variables were used a covariate.

Results:

Total 81 subjects subdivided according to Tobacco and Opium Status (TOS): Nonsmoking controls (NSC) 29, Tobacco Smoking control (SC) 27, Non Smoking OPium Abuser (NSP) 16, Tobacco Smoking Opium Abuser (SP) 9. Results below.



Rule	Level 1	Level 2	Level 3	Level 4	Level 5	P-Value	avg.CCA.CIMT
1	Waist< 99.5	BMI>=28.26175				0.00064	0.062
2	Waist< 99.5	BMI< 28.26175	CHOL< 150			0.00035	0.0625
3	Waist< 99.5	BMI< 28.26175	CHOL>=150	Chest/AbdomenPain=0	TOS=NSC,NSP,SP	0.000277	0.06692308
4	Waist< 99.5	BMI< 28.26175	CHOL>=150	Chest/AbdomenPain=0	TOS=SC	0.000371	0.07571429
5	Waist<99.5	BMI< 28.26175	CHOL>=150	Chest/AbdomenPain=1		0.001492	0.07923077
6	Waist>=99.5					0.001371	0.07857143

Conclusion:

CIMT on opium population have so far rarely been published from anywhere in the world and is a novel part of our study. Preliminary statistical analysis of the result of this pilot is apparently showing some reversal of smoking mediated cardiometabolic effects eg mild reduction in blood pressure and CIMT to nonsmoking levels. However whether this is a sampling artifact or a real biological phenomena will need to be tested on well designed larger studies.

A-065

Cardiovascular Risk Factors in first-degree relatives of young patient survivor of acute myocardial infarction

M. G. CASTELO[1], R. Dias Filho[2], A. Pereira[2], D. Gonçalves[1], C. F. Pereira[3], M. D. Freire[3], O. P. Denardim[3], F. Furtado[1], L. Belem[4], T. C. Sousa[1]. *[1]DASA, FORTALEZA, Brazil, [2]USP, São Paulo, Brazil, [3]DASA, São Paulo, Brazil, [4]UFC, FORTALEZA, Brazil*

Background:

The premature coronary heart disease (CHD) is strongly associated with familiar component. Many studies have established the importance of a positive history family as an independent risk factor of CHD. The screening of risk factors must be considered in first-degree relative of any patient who develops CHD in young age. The aim of this study was compare the metabolic profile of first-degree relatives (FDR) of young patient survivor of acute myocardial infarction (AMI) with a group of healthy people without family history for precocious CHD.

Methods:

The study was conducted from November 2010 to January 2015 in a tertiary hospital. We excluded cases of familial hypercholesterolemia. A total of 167 first-degree relatives of patient with early onset AMI (age<45y) was matched for sex and age with a group enrolled by 267 persons of both sex and without familiar history of

premature CHD. Laboratory analysis included fasting blood glucose, total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides and TSH. The patients were evaluated for the presence of metabolic syndrome. We defined metabolic syndrome using the 2007 International Diabetes Federation criteria.

Results:

There was a significant statistical difference between the FDR and control group for TSH (2,51±0,19 *vs* 1,84±0,10mUI/L; *p*=0,004); HDL-C (39,3±1,01 *vs* 49,4±1,3 mg/dL; *p*=0,001); triglycerides (163,1±9,9 *vs* 121,9±6,5 mg/dL; *p*=0,001); Body Mass Index (kg/m^2) (28,7±0,4 *vs* 26,8±0,4; *p*=0,003) and metabolic syndrome presence (46,5% *vs* 21,7%; *p*=0,001). No statistically significance difference was found between the two groups for TC (190,1±4,1 *vs* 185,9±3,6mg/dL; *p*=0,463); LDL-C (118,1±3,6 *vs* 111,4±3,4mg/dL; *p*=0,184) and fasting blood glucose(95,5±2,4 *vs* 92,9±1,2mg/dL; *p*:0,448).

Conclusion:

This study suggests that first-degree relatives of young patient with acute myocardial infarction have an unfavorable metabolic profile compared to those ones without family history for CHD ratifying the importance of early laboratorial evaluation in these people.

A-341

Growth Differentiation Factor-15 in Patients with Light Chain (AL) Amyloidosis Has Independent Prognostic Significance and Adds Prognostic Information Related to Risk of Early Death and Renal Outcomes

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Background: Growth differentiation factor-15 (GDF-15) has prognostic value in patients with cardiovascular disorders and adds prognostic information to other cardiac markers such as NT-proBNP and hs-TnT. Cardiac involvement is the most important determinant of prognosis in patients with AL-amyloidosis and cardiac biomarkers have major prognostic importance in AL. The aim of the study was to explore the value of GDF-15 in patients with AL-amyloidosis.

Patients and Methods: We measured the circulating levels of GDF-15, NT-proBNP and hs-TnT in 77 patients with newly diagnosed AL-amyloidosis, before and 3-months post frontline treatment. GDF-15 was measured by a novel pre-commercial immunoassay (Roche Diagnostics).

Results: Patients' median age was 68 years; most patients had cardiac (61%) or renal involvement (74%); 61% had NT-proBNP >1,284pg/ml and 46% had hsTnT >54ng/ml. Median levels of GDF-15 were 3,594pg/ml (range 626-71,475pg/ml); 95% of patients with AL had GDF-15 levels >1,200pg/ml. GDF-15 correlated with NT-proBNP (r=0.538, p<0.001), hs-TnT (r=0.447, p=0.02) and eGFR (r=-0.570, p<0.001). Patients with GDF-15 levels within the upper quartile (>7,575 pg/ml) had a very poor outcome (median overall survival (OS) 3-months) compared to patients with GDF-15 levels below the upper quartile (p=0.01). Higher cut-off levels for NT-proBNP and hs-TnT did not discriminate patients at high risk for early death more accurately. In a multiple logistic regression model which included GDF-15, NT-proBNP and hs-TnT, only GDF-15 in the upper quartile (HR: 8.427, 95% CI 1.73-41.1, p=0.008) was independently predictive of early death at 3-months. Similar results were obtained when these biomarkers were treated as continuous variables. Regarding OS, GDF-15 had independent prognostic significance in a multivariate model that included both NT-proBNP and hs-TnT. We also evaluated changes in the levels of GDF-15, NT-proBNP and hs-TnT in patients who received lenalidomide after 3-months of treatment. In these patients NT-proBNP often increases without obvious deterioration of cardiac function, thus complicating the assessment of cardiac response early, during the course of therapy. NT-proBNP levels increased substantially both in those with hematologic response (p=0.05) and in those without hematologic responses (p=0.013); similarly, hs-TnT levels increased in non-responders (p=0.006), while GDF-15 levels did not change significantly in both cases.

As GDF-15 reflects heart and renal defects, we further evaluated whether GDF-15 could be associated with the risk of progression to ESRD and need for dialysis. Using ROC analysis, GDF-15 >median was identified to better discriminate patients with shorter time in dialysis (29-months vs not reached, p=0.001; with 38% vs. 8% progressing to ESRD, respectively). eGFR <60ml/min/m2 was also a strong predictor of ESRD (p=0.004). However, in multivariate analysis which included GDF-15 >median, eGFR <60ml/min/m2 and proteinuria >5g/day, only GDF-15 was independently associated with a higher risk of ESRD requiring dialysis (HR: 4.25, 95% CI 1.01-18, p=0.045).

Conclusions: GDF-15 is a novel biomarker with prognostic implications for different outcomes in patients with AL-amyloidosis; it is associated with a high risk of early death, with OS and also with renal outcome. More importantly GDF-15 adds prognostic information independent of the traditional cardiac biomarkers and thus, its measurement in larger series of patients is recommended.

Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Endocrinology/Hormones

A-066

Development and Performance of a LOCI Testosterone Assay on the Dimension Vista System

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We describe a fully automated homogeneous competitive binding immunoassay for total testosterone (TTST)* on the Dimension Vista® System based upon LOCI® technology. The TTST method utilizes three reagents: two synthetic bead reagents and a biotinylated monoclonal antibody specific for testosterone. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitizer dye. The second bead reagent (Chemibeads) is coated with a testosterone analog and contains chemiluminescent dye. Sample is reacted with a displacer to release testosterone bound to endogenous sex hormone binding globulin, which then competes with Chemibeads for biotinylated antibody to form bead-analog-antibody immunocomplexes. Addition of Sensibeads leads to formation of bead pair complexes. Illumination of the complex by light at 680 nm generates singlet oxygen from Sensibeads, which diffuses into Chemibeads to trigger a chemiluminescent reaction. The resulting signal (measured at 612 nm) is directly related to analyte concentration. The assay uses a 10 µL sample volume of serum or plasma and has an analytical range of 8-1000 ng/dL undiluted. With dilution, samples up to 2000 ng/dL can be tested. Results are traceable to the CDC ID-LC-MS/MS reference method. Time to first result is 23 minutes. Precision was evaluated per CLSI EP5 using serum pools and commercial quality control materials. Repeatability and within-lab precision were < 4.9 %CV and < 7.0 %CV, respectively, across the assay range. Good agreement was observed in patient sample method comparison studies versus two different systems: Dimension Vista = 0.93 * ID-LC-MS/MS + 3.9 ng/dL (r = 0.99, n = 38), Dimension Vista = 0.90 * Roche ELECSYS® - 2.60 ng/dL (r = 0.99, n = 215). Minimal cross reactivity (< 10%) was observed with key compounds including: androstenedione, androsterone, 5a-dihydrotestosterone, corticosterone, 11-deoxycortisol, DHEA, DHEA-sulfate, 17b-estradiol, progesterone, cortisol, dexamethazone, danazol, 17a-methyltestosterone, 11b-hydroxytestosterone, and 11-ketotestosterone. **Conclusions.** We conclude that Dimension Vista Testosterone Assay with LOCI technology provides acceptable sensitivity, precision, accuracy, turnaround time, and dynamic range, and shows a high level of agreement with the Testosterone assays on Roche Elecsys and ID-LC-MS/M. *Product under development-Not available for sale

A-067

Hair cortisol as a biomarker of the HPA axis in pregnant women with asthma

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Background: Hair cortisol analysis has been used to assess the effect on the hypothalamus-pituitary-adrenal (HPA) axis by a variety of psychiatric and physical stressors. Because hair grows on average 1 cm/mo, such analysis allows detecting changes over time. For patients with Cushing syndrome, the hair cortisol level paralleled the high endogenous levels due to the disease, and it is 86% sensitive and 98% specific for the detection of cycling Cushing syndrome. Recently, we observed that hair cortisol of children with asthma was two-fold lower when taking inhaled corticosteroid than prior to the medication. Due to the importance of cortisol in the fetal maturation process and the stress response, the objective was to examine whether hair cortisol is a sensitive biomarker to assess the effects of asthma on the HPA axis in pregnant women.

Methods: A prospective case-controlled study was carried out to collect hair samples from pregnant women with and without asthma. Women were eligible to participate if they did not use topical corticosteroids on their scalp or have a pre-existing condition characterized by high cortisol levels. Hair samples were segmented to provide cortisol results corresponding to pre-conception (PC), trimesters 1-3 (T1-3), and post-partum (PP) based on the average growth rate of 1 cm/month. The hair cortisol levels were measured using a validated ELISA method (Spearman rho = 0.92, $p < 0.0001$, when compared to a hair cortisol LC-MS methods). The intra- and inter-day coefficients of variation were 1.7% and 2.5%, respectively. The hair cortisol results were compared within and between the two groups of women using the appropriate parametric or non-parametric tests.

Results: Hair samples for 93 pregnant women, 31 without asthma and 62 with asthma, were analyzed thus far. In healthy controls, there was an increase in hair cortisol over the course of pregnancy, with statistically significant changes occurring up to T2 or T3. This trend, also shown by other groups, was dampened in women with asthma. Women with asthma had significantly lower hair cortisol levels in T2 and T3 ($AUC_{T2} = 0.664 \pm 0.071$ (SEM), $p = 0.03$; $AUC_{T3} = 0.795 \pm 0.088$ (SEM), $p = 0.01$).

Conclusions: Hair cortisol successfully detected the expected increase during the course of a healthy pregnancy. In contrast, asthma was associated with a diminished ability to increase cortisol levels in late pregnancy. Children born to women with asthma have been found to have an increase in congenital malformations, endocrine or metabolic disorders, and digestive system diseases. Future research will need to establish the potential role of the changes in cortisol in pregnancy on pregnancy outcomes and fetal well-being.

A-068

Screen with Reflex to Better Test Utilization: A Cost Analysis of Thyroglobulin Testing Strategies

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Determination of thyroglobulin (Tg) concentration is important for monitoring recurrence of thyroid cancer. In traditional immunoassay detection of Tg, accurate quantitation may be affected if Tg antibodies (TgAb) are present in the patient sample. Quantitation of Tg by liquid chromatography – tandem mass spectrometry (LC-MS/MS) accurately measures Tg in TgAb-positive patients by eliminating this possible interference. Typically, Tg and TgAb quantitation can be ordered separately or as part of a reflex in which TgAb status is determined first. In reflex testing, if the patient sample is negative for TgAb, then Tg is quantified using an immunoassay; if positive, Tg is determined using LC-MS/MS. This may have important implications for overall cost since immunoassay testing is often less expensive than LC-MS/MS analysis. To examine this we reviewed ordering patterns for almost 100,000 unique patients after reflex testing became available in our laboratory. Cost analysis was performed using an average of list prices from three different laboratories. For this data set, 90,312 orders for reflex testing occurred compared to 11,279 separate orders for Tg by LC-MS/MS. From the reflex testing subset, 11% of the samples were TgAb-positive and therefore reflexed to LC-MS/MS for Tg quantitation. For TgAb-negative samples, the reflex to immunoassay for Tg quantitation resulted in a total cost savings of over $9 million compared to the cost if LC-MS/MS were utilized when not required for accurate quantitation. There were also 49,018 standalone orders for TgAb, of which approximately 5% also ordered Tg by LC-MS/MS. In this subset, 10% of samples were positive for TgAb, showing good agreement with the percent TgAb-positives seen in the reflex testing subset. Of this group, 64% represented separate orders on the same date. It is possible these clinicians were unaware of the reflex testing option offered by the laboratory. Since the overwhelming majority (90%) of samples in this subset were TgAb-negative, had these samples been ordered as part of a reflex test and sent to immunoassay for Tg quantitation, a total savings of almost $300,000 could have been realized. For patients known to be TgAb-positive, reflex testing is unnecessary and Tg is best quantified using LC-MS/MS. In the subset of separately ordered TgAb and Tg by LC-MS/MS with a previously known TgAb result, 12% were TgAb-positive. Less than half of these samples represented TgAb results obtained within the past six months. In summary, the majority of clinicians utilized the reflex testing option for Tg which resulted in tremendous cost savings since, according to our data, only approximately 10% of patients were TgAb-positive. A smaller percentage of clinicians ordered TgAb and Tg separately by LC-MS/MS when the antibody status was either unknown (ordered the same day) or was previously determined to be negative, resulting in unnecessary utilization of the more expensive LC-MS/MS testing. These data provide a current example that identifying situations in which using more expensive testing methods (*e.g.* LC-MS/MS) is most appropriate leads to more economical use of valuable health care resources.

A-069

Characterization of indirect effect of canagliflozin (Invokana), a sodium-glucose cotransporter 2 inhibitor, to inhibit reabsorption of 1,5-anhydroglucitol by sodium-glucose cotransporter 4

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Background: Renal reabsorption of 1,5-anhydroglucitol (AG), a non-metabolizable glucose analogue acquired from diet, is competitively inhibited by elevated glucose, often leading to a depleted state in diabetes. Recovery of plasma AG from a depleted state correlates with improved glycemic control. Drugs that inhibit glucose reabsorption in the kidney (sodium-glucose cotransporter 2 (SGLT2) inhibitors) can negate this correlation, however, because they also promote a decrease in AG. The mechanism is indirect: whereas AG reabsorption occurs via SGLT4, inhibition of SGLT2 elevates urine glucose which in turn competitively inhibits AG reabsorption by SGLT4. Using literature data for the effect of the SGLT2 inhibitor canagliflozin to decrease AG, our objective was to quantitatively characterize the effect on AG reabsorption, and therewith to estimate the corresponding half-life (t1/2) for changes in AG upon initiation of canagliflozin therapy (CT). The utility of t1/2 characterization is that AG measurements might serve as an early adjunct marker for canagliflozin activity.

Methods: Primary data (Balis et al., J Diabetes 2014;6:378-80) were serum AG concentrations ([AG]) 26 weeks post initiation of CT (300 mg/day): [AG] = 1.4 ± 0.7 µg/mL (reference range: [AG] = 7.2-33.3 µg/mL). Analysis used an established two-compartment AG mass balance model (Am J Physiol Endocrinol Metab 1997;273:E821-E830). Changes in total body mass of AG (T) reflect any difference between ingestion and excretion rates: $dT/dt = ki - \alpha T$ (Eqn.1), where ki = AG ingestion rate (mg/day), and α = GFR (1-f)/(1+K)/V, where GFR = glomerular filtration rate (mL/min), K is the ratio between tissue and plasma compartments (K = 2.1), V is the plasma volume (nominally 3 L), and f is the fractional reabsorption in kidney of filtered AG (in normoglycemia, f = 0.9984). For steady-state (ingestion rate = excretion rate), ki = [AG] GFR (1-f) (Eqn.2). Normal distributions for ki (4.62 ± 1.62 mg/day) and GFR (80-120 mL/min) are known. Assuming that model parameters other than f are unaffected by CT, then values for f in CT are computed from Eqn.2 using the [AG] values reported for CT patients. Determination of f then allows calculation of the expected t1/2 for changes in [AG] upon initiation of CT (derived from Eqn.1): $t1/2 = -\ln(0.5)/\alpha$.

Results: The distribution [AG] = 1.4 ± 0.7 µg/mL (average ± 1sd) observed in CT patients is consistent with average f = 0.977 (range: f = 0.984-0.953). Compared to normoglycemia (f = 0.9984), the decrease in f was <5%. CT nonetheless decimates [AG] relative to the reference range ([AG] = 7.2-33.3 µg/mL). Decrease in [AG] post CT therapy is predicted to be rapid, with average t1/2 = 1.95 days (range: 2.8-0.95 days).

Conclusions: According to model calculations, modest decreases (<5%) in fractional reabsorption of AG account for the drastic decrease in [AG] observed during CT. Decreases are predicted to be rapid (t1/2 < 3 days) after initiation of CT. Although CT negates the usual premise of AG measurement (that [AG] should increase with improved glycemic control), the rapid effect on [AG] means that AG measurement could instead provide an early independent measure of effective CT.

A-070

Development of quantitative Progesterone assay for fully automated analyzer LUMIPULSE® G1200

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Background: Progesterone (PRG) is one of an endogenous steroid hormone involved in the menstrual cycle, pregnancy, and embryogenesis. It is generally used to confirm the existence of ovulation and the corpus luteum function. In this time, we developed new reagent (Lumipulse PRG-N) which has excellent correlation with ID-GC/MS and reference materials and improved cross reactivity to some drugs or PRG derivative. Lumipulse PRG-N is one-step immunoassay, and PRG in specimen samples and PRG coated on the micro particle competitively react with ALP labeled anti PRG antibody. It is finally detected based on CLEIA technology. Here we show the excellent fundamental performance with fully automated chemiluminescence analyzer LUMIPULSE G1200.

Methods: The sample types used for this study were serum or heparin Li-plasma. Correlation with ID-GC/MS, commercial competitive kit, matched pair correlation between serum and plasma, cross-reactivity to drugs, within-run and between day precision, limit of quantification (LoQ) were evaluated following recommendation from CLSI documents EP-5, EP-7, EP-12, EP-14 and EP-17. All evaluations were executed with LUMIPULSE G1200 (FUJIREBIO INC.).

Results:

Correlation with ID-GC/MS using 40 specimen samples was excellent (slope: 1.02, regression: 0.98) and the measurement value in Lumipulse PRG-N calibrators was traceable to two different kinds of reference materials (ERM-DA377 and BCR348R). The significant correlation with the commercial available kit with 79 specimen samples was observed (Cobas, slope: 0.85, regression: 0.991, ARRCHITECT, slope: 0.98, regression: 0.983). Correlation between serum and heparin Li-plasma with 60 matched pair samples was excellent (slope: 1.05, regression: 0.997). Within-run and between day precision % CVs for our assay ranged from 2 to 5% when 3 different conc. of samples were tested, LoQ was calculated at 0.17 ng/mL by precision profile. As a result of evaluation with total 19 kinds of drugs and PRG derivatives, significant cross reactivity with almost all cross reactants were not observed.

Conclusion: These results demonstrated that Lumipulse PRG-N was a precise and highly useful for measuring PRG in serum and heparin Li-plasma. Also this assay is perfectly traceable to ID-GC/MS and reference materials.

A-071

Pediatric and Adult Reference Intervals for Key Endocrine and Special Chemistry Markers based on the Canadian Health Measures Survey (CHMS)

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Background: Accurate, up-to-date reference intervals are essential for correct interpretation of clinical laboratory test results. Concentrations of circulating biomarkers can vary profoundly with age and gender, making it essential that reference intervals are stratified accordingly. The Canadian Laboratory Initiative for Pediatric Reference Intervals [CALIPER] has been establishing a new reference interval database for pediatric disease biomarkers, to address the current gaps in pediatric reference intervals. Recently, CALIPER has collaborated with Statistics Canada to access data obtained through the Canadian Health Measures Survey (CHMS) and develop pediatric, adult, and geriatric reference intervals for 13 endocrine and special chemistry biomarkers.

Methods: Approximately 12,000 Canadians, 3-79 years of age, participated in the CHMS and provided health information, physical measurements, urine and serum. Thirteen immunoassay biomarkers were measured by the CHMS using the Siemens Immulite 2000, Siemens Advia Centaur XP, Ortho Vitros 5, 1 FS, or DiaSorin Liaison analyzers. CALIPER performed statistical analysis in accordance with CLSI C28-A3 guidelines, using SAS and R software. Subjects were excluded if they were pregnant, using prescription medication, or had a serious medical issue or chronic illness. Scatter and distribution plots were created to visually inspect data and remove extreme outliers. Suspected gender and age partitions were then confirmed by the Harris and Boyd method, the normality of each partition was assessed using Q-Q plots and then the data was transformed using the Box-Cox method. Outliers were removed by the Tukey test or adjusted Tukey test for partitions that were normally distributed or skewed, respectively. The nonparametric or robust method were used to calculate reference intervals for each partition, depending on if the sample size of the partition was >120 or >40 but < 120, respectively. Lastly, 90% confidence intervals were calculated for the endpoints of each reference interval.

Results: Dynamic changes in concentration were observed across pediatric to geriatric age groups. Age-partitioning in reference intervals was required for all 13 analytes and additional gender partitions were necessitated for apolipoprotein (Apo)-AI, homocysteine, ferritin, and high sensitivity C-reactive protein (hsCRP). Apo-A1 levels were slightly higher in males, but remained relatively constant throughout life, while vitamin B12 steadily decreased throughout childhood and then remained constant. ApoB, homocysteine, ferrition, 25-OH vitamin D, RBC folate, serum folate, HbA1c, and insulin all increased with age. Microalbumin, hsCRP, and PTH all showed variable fluctuations across the age range.

Conclusions: The collaboration between CALIPER and the CHMS has enabled a unique examination of the complex biological changes in 13 immunoassay biomarkers over a wide age range. Our rigorous statistical approach, well-defined exclusion criteria and outlier removal procedure has resulted in a robust dataset to permit detailed examination of the normal fluctuations in biomarker levels occurring in apparently healthy individuals. Our analysis not only provides insight into important biological changes that occur with development and aging, but the reference intervals developed here have important clinical implications for improved diagnostic accuracy and patient care.

A-072

Comparison of an Aldosterone Chemiluminescent Immunoassay to a Radioimmunoassay

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Background: Aldosterone is a hormone that regulates electrolyte balance and blood pressure. Measurement is used for diagnosing and differentiating primary aldosteronism from the more prevalent secondary aldosteronism. We evaluated the performance of the LIAISON® Aldosterone CLIA (DiaSorin Inc.) and compared it to the recently discontinued Siemens Coat-A-Count® Aldosterone RIA (Siemens Healthcare Diagnostics, Inc.).

Methods: Specimens included deidentified residual serum, urine and heparinized plasma specimens submitted for routine testing, banked adult and pediatric sera and fresh 24-hour urine. Samples were measured according to each manufacturer's protocol. Performance characteristics evaluated included method correlation, analytical sensitivity, linearity, precision and room temperature stability. Serum and urine reference intervals were also verified.

Results: For serum, Deming regression of the CLIA versus the RIA was y=0.85x+5.4, Spearman r=0.959 (n=87). For urine, Deming regression of the CLIA versus the RIA was y=0.60x+113, Spearman r=0.952 (n=40). Utilizing the sample diluent and low aldosterone concentration samples, the CLIA's analytical sensitivity was 0.34 and 1.57 ng/dL for serum and urine, respectively (manufacturer claims: 1.45 and 2.0 ng/dL, respectively). The CLIA's linearity claims for both sample types over the analytical measurement range of 3.0-100 ng/dL were verified using dilutions of highly concentrated aldosterone serum and urine specimens (five pools, four replicates per pool per sample type). Linear regression for serum was y=1.002x+0.253, r^2=1.000; regression for urine was y=1.008x+0.154, r^2=1.000. Utilizing two serum and two urine pools, precision was assessed over 5 days, 1 run per day, 4 replicates per run (20 total observations per concentration). For serum, repeatability CVs were 3.4 and 2.2%, and within-laboratory CVs were 5.7 and 4.4% for Levels I (14.1 ng/dL) and II (36.1 ng/dL), respectively. For urine, repeatability CVs were 2.1 and 3.3%, and within-laboratory CVs were 5.1 and 5.3% for Levels I (15.5 ng/dL) and II (62.5 ng/dL), respectively. Aldosterone was stable at room temperature for a minimum of 8 hours in serum (CVs≤4.0%) and 4 hours in unpreserved urine (CVs≤3.2%). Using banked sera, age-partitioned serum aldosterone reference intervals previously established using the RIA, were verified for the CLIA. For urine, the manufacturer's suggested reference interval of 1.19-28.1 µg/d was verified utilizing fresh 24-hour urine samples from 24 healthy volunteers. Although heparinized plasma is not listed as an acceptable sample type for the CLIA, a comparison study versus the RIA generated a correlation of y=0.87x+2.7, Spearman r=0.952 (n=14). Additionally, paired serum and heparinized plasma specimens taken during the same blood draw produced a correlation of y=1.00x+0.07, Spearman r=0.981 (n=19) for the plasma versus serum.

Conclusions: Comparisons between the LIAISON Aldosterone CLIA and the discontinued Siemens Coat-A-Count Aldosterone RIA using serum and urine samples had correlation coefficients >0.950. The low slope values may possibly due to calibration differences. Despite this, verification of RIA reference intervals indicated similar clinical classification for both assays. These data, along with other acceptable performance characteristics, suggested the CLIA may be a suitable alternative for the RIA. Additionally, heparinized plasma may be considered an acceptable sample type for the CLIA.

A-073

Analytical Performances and Method Comparison Studies of the Beckman Coulter Access 25(OH) Vitamin D Total Assay

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Back ground and Objectives:

Vitamin D is a lipid-soluble steroid hormone that is produced in the skin through the action of sunlight or is obtained from dietary sources. The role of vitamin D in maintaining homeostasis of calcium and phosphorus is well established. Chronic severe vitamin D deficiency in infants and children causes bone deformation commonly known as rickets, while in adults, proximal muscle weakness, bone pain and osteomalacia may develop. In this study, we assessed the analytical performance of the new Access 25(OH) Vitamin D Total (Beckman Coulter, Brea, USA) assay for Access 2 and DxI platforms.

Methods:

The Access 25(OH) Vitamin D Total assay is a competitive binding immunoassay that uses a vitamin D analogue conjugated to alkaline phosphatase to compete for binding sites on a monoclonal anti-25(OH) vitamin D antibody attached to paramagnetic microparticles. The assay was evaluated for linearity, imprecision and analytical sensitivity using three different reagent lots on three instruments per platform. Equimolar recognition of 25(OH) vitamin D_2 and D_3, cross-reactivity and interfering substances were tested on one instrument and one reagent pack lot on the Access 2 and UniCel DxI 800 platforms. Method comparison studies were performed on 110 samples ranging from 20.0 to 246.5 nmol/L vs. Reference Measurement Procedure (RMP).

Results:

The Access 25(OH) Vitamin D Total assay was found to be linear across the measuring range of 17.5 to 300 nmol/L with an LoB of 3.75 nmol/L, an LoD of 5.0 nmol/L, and an LoQ of 15.0 nmol/L. Within run imprecision ranged from 3.0% to 4.7% on DxI 800 and 1.5% to 3.8% on Access 2, with a total imprecision of 6.6% to 9.3% on DxI 800 and 6.8% to 7.7% on Access 2.

The assay demonstrated equimolar recognition of 25(OH)D_2 and 25(OH)D_3 (Dose ratio 25(OH)D_2/25(OH)D_3: 98% on UniCel DxI 800 and 102% on Access 2), while maintaining good sensitivity and low cross-reactivity with vitamin D metabolites. The Access 25(OH) Vitamin D Total assay was found to have a good correlation with the Joint Committee for Traceability in Laboratory Medicine (JCTLM) approved isotope dilution mass spectrometry (ID-LC-MS/MS) RMP developed at Ghent University[1]. Linear regression results were: y=0.99x-8.46 (r=0.94) on DxI and y=1.01x-7.17 on Access 2 (r=0.95).

Conclusion:

The Access 25(OH) Vitamin D Total assay demonstrated good analytical performance and precision on the Access 2 and DxI 800 platforms. In addition, the assay has equimolar recognition of vitamin 25(OH)D_2 and 25(OH)D_3 and shows a good agreement with the 25(OH) Vitamin D RMP method developed at Ghent University. These attributes indicate the assay is well suited for the automated routine assessment of 25 (OH) vitamin D statuses in patients.

1) Thienpont L, et al. Standardization of measurements of 25-Hydroxyvitamin D3 and D2. Scand J Clin Lab Invest 2012; 72 (Suppl 243): 41-49.

A-074

Novel Anti-Müllerian Hormone ELISAs: Help Diagnose Polycystic Ovary Syndrome*.

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Objective:

The aim of this study was to measure circulating anti-Müllerian hormone (AMH) levels in women with polycystic ovary syndrome (PCOS) and normal controls using well-characterized AMH ELISAs.

Relevance:

There is a growing interest in the role and measurement of AMH in PCOS. It is a syndrome characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovary morphology. Since the assessment of ovarian morphology requires ultrasonography, there has been considerable interest in identifying biochemical proxies for PCOS-associated changes in folliculogenesis. AMH may be such a proxy.

Within the ovary, highest levels of AMH are expressed by the granulosa cells of small antral follicles <4 mm in diameter. A high circulating AMH concentration identifies women with an unusually high number of small antral follicles. Classically, these will be women with polycystic ovaries. Accordingly, it may be possible to use AMH as a diagnostic tool to differentiate PCOS from its age-matched healthy subjects. Current commercial AMH immunoassays are designed to measure pro-mature AMH complex and may not detect the cleaved pro-region fragment if present in circulation. The development of new immunological methods to measure AMH isoforms in circulation is needed to better understand the role of AMH in PCOS.

Methods:

Two independent ELISA methods (24/32 & 10/24) based on antibody pairing against linear epitopes in the mid-region capture (358-369aa) and mature-region detection (491-502), and pro-region capture (36-47) and mid-region detection (358-369) have

been developed to measure circulating levels of AMH. Serum from 368 PCOS and 192 aged matched control subjects were studied and the diagnostic accuracy was calculated dividing the sum of true positives and true negatives by the total number of subjects.

Results:

The limit of detection of 24/32 and 10/24 ELISAs were 1.0 and 0.5 pg/mL, respectively. Total imprecision measured on two controls (70.4 pg/mL, 221.4 pg/mL) using 24/32 and 10/24 AMH ELISAs over 22 runs were 6.6%, 6.8% and 4.1%, 5.0%, respectively. Linearity of dilution plot (multiple dilutions of 5 samples) resulted in a slope of 1.0 and a p value of <0.0001 in both ELISAs. The median AMH levels for the 24/32 ELISA and 10/24 ELISAs showed significant difference between the control and the PCOS subjects (10.14 vs 2.71 ng/ml, and 6.05 vs 1.78 ng/mL, respectively). ROC analysis for each ELISA was used to establish the cut-offs for diagnosing PCOS subjects (characterized by NIH criteria). The sensitivity, specificity and diagnostic accuracy of 0.84, 0.83, 83.6 at a cut-off of 5.0 ng/mL and 0.85, 0.83, 84.3 at a cut-off of 3.0 ng/mL were observed for 24/32 and 10/24 ELISA, respectively. Higher prevalence of PCOS was observed in sisters of PCOS subjects (43 out of 113 subjects) using the ELISAs.

Conclusion:

Highly sensitive, specific and precise AMH ELISAs have been developed to measure circulating forms of AMH in PCOS subjects. AMH levels in PCOS subjects were highly elevated and were significantly different than the control group. The diagnostic accuracy of 85% was obtained by the novel ELISAs where subjects were characterized by NIH criteria.

* Research Use Only.

A-075

Use of Micro-Liquid Chromatography/Tandem Mass Spectrometry Method to Assess Diurnal Effects on DHEA

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Background: The steroids cortisol, testosterone, 11-deoxycortisol, corticosterone, cortisone, androstenedione, and 17-α-hydroxyprogesterone have all previously been shown to have diurnal variation when measured using micro-liquid chromatography/tandem mass spectrometry (LC/MS-MS) with significantly higher circulating concentrations in the morning (1). Our aim was to assess if diurnal variation could also be observed in the steroid DHEA when measured by LC/MS-MS.

Methods: We measured DHEA using LC/MS-MS in plasma samples from 19 healthy adults drawn at midnight and 0800 the following morning. We used an Agilent 6490 triple-quadrupole LC/MS-MS equipped with an Agilent atmospheric pressure photo ionization (APPI) source and Agilent 1200 HPLC system. 100µL of human plasma was mixed with 150µL of acetonitrile containing deuterated internal standard and vortexed for 30 seconds, then centrifuged for 10 minutes at 13,000 RPM. 150µL of the supernatant was diluted with 250µL of HPLC grade water and vortexed for 10 seconds. Then 300µL of sample was injected into the LC where both DHEA and internal standard undergo an on-line extraction, binary gradient separation and elution. An Agilent Poroshell 120 SB-C8 column was used for chromatographic separation. Quantification by multiple reaction-monitoring (MRM) analysis was performed in the positive mode. The transition selected was: mass-to-charge (m/z) 271.3 to 253.2. Nitrogen served as both the source and collision gas. Circulating DHEA concentrations at midnight and 0800 were compared using paired-sample Student *t*-tests.

Results: Our findings demonstrated a statistically significant difference between DHEA circulating concentrations measured at midnight and 0800 ($p<0.0001$). Mean DHEA increased 60% from 230.7ng/dL at midnight to 566.4ng/dL at 0800.

Conclusion: We demonstrated significant diurnal variation in DHEA concentrations when measured by LC/MS-MS. Time-specific reference values must be generated in order to understand the clinical relevance of DHEA measurements by LC/MS-MS.

References: Stolze et al. *Clinical Chemistry*, 2015; 61(3)

A-076

LC-MS/MS Study of 25-OH Vitamin D2 and D3 with PerkinElmer Vitamin D kit Using both Derivatized and Non-derivatized Methods

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Background:

25-OHase enzyme converts Vitamins D2 and D3 to 25-OH Vitamin D in the liver. Quantification of these metabolites is widely used as a means of assessing vitamin D deficiency status because of their clinical significance in a variety of disorders, which lead to alterations in the concentrations of circulating hormones. This work presents a fast, reliable, and accurate LC-MS/MS method on an IONICS 3Q 120 triple quadrupole mass spectrometer for studying 25-OH Vitamin D2 and D3 for research purposes with PerkinElmer Vitamin D kit using both derivatized and non-derivatized methods.

Methods:

The PerkinElmer Vitamin D kit is intended for quantitative determination of 25-OH Vitamin D2 and D3 in human serum and plasma samples. This kit uses a combined solvent extraction and protein precipitation method. It can be used in two alternative ways; non-derivatized, or derivatized. The calibrators are isotope labeled as 2H6-25-OH Vitamin D2 and 2H6-25-OH Vitamin D3 with six levels ranging from 5.4-164.5 ng/mL for 2H6-25-OH Vitamin D2 and 4.6-139 ng/mL for 2H6-25-OH Vitamin D3. Three levels of QC standards were provided, 10, 42.3, and 87ng/mL for 2H6-25-OH Vitamin D2, and 8.7, 35.8 and 73.6ng/mL for 2H6-25-OH Vitamin D3. The IONICS 3Q 120 mass spectrometer was equipped with a heated coaxial flow ion source and "Hot Source-Induce Desolvation" interface for the best ionization and sampling efficiencies. Electrospray ionization was used for this analysis. A Shimadzu Prominence XR UFLC system was used. The column was from Kinetex (C18, 100x2.1mm, 1.7um). The injection volume was 10 µL. A gradient method was created with a flow rate of 0.3mL/min and a total LC cycle time of 4.5 minutes.

Results:

In a 4.5-minute LC run, good chromatogram peak shapes were obtained for both 2H6-25-OH Vitamin D2 and D3. No carryover was detected in a blank injection immediately following the upper level calibration sample. The calibration curves showed good linearity with a coefficient $R2>0.993$ for 2H6-25-OH Vitamin D2 and D3 with non-derivatized and derivatized methods. At the lowest levels for both 2H6-25-OH Vitamin D2 and D3, the accuracies were between 97-102% and CVs were < 10%. For the lowest level in the kit, the S/N ratios of 2H6-25-OH Vitamin D2 were about 70 and 110 for non-derivatized and derivatized, respectively; the S/N ratios of 2H6-25-OH Vitamin D3 were about 40 and 92 for non-derivatized and derivatized , respectively. For 2H6-25-OH Vitamin D2 and D3 in the QC samples with both non-derivatized and derivatized analysis, the accuracies were between 93-109% and the CVs were < 9%.

Conclusion:

A rapid, accurate, and reproducible LC-MS/MS research method was developed on IONICS 3Q 120 mass spectrometer for evaluating the Perkin Elmer Vitamin D kit. The S/N results at the lowest levels indicate that the expected LLOQs for 2H6-25-OH Vitamin D2 and D3 would be at least 4 times lower(~1ng/mL) for non-derivatized and about 10 times lower(~0.5ng/mL) for derivatized methods. Therefore, this LC-MS/MS method with IONICS 3Q 120 mass spectrometer is capable to provide high enough sensitivity, accuracy and reproducibility for quantifying 25-OH Vitamin D2 and D3 with PerkinElmer Vitamin D kit.

A-077

Determining an Effective Point-of-Care β-Hydroxybutyrate Concentration to Initiate Earlier Treatment of Pediatric Diabetic Ketoacidosis

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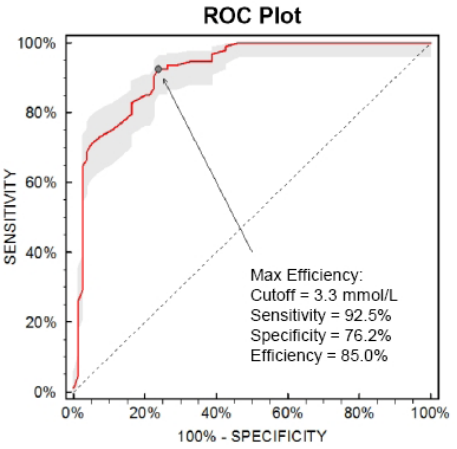
Background: Diabetic ketoacidosis (DKA) occurs when ketones accumulate in the blood due to low insulin concentrations. DKA is life-threatening and the leading cause of hospitalization and morbidity in Type 1 diabetic patients. Patients presenting to the ED with suspected DKA need quick assessments and treatments. Our current practice uses a CO_2 value to determine if a DKA Clinical Practice Guideline (CPG) is to be activated. A $CO_2 \geq 16$ mmol/L suggests that DKA is unlikely. However, a $CO_2 < 16$ mmol/L indicates that the patient is acidotic and the DKA CPG is activated. As the

laboratory turnaround time for CO_2 values can be 45 minutes, we wanted to determine if a point-of-care (POC) βHB value could be used to initiate DKA treatment earlier. **Objective:** Determine a best βHB concentration for beginning earlier treatment of patients with suspected DKA. **Method:** A retrospective chart review was done on patients with POC βHB and laboratory CO_2 values within one hour of each other, prior to the initiation of treatment. 173 patients met these criteria. ROC analysis was performed for βHB with CO_2 concentrations used as the gold standard for the diagnosis of DKA. CO_2 values < 16 mmol/L were considered positive and values ≥ 16 mmol/L were considered negative. **Results:** ROC analysis indicated that a βHB value of 3.3 mmol/L predicts DKA with 92.5% sensitivity and 76.2% specificity. The ROC-AUC was 0.922 with an efficiency of 85%. The mean βHB value for the negative cases was 2.07 mmol/L (SD 1.68) and for the positive cases was 5.32 mmol/L (SD 1.49). βHB ranges for negative and positive cases were 0.1-8.0 mmol/L and 1.8-8.7 mmol/L, respectively. **Conclusion:** Measuring blood ketones at POC and using a βHB cutoff of 3.3 mmol/L to initiate a DKA CPG provides treatment sooner without adverse effects or significant cost increases.



A-078

Evaluation of the Cisbio Bioassays Aldosterone (ALDO-ELISA) and Active Renin (RENIN-ELISA) Assays for Determination of the Aldosterone-Renin Ratio for Screening in Primary Hyperaldosteronism

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Background: The screening of suspected primary hyperaldosteronism (PA) is assessed widely by determination of the aldosterone-renin ratio (ARR). New ELISA methods for serum/plasma aldosterone and plasma renin concentration have become available to replace traditional radioimmunoassays (RIA). This study evaluates two new commercial assays from Cisbio Bioassays for aldosterone and active renin against RIA in a clinical setting, and proposes an ARR cut-off for the screening of PA.

Methods: The study examined 120 patients including 61 normotensive individuals, 39 with essential hypertension, and 20 with a diagnosis of PA who were tested at University Health Network, Toronto, Canada, between May 2014 - February 2015. The Cisbio Bioassays ALDO-ELISA and RENIN-ELISA kits use a 96-well plate format which employs colorimetric detection. For method comparison studies, the predicate RIA methods were the Siemens Coat-A-Count® Aldosterone Assay and Cisbio Bioassays Renin III Generation assay. Paired serum samples and EDTA plasma samples were used for aldosterone and active renin measurements, respectively. Clinical correlation was performed by chart review and an ARR cut-off was determined from Receiver-Operator Characteristic (ROC) curve analysis.

Results: Comparison of serum aldosterone and plasma renin concentrations for the Cisbio Bioassays ELISA methods versus RIA methods showed good agreement with correlation coefficients (r) of 0.918 and 0.976, respectively. For screening of PA, an ARR threshold of 91.8 (pmol/mIU) with a sensitivity of 85% (95% CI: 64.0-94.8) and specificity of 98% (95% CI: 93.0-99.5) was obtained from ROC curve analysis. Using the Cisbio Bioassays ELISA methods, the ability of the ARR to detect PA was very good with an area under the curve (AUC) of 0.963 (95% CI: 0.92-1.01). The positive and negative predictive values at the threshold were 89.5% and 97.0%, respectively.

Conclusion: The Cisbio Bioassays ALDO-ELISA and RENIN-ELISA assays gave comparable performance to the predicate RIA assays for the quantitation of aldosterone and active renin, respectively. Preliminary results suggest that screening for PA can be achieved at an ARR cut-off near 92 (pmol/mIU) using the new ELISA methods.

A-079

Human Serum Leptin Assay on A Multiplex Immunoassay Platform

L. Nguyen, S. Li, B. Yue, W. Li. *NMS Labs, Willow Groove, PA*

***Background*:** Leptin is synthesized in adipose tissue and binds to specific receptors in the hypothalamus to control appetite and energy intake. It has been proposed to be an essential signaling factor to regulate body weight homeostasis and energy balance. Increased leptin concentration suppresses appetite and elevates thermogenesis. Leptin deficiency leads to hypopituitarism and massive obesity.

Objective: The goal of the study was to improve and validate a sensitive and reliable method to accurately quantify leptin concentration in human serum using a Bio-Plex Pro Human Diabetes Assays Kit (Bio-Rad) on a Bio-Plex 200 system (Bio-Rad).

***Methods*:** The assay employs a similar quantitative ELISA format on magnetic beads. Capture antibody is covalently bound to the beads and reacts with leptin in serum. After washing to remove unbound proteins, a biotinylated detection antibody is added to create a sandwich complex. The final detection complex is formed when streptavidin-phycoerythrin (SA-PE) conjugate is added. Laser excites phycoerythrin to generate a reporter fluorescence signal. The concentration of leptin bound to each bead is proportional to the median fluorescence intensity of reporter signal. The leptin assay is performed according to the instructions supplied by the manufacturer with some modifications. Serum (50 µL) is first diluted with standard diluent 1+3 and then diluted with sample diluent 1+9 before being aliquoted in duplicate onto a 96-well microplate. The calibrator is first serially diluted with standard diluent and then each level is diluted with sample diluent 1+9 before being aliquoted onto the microplate in duplicate. Data analysis is performed with Bio-Plex Manager software version 6.1.

***Results*:** Although the Bio-Plex 200 system has a signal output (fluorescence intensity) up to ~25,000 for the leptin assay, the linear signal output is around 10, 000, due to signal saturation. Thus, a linear calibration curve is opted instead of a five-parametric sigmoidal dose-response curve fitting recommended by the manufacturer. The linear calibration greatly improves accuracy and precision with a reasonable linear dynamic range and it is necessary to dilute samples of high concentration level.

The within-run coefficients of variance (CV) are < 6.4% for three levels of quality control samples while between-run CVs are <8.9%. Compared to a quantitative chemiluminescent immunoassay (CIA), the correlation (Excel, simple linear regression) is as follows: $Y = 1.876 * X_{CIA} + 2.7656$, r =0.9068, n=46. The correction is $Y = 2.495 * X_{CIA} + 0.2209$, r =0.9324, n=35, leptin by CIA <10.5 ng/mL, with removal of the high concentration levels. Mean recovery using metrological traceable standard solution (Sigma, L4146, leptin human, >99% by SDS-PAGE, recombinant, expressed in E. *Coli*, lyophilized powder) was 98.5%.

***Conclusions*:** The leptin assay on the Bio-Plex platform (multiplex immunoassay) is successfully validated and is suitable for clinical use in reference laboratory settings. Caution must be exercised when comparing absolute concentration levels from different manufacturers and platforms. It is also necessary to note the assay performance dependence on assay platform, sample type and concentration levels when clinically interpreting the results. Reference intervals for leptin were established.

A-080

Serum 5α-Dihydrotestosterone Measurement by 2D-LC-MS/MS

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Backrgounds: In humans, circulating androgen 5α-dihydrotestosterone (DHT) exerts major biological effects on skin and prostate. DHT is a more potent androgen than testosterone (T) and is the primary androgen in the prostate. The DHT concentration also increases with androgen replacement therapy because of T conversion to DHT. The serum DHT concentration and DHT/T ratio are clinically useful for monitoring 5α-reductase deficiency, treatment of benign prostate hyperplasia or prevention of prostate cancer by 5α-reductase inhibitors. The challenges to develop a sensitive, accurate and specific bioanalytical method for DHT include low concentration levels and endogenous T metabolites that may interfere. Radioimmunoassay for DHT measurement requires intensive sample workup and lacks specificity due to cross-reactivity.

Objective: We intend to develop a simple high-throughput assay utilizing two-dimensional liquid chromatography-tandem mass spectrometry with required performance for routine clinical use.

Method: Sample aliquot spiked with internal standard was extracted using a mixture of ethyl acetate and hexane. After vortex mixing, centrifugation, phase separation, complete solvent evaporation, DHT is derivatized with picolinic acid at room temperature and then injected into a 2D-LC-MS/MS system without further purification. An API-5000 triple-quadrupole mass spectrometer (AB Sciex) is coupled to a Shimadzu HPLC system of two sets of binary pumps for 2D-LC-MS/MS. The 1st D-LC uses an Agilent Zorbax 300SB-C3 guard column (12.5 x 2.1 mm) for online extraction and cleanup with 0.5% formic acid in water and methanol as mobile phase while the 2nd D-LC uses a Phenomenex Kinetex C18 (100 x 3.0 mm) for analytical separation using 0.1% formic acid in water and acetonitrile as mobile phase. A six-port switching valve is switched at 1.7 min and 2.2 min to transfer compounds of interest from 1st D to 2nd D in heart-cutting fashion without back flash. The API 5000 is operated in positive electrospray ionization and multiple reaction monitoring (MRM) mode with two MRMs monitored for each analyte or internal standard.

Results: The method was fully validated. The lower limit of quantitation (LLOQ) was validated at 5pg/mL with accuracy >93.8% and total %CV < 8.7%, while the upper limit of quantitation (ULOQ) was validated at 2500pg/mL. Within-run CVs were < 3.0% for three levels of QC samples while between-run CVs were <2.9% and a total CVs <5.6%. The extraction recovery was ~96.2% with matrix effect at ~71.3% and process efficiency of 68.6%. The correlations compared with a reference method (EP Evaluator, Deming Regression, 99% confidence interval to exclude outliers) are as follows: Y = 1.11 * X Reference method + 9.402, r =0.9983, n=37/40, SE =14.053.

Conclusion: The 2D-LC-MS/MS setup allows extensive clean-up and transfers only a small part of elution profile of the 1st dimension containing targeted analyte to the 2nd dimension for high efficiency separation. A simple and sensitive method to accurately quantify DHT in serum by 2D-LC-MS/MS was developed and validated, with a LLOQ of 5 pg/mL and suitable for routine clinical laboratory use.

A-081

The Use of Clinical Equivalence Measured at Different Allowable Total Error to Compare Enzymatic, Immunoturbidimetric & HPLC Methods for the Determination of HbA1c Levels In Patients With Normal and Abnormal Hemoglobin.

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Background: High-performance liquid chromatography (HPLC) is considered the acceptable standard measurement procedure for hemoglobin A1c (HbA1c). The objective of this study was to compare and correlate the analytical performance by measuring clinical equivalence(CE) measured at different allowable total error (TEa) of 3 methods for measurement of HbA1c, including HPLC (Tosoh® G8), immunoturbidimetric (Cobas® 6000) and enzymatic (Architect® c4000) methods in patients with normal and abnormal Hb.

Method: Measurements of HbA1c by the three methods were made in blood from 151 patients with normal and 103 patients with abnormal Hb. Intra and inter assay precision of each method was evaluated with control specimens. Results from the Architect® c4000 and from Cobas® 6000 were compared with those of Tosoh® G8 HPLC method to determine correlation & CE at different TEa.

Results: The average HbA1c levels measured by Architect® 4000, Cobas® 6000 and Tosoh® G8 were 6.86 ± 2.16, 6.75±1.27 and 6.96±1.99% for normal Hb samples and 5.81±1.61, 5.70±1.22 and 5.74±1.37% for abnormal Hb samples, respectively. Ten abnormal Hb samples could not be read by at least one machine (6 by Tosoh® G8, 5 by Architect® 4000 and 8 by Cobas® 6000), therefore were not included in the correlation & CE studies. Comparing Architect® c4000 to Tosoh® G8 reviled r=0.9944, y=1.093x-0.720 and CE at an TEa as low as 7% in the normal Hb samples, and r=0.9710, y=1.197x-0.962 and CE at TEa as low as 13% in the abnormal Hb samples. Comparing Cobas® 6000 to Tosoh® G8 reviled r=0.9932, y=0.865x+0.740 and CE at TEa as low as 9% in the normal Hb samples, and r=0.9716, y=0.887x-0.610 and CE at TEa as low as 15% in the abnormal Hb samples. The difference in lowest TEa to achieve CE was mainly observed with HbA1c <5.7 and >6.4%.

Conclusion: Both Architect® c4000 and Cobas® 6000 showed acceptable data quality & correlation with Tosoh® G8 and achieved CE at fairly low TEa for the measurement of HbA1c in patients with normal and abnormal Hb, with Architect® c4000 achieving CE at lower TEa than Cobas® 6000 in both low and high ranges.

A-082

Zooming In on the Low End: Functional Sensitivity of Automated Testosterone Immunoassays

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Background: Measurement of testosterone provides an overall assessment of androgen status and aids in diagnosis of several endocrinopathies in men, women, and children. Testosterone concentrations are commonly determined using commercial immunoassays. However, there are well-documented concerns regarding the reliability of these assays at low testosterone concentrations. This is particularly troublesome for patient populations where low testosterone concentrations are expected, such as women and children. While mass spectrometry methods have proven more accurate, adopting these methods is not feasible for all laboratories. The objective of this study was to assess the functional sensitivity (FS) of 5 automated testosterone immunoassays.

Methods: Residual serum samples were obtained following measurement of testosterone using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples with similar results were pooled together to prepare 14 human serum pools (pool concentrations 1.2 - 962.5 ng/dL by LC-MS/MS) which were aliquoted and stored at -70°C until use. All pools were tested for total testosterone using the Abbott ARCHITECT $i2000_{SR}$, Beckman Coulter DxI, Roche MODULAR E170, SIEMENS Centaur and IMMULITE 2000 using 2 reagent lots and at least 2 calibrations. Each pool was assayed once per day, 2 days per week for 6 weeks, totaling 12 replicates per pool. Three pools were excluded from analysis on the E170 because the results were below the analytical measurement range. FS was estimated by fitting a power function to the imprecision data and calculating the testosterone concentration that corresponded to a CV of 20% using Excel. Statistical significance between lots (p-value) was calculated using GraphPad Prism.

Results: FS for the DxI, E170, Centaur, and IMMULITE was 10.4, 22.1, 35.5, and 100.5 ng/dL, respectively. The LoQ for LC-MS/MS was previously determined to be 1.0 ng/dL. For the ARCHITECT assay, all pools tested had CVs below our FS definition of 20%, thus true FS could not be calculated. However, the lowest pool measured on the ARCHITECT had a testosterone concentration of 4.0 ng/dL (1.2 ng/dL by LC-MS/MS) with a CV of 2.6%, which confirmed the manufacturer's limit of quantitation (LoQ) claim of ≤4.3 ng/dL. The E170 was the only other method with a manufacturer's LoQ claim (12.0 ng/dL), although our study was not able to meet that claim. Statistically significant lot-to-lot differences were observed for all methods except the Centaur, which contributed to the final CV used in FS calculations. With few exceptions, the ARCHITECT, DxI, E170 and Centaur over-recovered testosterone with an average % recovery of 131, 115, 107, and 168, respectively; whereas the IMMULITE under-recovered (81%).

Conclusions: Significant differences in FS exist among testosterone methods. Some methods showed acceptable performance while others would benefit from assay improvement at lower testosterone concentrations. Lot-to-lot differences contributed to the variability observed, with some methods being more affected by this variability than others. Importantly, in comparison to LC-MS/MS, all methods had a greater tendency to over-recover testosterone except for the IMMULITE. It is critical to understand the accuracy and precision limitations of commercial immunoassays at low testosterone concentrations. This must be considered when evaluating populations where low testosterone concentrations are expected, such as women and children.

A-083

Performance Evaluation of a Prototype Insulin Assay* on the VITROS® ECi Immunodiagnostic System

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Background: Insulin concentrations in the blood are indicative of endogenous insulin produced by the pancreas. Insulin measurement is important in the management of people with diabetes mellitus and the treatment of insulin resistance. We have developed a prototype assay using monoclonal antibodies provided by Mercodia AB, for the quantitative measurement of insulin in serum for use on the VITROS® ECi Immunodiagnostic System.

Methods: Precision was evaluated by testing a 5 member panel in triplicate 2 times per day for 5 days. Cross reactivity with proinsulin and c-peptide was assessed up to 1000ng/ml; and bovine and porcine insulin were assessed up to 1000µIU/mL. A total

of 134 samples that spanned the assay range were tested in the prototype assay and an aliquot was sent out for testing on a commercially available automated comparator method. The sample set included random samples, fasting samples, post meal samples collected from in house volunteer participants as well as archived samples purchased from a vendor. Reagent stability was evaluated out to 13 weeks.

Results: The total %CVs ranged from 1.1% to 2.4% for precision panel members ranging in concentration from 8 to 218µIU/mL. At 1000ng/mL, the observed % cross reactivity for proinsulin and c-peptide was 0.08% and 0.14%, respectively. At 1000µIU/mL, the observed % cross reactivity for bovine and porcine insulin was 81% and 107%, respectively. For the method comparison, Deming regression analysis yielded a slope of 1.00, intercept of -0.58 and Pearson Correlation Coefficient of 1.00. The overall mean bias for the prototype method was -1.8% as compared to the commercially available automated comparator method. For the stability study, a calibration curve was run at baseline and 5 stability panel members were predicted off this curve at baseline, 2, 4, 6, 8, 12, and 13 weeks using the same preparation of working strength reagents. The largest observed change in predicted concentration at 13 weeks was -2.1%.

Conclusion: Preliminary performance data demonstrate that the prototype assay has acceptable precision, cross reactivity with proinsulin and c-peptide, stability and excellent correlation with a commercially available method.

*Under development

A-084

LC-MS/MS method for the measurement of free 25-OH vitamin D3

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Background: The measurement of total 25-OH Vitamin D_3 is suboptimal with serum concentrations correlating poorly with PTH. For this reason we wished to explore the possibility of quantifying the free fraction employing ultrafiltration at 37°C and LC-MS/MS.

Methods: An AB SCIEX TRIPLE QUAD 6500 tandem mass spectrometer equipped with Atmospheric Pressure Chemical Ionization (APCI) source and Shimadzu HPLC system was employed to perform the analysis using isotope dilution with deuterium labeled internal standard 25-OH Vitamin D_3-d_6. 600 µL of 20 pg/mL internal standard in MeOH was added to the collection cup of a Sartorius VIVASPIN 2 HY ultrafiltration device (10,000 MW cut-off) in advance. 500 µL of human plasma/serum was pipetted to the VIVASPIN 2 ultrafiltration device for centrifugation at 2200 g and 37°C for about 8.5 minutes, when just 300 µL of sample was filtered through the ultrafiltration device. After centrifugation, ultrafiltrate and internal standard mixture was transferred directly to a glass sample vial and vortexed for 10 seconds. 300 µL aliquot was injected onto an Agilent Poroshell 120 SB-C8 column where both 25-OH Vitamin D_3 and internal standard undergo an on-line extraction, gradient chromatographic separation and elution. Quantitation by multiple reaction-monitoring (MRM) analysis was performed in the positive mode. The transitions selected were: mass-to-charge (m/z) 383.3 →229.2 for 25-OH Vitamin D_3 and 389.3 →211.2 for 25-OH Vitamin D_3-d_6. Nitrogen served as curtain and collision gas. The main working parameters of the mass spectrometer were: collision gas 7, curtain gas 35, ion source gas (GS1) 60, nebulizer current 3, probe temperature 350 °C, entrance potential 10 V, and dwell time 50 msec.

Results: The between-day coefficients of variation (CVs) were below 10% for free 25-OH Vitamin D_3 at all concentration tested. Accuracy ranged between 90% and 110%. Good linearity was also obtained within the concentration range of 1-25 pg/mL for free 25-OH Vitamin D_3 ($r \geq 0.995$). The range of results from 34 healthy volunteers was 1.5 to 17.9 pg/mL. This cohort was supplemented with 8 patients with elevated parathyroid hormone (PTH). The free 25-OH Vitamin D_3 concentration correlates excellently with the concentration of PTH and poorly with the total 25-OH Vitamin D_3 concentration. A poor correlation was observed between total 25-OH Vitamin D_3 and PTH.

Conclusion: We describe the first simple, accurate, and fast isotope dilution tandem mass spectrometry method for the measurement of free 25-OH Vitamin D_3 in human serum/plasma samples employing a high sensitivity tandem mass spectrometer. We can now evaluate the role of free 25-OH Vitamin D_3 in patients with bone and/or a variety of malignant diseases.

A-085

Development and performance evaluation of whole PTH (1-84 PTH) assay on LUMIPULSE® G1200

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Introduction: Parathyroid hormone (PTH) consists of 84 amino acids, secreted from parathyroid glands. PTH influences calcium and phosphorous homeostasis directly through bone and kidney. PTH concentration in the blood is measured to diagnose hypercalcemia and hypocalcaemia for renal disorder, and to assess parathyroid function in bone and mineral disorders. In the ordinary physical condition, PTH undergoes cleavage. This results in various C-terminal fragments in the blood. And it has been known PTH (1-84) but not these fragments has the bioactivity. We recently developed and launched a fully automated chemiluminescent enzyme assay: Lumipulse whole PTH assay using anti-(1-4) and anti-(39-84) antibodies. This assay detects PTH (1-84), and shows no cross reactivity with any PTH fragments.

Objective: The aim of this study is to evaluate the analytical performance of this new assay.

Materials and Methods: Lumipulse whole PTH is a one-step sandwich assay which uses two goat polyclonal antibodies. On the LUMIPULSE G1200 system, it uses 50 µL of serum or plasma specimen incubated with an ALP-conjugated N-terminal antibody and magnetic particles coated with C-terminal antibody simultaneously. Almost all specimens used in this study were commercial Heparinized or EDTA plasma spiked with PTH. In the correlation study and the reference range study, specimens from the clinical side and healthy donors were utilized.

Results: The intra- and inter-assay precision were assessed by using three plasma spiked with PTH antigen and buffer based PTH solution. The results were 0.6-2.2% and 1.4-1.7%, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were determined as follows: A zero calibrator was measured in replicate of 20, and its average and SD were determined. LOD was calculated as the average +3SD. LOQ (functional sensitivity) is defined as the low range measured value which is its CV (n=20) of 10%. The results were 0.2 pg/mL and less than 1.6 pg/mL, respectively. Measuring range was determined to show a linear standard curve between 4-5000 pg/mL. The dilution linearity was assessed by serially diluted PTH specimens. The specification was the recovery within 100±10%. It was met up to 1:10 dilution. Cross reactivity was evaluated by adding 50,000 pg/mL of each PTH fragment into a specimen. N-terminal, middle and C-terminal fragments exhibited 0.000~0.057% cross reactivities according to CLSI EP7-A2 calculation. These are negligible level for whole PTH measuring in blood. Correlation study of Lumipulse whole PTH with IRMA whole PTH was evaluated. Using Passing-Bablok regression, it showed a slope of 0.98 (95% CI = 0.96 to 1.01) with a correlation coefficient of 0.99 and an intercept of 0.95. The reference range was calculated using 171 healthy volunteer donors by the non-parametric method with reference to CLSI EP28-A3c. The result was 6.8 (90% CI = 5.6 to 7.6) to 38.2 pg/mL (90% CI = 34.1 to 63.3).

Conclusions: Lumipulse whole PTH is the 3rd generation fully automated assay on LUMIPULSE G1200. And the results described above demonstrate its high analytical performances.

A-086

Hypocretin (orexin A / orexin B) and sleep quality in patients with syphilis or HIV positive

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Background:

Hypocretin (Hcrt) or Orexin is a neuropeptide that binds to two major receptors: Hcrt 1 (Orex A) and Hcrt 2 (Orex B). Their neurons are located in the lateral hypothalamus, dorsomedial and perifornical area. The deficit Hart directly affects on the sleep-wake cycle, homeostasis, search natural reward in drug abuse and appetite. Narcolepsy, a sleep disorder whose main symptom excessive sleepiness (ES) and is characterized by low levels Hcrt in the cerebrospinal fluid (CSF). However there are reports in

the literature that the variation in the levels of this also can occur in other infectious diseases such as affecting the CNS (Central Nervous System).

Our objective was to investigate the Hcrt levels in the CSF of patients with active or treated syphilis or HIV positive patients and associate these levels the presence of ES.

Methods:

142 consecutive patients in the hospital of sexually transmitted diseases, aged above 18 years, n = 27 women, n = 22 treated syphilis or active n = 41 with previously diagnosed HIV positive and had CSF collection request (lumbar or cisternal) for tests, were evaluated.

The Epworth questionnaire was used to assess the presence of ES in recent months. The CSF sample was taken to measure the concentrations of Hcrt (1 and 2) by enzyme immunoassay method (EIA) using the reagent: Cusabio Human Orexin A e B (ELISA Kit Catalog Number CSB-E08859h).

Results:

Patients with active syphilis or treated showed significantly lower values Hcrt 1 = 63.5. ± 15.8 ng/mL vs 138.0 ± 46.4 ng/mL($p = 0.043$) and Hcrt 2 = 547.5 ± 34.7 ng/mL vs 528.8 ± 55.6 ng/mL, $p = 0.125$) compared to those without syphilis. For HIV positive patients also had significantly lower levels of Hcrt 1 (55.8 ± 24.3 ng/mL vs 106.6 ± 33.1 ng/ml, $p = 0.006$) and Hcrt 2 (517.8 ± 145.3 ng/mL vs 488.2 ± 93.4 ng/ml, $p = 0.08$) when compared to HIV negative ones.

For ES level in patients with active or treated syphilis presented (5.4 ± 3.9 vs 7.7 ± 5.7, $p = 0.032$) compared to negative patients. And was not seen differences between the degree of ES in HIV positive patients (7.3 ± 4.5 vs 7.8 ± 6.1, $p = 0.56$) when compared to HIV negative. The Spearman correlation test between Hcrt 1 and the ES scale was $r = 0.79$, while for Hcrt 2 was $r = 0.46$. te superior do formulário

Conclusion:

Our results demonstrated that Hcrt 1 or orexin A presented is reduced in patients with syphilis and HIV positive, it seems to be related to the degree of ES, but the ES levels found were not severe, and although significantly different, are within the limits of normality, this way, the relationship between reducing agents Hcrt and ES in patients with CNS infection needs further evaluation.

A-087

Development of a Vitamin D Total Assay* Using LOCI Technology on the Dimension EXL Integrated Chemistry System

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Background:

The Siemens Dimension® EXL™ Integrated Chemistry System incorporates multiple detection technologies, including LOCI® technology, which enables high-sensitivity immunoassay formats. Siemens is currently developing a Vitamin D Total assay for serum and plasma.

Methods:

The Dimension EXL Vitamin D Total assay (VITD) is a homogeneous competitive chemiluminescent immunoassay based on LOCI technology. The assay measures the total 25(OH)vitamin D concentration [comprising both 25(OH)vitamin D_2 and 25(OH)vitamin D_3] in both serum and plasma. Vitamin D Total LOCI reagents include a releasing reagent, biotinylated monoclonal antibody, and two synthetic bead reagents. Patient sample is incubated with the releasing reagent to release 25(OH)vitamin D molecules from the vitamin D-binding proteins. The reaction mixture is then incubated with biotinylated antibody to form a 25(OH)vitamin D/biotinylated antibody complex. Chemibeads coated with a 25(OH)vitamin D_3 analog and chemiluminescent dye are added to remove the excess free biotinylated antibody. Streptavidin-coated Sensibeads containing a photosensitive dye are added to bind the biotinylated antibody. Aggregates of the Chemibead analog/biotinylated antibody/streptavidin Sensibeads are formed as a result. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen from the Sensibeads, which diffuses into the Chemibeads and triggers a chemiluminescent reaction. The resulting chemiluminescent signal is measured at 612 nm and is inversely proportional to the concentration of total 25(OH)vitamin D in the sample.

Results:

The method requires 8 µL of serum or plasma. Time to first result is 32 minutes, with stable calibration for 7 days. Three-day open-well stability and 30-day onboard unopened stability have been achieved. Calibrator values are traceableto the Ghent ID-LC/MS/MS 25(OH)vitamin D reference measurement procedure. The VITD method is linear from 4 to 150 ng/mL. Reproducibility was assessed using the CLSI EP5-A2 protocol with serum samples ranging from 16 to 49 ng/mL. Repeatability CVs ranged from 2.2 to 2.5%. Within-lab CVs ranged from 3.3 to 3.9% respectively. Split sample correlation between this method and LC-MS/MS (VDSCP-certified) produced the following statistics: slope = 1.0115, intercept = −2.00 ng/mL, $r = 0.9172$, and $n = 112$ over a concentration range of 7.8-71 ng/mL. Minimal cross-reactivity is observed with 1,25$(OH)_2$vitamin D_2 and D_3 at 500 pg/mL, 3-epi-25$(OH)D_3$ at 100 ng/mL, and vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) at 1000 ng/mL. This assay is equimolar for 25(OH)vitamin D_2 and D_3.

Conclusion:

The Dimension EXL Vitamin D Total assay demonstrates acceptable precision, accuracy, and turnaround time for total 25(OH) vitamin D measurement on the Dimension EXL system.

*Under development. Not available for sale.

A-088

Prototype of accurate and precise immunoassay for low estradiol concentration determination

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Background:

Estradiol immunoassays are prone to inaccuracy at low estradiol concentrations which is detrimental when addressing the clinical status of children, men, postmenopausal women, and women receiving aromatase inhibitors. The aim of the abstract is to present a prototype immunoassay that is able to accurately and precisely measure estradiol concentrations from 16 pg/mL to 5000 pg/mL.

Methods:

Ninety samples covering the physiological variability (male, female, post-menopausal) and the range of estradiol concentrations (1.2-4368 pg/mL) were assayed on a JCTLM approved reference measurement procedure (ID-GC/MS), Siemens Centaur XP (S), Roche Cobas II (R) and Beckman Coulter Access prototype (B) immunoassays. Correlation was assessed using Spearman rank correlation. Method comparison was assessed using Passing Bablok linear regression and Bland-Altman percentage bias. For all analysis, ID-GC/MS estradiol measurement was used as the reference. Limit of Quantification (LoQ) was assessed according to CLSI EP17-A2 by measuring the total imprecision of 9 samples with a minimum of 5 replicates each day over 5 days on both Roche Cobas (R) and Beckman Coulter Access prototype (B) immunoassays.

Results:

The Spearman rank correlation was high for all methods at 0.967, 0.989 and 0.993, for methods R, S and B, respectively. Over the 90 samples, each method failed to quantitate some of the 17 samples below 10 pg/mL due to lack of sensitivity, with 8, 4 and 3 missed samples for methods R, S and B, respectively. The LoQ was determined at 23 and 16 pg/mL for methods R and B respectively which explains the higher number of missed samples on method R. In addition to random variability, methods R and S exhibit a systematic bias at low estradiol concentrations as highlighted by the intercept of the Passing Bablok linear regression of 13, 11 and 2 pg/mL for methods R, S and B, respectively, the last being not statistically different from 0 pg/mL. Focusing on three representative samples at 8.7, 4.6 and 1.6 pg/mL as determined by the reference method the systematic bias is particularly elevated for method R, the Bland-Altman percentage bias ranging +577% to +1900% on those samples assayed at 58.7, 45.2 and 31.6 pg/mL respectively. For method S, the Bland-Altman percentage bias is still elevated, ranging +284% to +824%, the samples assayed at 33.3, 19.7 and 14.6 pg/mL respectively. For method B, the Bland-Altman percentage bias is acceptable, ranging +15% to -58%, the samples assayed at 10, 1.9 and 0.8 pg/mL respectively.

Conclusion:

The Beckman Coulter Access prototype assay is able to accurately and precisely measure low estradiol concentrations which represents more than 50% of routine clinical measurements in general laboratories. In this study the prototype assay was the only immunoassay that was accurate and precise down to 16 pg/mL while other immunoassays were not.

A-089

Early performance evaluation of a new Intact PTH assay* on the Siemens ADVIA Centaur Systems in comparison to a previous assay generation

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Background: Parathyroid hormone (PTH) plays a major role in the regulation of mineral metabolism and skeletal physiology. The continuous advancement of Acridinium Ester (AE) chemiluminescence technology by Siemens Healthcare Diagnostics led to the development of a novel two-site monoclonal sandwich intact PTH assay**.** The first antibody in the Lite reagent, is a mouse anti-human PTH (N-terminal 14-28 region) antibody 2007 26/032 clone labeled with acridinium ester. The second antibody is a biotinylated mouse anti-human PTH (C terminal 52-59 region) antibody clone that is preformed to streptavidin coated paramagnetic latex particles in the solid phase. This is an evaluation of a new monoclonal ADVIA Centaur® intact PTH assay in comparison to the previous polyclonal assay generation in a dialysis laboratory setting, DaVita dialysis center.

Methods: Two hundred and nineteen (219) samples from fresh EDTA plasma specimens were assayed over four runs, one run per day, at DaVita Labs in Deland, FL. The range of specimens was selected to span the measuring limits of the new Investigational Use Only (IUO) PTH assay*. Data were collected from two assays run in parallel - the IUO assay and the current ADVIA Centaur iPTH assay, and analyzed according to CLSI EP-09 using a procedure for method comparison.

Results: All tested controls recovered within their expected dose ranges in each of the four analytical runs. Linear regression statistics were calculated for both assay for 219 patient samples and analyzed using Deming weighted regression. Two different reagent lots of the IUO assay yielded 95% CI for slopes 1.07 to 1.09 and 0.99 to 1.01, respectively, indicating good agreement with the current ADVIA Centaur iPTH assay. The repeatability CVs for the IUO reagent averages 1.6% across the assay range. Limit of Detection (LoD), measured according to CLSI EP17-A2 is found to be 1.6 pg/mL. In addition, the IUO assay has an increased measuring interval 3,000 pg/mL. No high dose hook effect was present at PTH concentrations up to 100,000 pg/mL.

Conclusion: A method comparison between the two assays shows good agreement, indicating users will benefit from an improved overall performance with no apparent shift in the PTH results.

*Under development. Not available for sale.

A-090

Analytical and Clinical Performance of IMMULITE 2000 TSI Assay*

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Background: Graves' disease (GD) is an autoimmune disorder and the most common cause of hyperthyroidism. In GD, thyroid stimulating immunoglobulins (TSI) bind to the TSH receptor and mimic TSH stimulation of the thyroid gland. The TSH receptor contains a large extracellular domain that presents epitopes for a variety of autoantibodies, including TSI and thyroid blocking immunoglobulins (TBI). In contrast to TSI, TBI bind to the TSH receptor and inhibit TSH stimulation of thyroid cells, leading to hypothyroidism. The IMMULITE® 2000 TSI assay is designed for the specific, quantitative detection of TSI in serum and plasma. The clinical utility of a TSI assay includes a determination of the autoimmune etiology of thyrotoxicosis, monitoring Graves' patient therapy, prediction of remission or relapse, confirmation of Graves' ophthalmopathy, and prediction of hyperthyroidism in neonates.

Methods: The IMMULITE 2000 TSI assay is an automated chemiluminescent immunoassay with a time to first result of 65 minutes. It employs a pair of recombinant human TSH receptor chimeras in a bridging format. The assay is traceable to the WHO 2nd International Standard for Thyroid Stimulating Antibody, NIBSC Code: 08/204.

Results: The detection limits of the assay were determined in accordance with CLSI EP17-A2 as follows: LoB = 0.03 IU/L; LoD = 0.06 IU/L; LoQ = 0.10 IU/L. The analytical measuring range of the assay is 0.10-40 IU/L. A total of 842 serum samples from apparently healthy males and females were analyzed. The results suggest a nonparametric upper 97.5th percentile of 0.07 IU/L. The assay precision was evaluated according to CLSI EP5-A2. The repeatability %CV varied from 3.5% to 7.0% across the assay range. The IMMULITE 2000 TSI assay was compared to the THYRETAIN TSI Reporter BioAssay using 244 serum samples from GD and other thyroid or autoimmune disease patients with the following results: Positive Agreement: 100% (129/129); Negative Agreement: 92.2% (106/115); Overall Agreement: 96.3% (235/244)**.** Serum samples from 236 treated and untreated GD patients, 138 individuals with other thyroid or autoimmune diseases and 200 apparently healthy individuals were evaluated against clinical diagnosis. The TSI values for the patients with other thyroid or autoimmune diseases had an upper limit of 0.39 IU/L. At 0.55 IU/L cut-off, the clinical sensitivity and specificity were 98.3% (232/236) and 99.7% (338/339), respectively.

Conclusion: The IMMULITE 2000 TSI assay is a sensitive quantitative immunoassay for the specific detection of TSI in the routine diagnosis and assessment of GD patients.

**This product is under development and not yet commercially available.*

A-091

Assessment of the Upper Reference Limit of Estradiol III using the software StatisProTM

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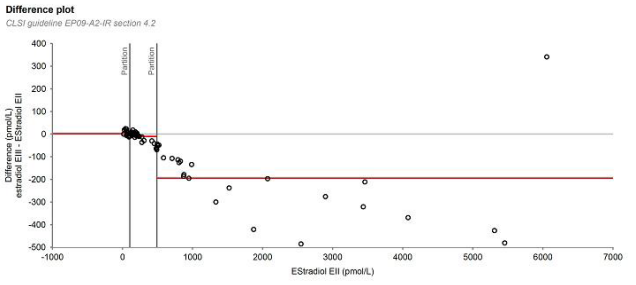
Background. To estimate the reference interval of sexual hormones in females is difficult since they are strongly linked to age and menstrual phase. Recently Roche introduced an assay for Estradiol (EIII) employing two biotinylated monoclonal antibodies (rabbit) that superseded EII assay employing a biotinylated polyclonal antibody (rabbit). Upper Reference Limits (URLs) proposed for EIII in male adults and in females in follicular and luteal phases and menopause are vey similar to those proposed for EII but that proposed for ovulatory phase is significantly lower (30 %). The aim of our study was to assess the capability of StatisPro™ to verify the EIII reference intervals proposed by the manufacturer.

Methods: EII and EIII were measured using Modular E-170 analyzer (Roche, Mannheim, Germany) in 77 serum samples consecutively collected in routine workload. The measurements were carried out in singleton following the EP9 2-IR CLSI standard and the calculations and the graphs were carried out using StatisPro™ (CLSI and Analyse-it, Wayne, USA) and Medcalc (Ostende, Belgium).

Results: The regression equation was EIII= -22-65 + 0.940*EII; Passing and Bablok regression 2.952 + 0.893 EII and the correlation was 0.996 (95% Confidence Interval: 0.994-0.997). The partitioned biases were 2,00 (SD 8.59) at E < 106 pmol/L (n=25); -10.4 (SD 10.61) at E ≥106 and < 490.1 pmol/L (n=27); -194.57 (SD 175.69) at E ≥490.1 pmol/L (n=25) with Sy.x=109.93.

Conclusion: Calculate or, at least, verify the reference intervals of hormones that present large variations dependent on sex, age and menstrual phases is often virtually impossible for laboratorians. StatisPro™ can be really useful since carries out all the calculations and graphs needed for demonstrating the comparability of the results along the concentration span of results yielded by different firms or different reagents. EIII lower URL in ovulatory phase is consistent with the negative bias at high concentration.



A-092

Circulating Bilirubin As A Marker Of Adverse Coronary Heart Disease Risk Profile In First Degree Relatives Of Patients With Type 2 Diabetes Mellitus

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Introduction & Objectives :

Recent studies have shown that circulating total bilirubin (Tbil), often considered to be a toxic byproduct of haem catabolism, is inversely associated with risk for coronary heart disease (CHD) and diabetic nephropathy. The objective of this study was to

examine the associations of TBil with low grade inflammation, circulating adipokines, insulin resistance (IR), metabolic syndrome (MetS) and incident diabetes in first degree relatives (FDR) of diabetic subjects.

Methods

Fasting Tbil, adiponectin, leptin, leptin receptor (sOB-R), insulin, glucose, high-sensitivity CRP (hsCRP), lipid profile were determined in 590 (238M and 352F) FDR. Free leptin index (FLI), insulin sensitivity (%S) and resistance (Homeostasis Model Assessment (HOMA-IR)) were calculated. Patients were categorised by IR, MetS (International Diabetes Federation criteria) and bilirubin quartiles.

Results

Tbil showed significant ($p<0.05$) inverse correlations with BMI ($r = -0.24$), insulin ($r=-0.16$), HOMA-IR ($r=-0.12$), Triglycerides ($r=-0.14$), Apo B ($r = -0.12$), HbA1c ($r=-0.19$) and direct correlations with %S ($r = 0.12$), sOB-R ($r = 0.20$) and FLI ($r=0.24$). Subjects in the first TBil quartile had higher ($p<0.05$) BMI, waist circumference, triglycerides, HbA1c, insulin, HOMAIR, resistin, leptin, FLI, hsCRP and lower HDL-C and adiponectin compared to subjects in the 4th quartile. TBil decreased stepwise with increase in BMI and number of MetS components. The prevalence of MetS from 1st to 4th quartile were 40%, 40%, 13%, and 7%, respectively. The prevalence of IR from 1st to 4th quartile were 30%, 26%, 24%, and 20%, respectively. Binary logistic regression analysis showed odds ratio of the association of TBil with IR, MetS and incident diabetes were 0.88, 0.92 and 0.93 respectively.

Conclusions

TBil in the upper quartile of the "normal" reference interval is associated with healthy metabolic profile and factors that reduce CHD risk . In contrast, TBil in the lowest quartile is associated with increased CHD risk profile, suggesting that TBil may have anti-atherogenic properties. These findings suggest the need for laboratory medicine practitioners to redefine the "normal" reference range for TBil. There is also need for attending physicians to prudently review results of the routinely estimated TBil as low levels could be useful adjunct for the selection of high risk FDR for more aggressive intervention to lower the risk of progression to T2DM or development of CHD.

A-093

Development of a highly sensitive enzyme immunoassay for oxytocin

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Background:

The neurohypophyseal peptide hormone oxytocin acts in the central nervous system and plays an important role in various complex social behaviors, including affiliation, sexual behavior, social recognition, stress buffering, aggression, and trust. There is increased interest in measuring peripheral oxytocin levels to better understand the role of this peptide in human social behavior. The levels of oxytocin in plasma have been measured by radioimmunoassays and enzyme immunoassays (EIA), but the many reported methods lack sufficient sensitivity and specificity for oxytocin. We here report the production of a high affinity and high specificity antibody towards oxytocin and its use in a highly sensitive EIA using colorimetric and bioluminescence detection.

Methods:

Antibody: Anti-oxytocin antiserum was obtained by immunizing rabbits with oxytocin-bovine thyrogloblin conjugate prepared by the glutaraldehyde method.

Assay method: Oxytocin sample was added to a second-antibody-coated microtiter plate and reacted overnight at 4 °C. Biotinylated oxytocin was then added for 1 hour at 4 °C, followed by the addition of horse radish peroxidase (HRP)-labeled avidin and incubation for 1 hour at room temperature. The plate was then washed three times with buffer to separate bound/free and the activity of HRP bound to antibody was measured colorimetrically using o-phenylenediamine at 490 nm.

For bioluminescence detection, second-antibody-coated magnetic beads, unlabeled avidin, thermostable biotinylated luciferase, and luciferin as the substrate were used.

Examination of cross-reactivity

The cross-reactivities of three oxytocin-like peptides, [Arg8]-vasopressin (AVP), [Lys8]-vasopressin (LVP), and [Arg8]-vasotocin (VT) with oxytocin were examined by cross-reactivity tests.

Results:

The proposed method is based on the principle of competitive EIA using anti-oxytocin antibody from rabbit and biotinylated oxytocin as the labeled antigen. The labeled antigen comprised biotin chemically bound to oxytocin containing 0 to 5 lysines, providing bridge-link heterology. Six labeled antigens were prepared and used to develop a highly sensitive EIA. Rabbits were immunized with oxytocin bound through the N-terminus to the carrier protein bovine thyroglobulin. The produced antibody and the six biotinylated oxytocins were used in various combinations to probe the sensitivity of the EIA. The sensitivity of the EIA improved as the number of lysine residues increased; consequently, biotinylated oxytocin bridged with 4 or 5 lysines was used thereafter. A standard curve range for oxytocin was 2.5 to 1000 pg / assay. The detection limit of the assay was 2.5 pg and the reproducibility of each point in the standard curve had an average coefficient of variation value (n = 5) of 3.18%. The specificity of the assay was tested using three compounds with structures similar to that of oxytocin: AVP, LVP, and VT. Cross-reactivity with all three compounds was less than 0.01%, indicating that this antiserum is very highly specific for oxytocin. In addition, the detection limit for this EIA can be improved to 1.0 pg/assay using a bioluminescence detection method.

Conclusions:

The proposed method is sensitive and more specific than conventional immunoassays for oxytocin and can be applied to the determination of plasma oxytocin levels.

A-094

Macroprolactin: Its prevalence and the need to screen in a tertiary care hospital

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Background: Circulating human prolactin is known to exhibit molecular heterogeneity. The clinical significance of which remains unknown. Macroprolactin, a 150 kDa molecular weight form, may interfere in prolactin assays causing false hyperprolactinemia that may lead to unnecessary additional investigation. However, the practice of screening for the presence of macroprolactin is not widely adopted. We investigated the prevalence of macroprolactin among patients presenting to a large tertiary care teaching hospital and being investigated for fertility disorders.

Methods: Serum samples from patients presenting to Parkland Memorial Hospital with elevated prolactin levels, as determined by laboratory-based immunoassay analyzer (COBAS, Roche Diagnostics, Indianapolis, IN) were retrospectively collected and stored at -20C until further analysis. Stored samples were thawed and mixed with polyethylene glycol (PEG) at 1:1 (v/v) for a final PEG concentration of 12.5% (v/v). Samples were allowed to stand at room temperature for 20 minutes followed by centrifugation at 3000xg for 5 minutes. Prolactin levels prior to and following PEG treatment were measured using ELISA (Calbiotech, Spring Valley, CA). Prolactin percentage recovery following PEG treatment was calculated.

Results: A total of 40 patients' samples were collected during the study period. Prolactin levels as determined by the laboratory-based immunoassay ranged from 37 ng/mL to 244 ng/mL, median 72 ng/mL. This is compared with ELISA-based prolactin levels at 3 ng/mL to 197 ng/mL, median 81 ng/mL. Percentage recovery following PEG treatment for all samples ranged from 30.6% to 127.9 %, median 86%. Samples with percentage recovery less than 40%, greater than 60%, and those between 40 and 60% were considered positive for the presence of macroprolactin, negative for the presence of macroprolactin, and as borderline respectively.

Two samples with prolactin levels of 76 ng/mL and 181 ng/mL were positive for the presence of macroprolactin with a percentage recovery of 30.6% and 35.1% respectively. Four samples at 130 ng/mL, 154 ng/mL, 140 ng/mL, and 147 ng/mL exhibited borderline percentage recovery at 52.4%, 57.2%, 46.8%, and 55.9 % respectively. The remaining study samples had recovery greater than 60% and were thus considered negative for macroprolactin.

Conclusion: Macroprolactin was detected in 6% of samples from patients with prolactin levels greater than 42 ng/mL. Those positive for macroprolactin had prolactin levels elevated at 76 ng/mL and 181 ng/mL.

Although this small study suggests lack of relationship between prolactin levels and the likelihood of macroprolactin presence, further analysis is required to ascertain the relationship and to investigate the borderline samples. Samples with unexplained elevated prolactin levels should be screened for the presence of macroprolactin.

A-095

Development of immunoassay for detecting liver-type fatty acid binding protein (L-FABP) for LUMIPULSE® G1200

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Background: Liver-type fatty acid binding protein, L-FABP, is a 14kDa fatty acid binding protein localized at the proximal renal tubule of human kidney. L-FABPs are known to be excreted through urine during the occurrence of ischemia and/or

oxidative stress within the renal tubule prior to kidney tissue damage. Therefore, L-FABP is considered a useful marker in the early diagnosis of diseases that are associated with tubular dysfunction, such as chronic kidney disease (CKD) or diabetic nephropathy. In order to evaluate the progression of such diseases, quantitation of low levels of L-FABP during the early stages is necessary. We have developed the first fully-automated, high sensitive chemiluminescence immunoassay for LUMIPULSE G1200 system for detecting urinary L-FABP, and its performance is evaluated.

Methods: L-FABP assay for LUMIPULSE is a two-step sandwich chemiluminescent enzyme immunoassay (CLEIA) without a specific pretreatment process prior to the 1st immunoreaction. The resulting reaction signals are derived within 30 minutes/sample, and are proportional to the amount of L-FABP in the sample allowing quantitative determination of urinary L-FABP.

Results: The detection limit of the assay was 0.03 ng/mL, and the limit of quantitation was 0.16 ng/mL. A 20-day precision study was performed during a 31-day period using two controls and three panel specimens, and the imprecision was ≤ 3.4% total CV. Dilution linearity was evaluated using three test samples, and the recovery rate of up to 100-fold dilution was 95-102% for manual dilution and 99-103% for automated dilution within the calibration range of 0.5 - 400 ng/mL. For spike recovery study, varying amounts of L-FABP were added to urine samples containing low levels of L-FABP to create test samples with concentrations ranging from 70-350 ng/mL. The measured values, when compared to the expected values, ranged from 91-105%. The correlation coefficient and the regression slope of Lumipulse G L-FABP and commercially available enzyme-linked immunosorbent assay kit (CMIC Co., Tokyo, Japan) were 0.96 and 1.07, respectively (N=111). No interference was observed with unconjugated (21.0 mg/dL) or conjugated bilirubin (18.5 mg/dL), hemoglobin (490 mg/dL), NaCl (2 g/dL), glucose (1 g/dL), acetone (100 mg/dL), creatinine (1 g/dL), albumin (1 g/dL), ascorbic acid (500 mg/dL), ethanol (1 g/dL), or riboflavin (10 mg/dL).

Conclusion: The performance of L-FABP assay for LUMIPULSE G1200 was satisfactory, suggesting the possible usage of various types of urine samples including hematuria, albuminuria, and urine with high concentration of ascorbic acid. The evaluation results indicated that L-FABP assay for LUMIPULSE has the ability to precisely quantitate significantly low level samples, and the measurement range of 0.5 - 400 ng/mL may be the widest of existing commercially available assays.

A-096

CLINICAL VALUE OF MEASUREMENT OF THYROTROPIN-RECEPTOR ANTIBODIES (TRAb) IN PATIENTS WITH GRAVES' DISEASE

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Background:

Graves' disease (GD) is characterised by stimulating autoantibodies to the TSH-receptor (TRAb). A fully automated immunoassay for the quantitation of TRAb in serum is available on Roche Cobas e411. This study was conducted to evaluate this assay in routine clinical use in the differential diagnosis of thyroid disorders.

Methods:

92 patients who attended a university thyroid clinic were included. Based on TFT results, 48 were classified as hyperthyroid, 18 as hypothyroid and 10 as euthyroid. Five patients who had carcinoma of thyroid and 11 patients who were treated for GD were also included in this study. Of the 48 patients who were hyperthyroid, 34 were diagnosed as GD, four had multi-nodular goitre and 10 were hyperthyroid due to other causes. The sensitivity, specificity, positive and negative predictive values (PPV, NPV) of the TRAb test were calculated using published cut-off values.

Results:

At the cut-off level of 1.6 IU/L (Syme et al, 2011), the sensitivity, specificity, positive and negative predictive values were 88.2%, 100%, 100% and 90.2% respectively. TRAb was positive in three patients who were treated for GD. Using the manufacturer's cut-off value of 1.75 IU/L, the following performance characteristics were found: sensitivity 85.3%, specificity 100%, PPV 100% and NPV 86%.

Conclusion:

Sensitivities, specificities, PPVs and NPVs calculated using the two TRAb cut-off values of 1.6 IU/L and 1.75 IU/L are comparable. TRAb is a useful laboratory test in the differential diagnosis of hyperthyroidism and for the follow-up of patients with GD.

A-097

Atherogenic index - cardiovascular risk indicator at diagnosis in hypothyroid females with and without coexisting diabetes mellitus

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Background:

Thyroid disorders and Diabetes mellitus (DM) coexist with a frequency greater than chance can predict. Both disorders are potential causes of dyslipidemia and cardiovascular disease (CVD) risk. However there is scant data on coexisting hypothyroidism and DM at diagnosis in Indian female subjects. We aimed to evaluate dyslipidemia and atherogenicity of hypothyroid females with and without coexisting DM at diagnosis.

Methods:

We enrolled 110 newly diagnosed hypothyroid females and 50 healthy control females of age group 20 to 50 years from outpatient endocrine clinics of a government hospital. Hypothyroid females were categorized as group I (hypothyroid with DM) and group II (hypothyroid without DM). All study participants were analysed for body mass index (BMI), blood pressure, serum T_3, T_4, TSH, Insulin (ELISA), lipid profile, fasting blood sugar (FBS) (enzymatic) and apo - B, apo - A_1 (Immunoturbidimetric). DM was diagnosed when FBS >126 mg/dl. The data was analysed for insulin resistance (HOMA-IR) and atherogenic index (AI) (log TG/HDL). AI, apo-B/apoA_1 and Total cholesterol/HDL (TC/HDL) ratios indicated atherogenicity. Data was statistically analysed (mean± SD) using student's t-test and Pearson's coefficient of correlation.

Results:

The frequency of coexisting DM in hypothyroid females at diagnosis was 42.7%. The hypothyroid females presented with significantly raised BMI as compared to healthy controls (HC) (22.16±3.33 v/s 28.73±5.12 p<0.0001). They showed gross dyslipidemia as compared to HC (HC v/s Hypothyroid TC 175.49±16.25 v/s 231.69±20.84 mg/dl; Triglyceride (TG) 126.49±23.1 v/s 184.04±40.07mg/dl; LDLc 110.41±17.9 v/s 133.04±37.2 mg/dl, p<0.0001; HDLc 41.18±5.60 v/s 42.75±4.83 mg/dl p=0.07). Hypertension was observed in group I and group II, with mean SBP and DBP higher (p<0.0001) in group I as compared to group II (137.83±12.24 v/s 126.66±9.39 mm Hg, p<0.0001) (92.09±8.06 v/s 84.75±6.14 mm Hg, p<0.0001), FBS in group I was significantly higher than in group II (205.48±4.25 v/s 92.58±1.68; p<0.0001), insulin resistance was observed in group I as compared to group II (31.37±1.30 v/s 6.30±4.06, p<0.0001), T_3, T_4 significantly were reduced in group I than in group II (T_3 0.29±0.19 v/s 0.61 v/s 0.12; T_4 2.47±1.07 v/s 3.68±1.05 p<0.0001) TSH significantly higher in group I than in group II (43.13±15.46 v/s25.56±24.8 p<0.0001). AI of group I was significantly higher than HC (0.64±0.06 v/s 0.41±0.19, p<0.0001) and group II (0.64±0.06 v/s 0.54±0.07, p<0.0001). The CVD risk ratios were significantly raised in group I than group II (apoB/apo A_1: 1.38±0.29 v/s 1.23±0.36, p=0.02; TC/HDL: 5.82±1.18 v/s 4.49±0.90 p<0.0001). Pearson's coefficient of correlation in group I showed significant correlation of AI with SBP (r = -0.93, p = 0.0001), HOMA-IR (r= 0.389, p = 0.0069), FBS (r=0.306, p=0.03) and non-significant association (NS) with DBP (r = -0.097, p=0.54). Group II showed NS association of AI with SBP (r=-0.23, p =0.061), DBP(r=-0.21, p =0.08), HOMA-IR (r=0.15, p = 0.201) and FBS (r=0.28, p=0.056).

Conclusion:

The atherogenic index of plasma is better correlated with CVD risk parameters in hypothyroid females with DM. Thus AI of plasma can be an important parameter for the risk assessment of atherosclerosis in hypothyroid females at diagnosis.

A-098

Verification of CALIPER reference interval for T3 and T4 in Brazilian pediatric population

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Background: The Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) established reference intervals (RI) stratified by age and sex for biochemical and hormonal laboratory markers, developing a valuable source of RI for healthy pediatric population. These intervals were determined in the Abbott ARCHITECT System®, providing RI for its users, but useful also for other laboratories, since validated to use for local population and for specific immunoassay platforms. This study objective is to assess, as recommend by CLSI, the applicability of T3 and T4 (free - FT3, FT4, and total - TT3, TT4) CALIPER RI for pediatric Brazilian population, using laboratory database, Lab Rede® - Minas Gerais, Brazil.

Methodology: Results were collected for children of both sexes, 1-11 months (jan/2013-dec/2014) and 1-18 years (jan-dec/2014). Each analyte was studied with related parameters: TT3 (n=2339) and FT3 (n=565) associated at normal TSH and FT4; TT4 (n=1611) and FT4 (n=1881) associated at normal TSH and TT3. Was used ARCHITECT i2000 platform (Abbott Park, IL, USA), chemiluminescent microparticle immunoassay, serum stored at 2-8°C. The data distribution by age and gender of CALIPER were submitted to EP Evaluator® program to RI verification and statistical analysis. **Results:** The results for groups (central interval of 95%) and their CALIPER RI were approved for FT4 (2 groups) and partially approved for FT3, disapprovingly 1 group on 5. For TT3 and TT4 most of CALIPER RI were rejected, only 1 group was approved on 6, for both. Because insufficient results the verification of FT3 and TT4 in children less than 1 year was disregarded.

Conclusions: It is a challenge to obtain RI for the pediatric population, so the use of database sampling constitutes a viable option for checking the ranges proposed at scientific literature. The CALIPER RI are applicable to the studied population for FT4 and most groups of FT3.

T3 and T4 CALIPER RI verification in Brazilian pediatric population									
TT3 ng/dL Ages (n)	CALIPER RI	RI Verification	Outside	CI 95%	TT4 mcg/dL Ages (n)	CALIPER RI	RI Verification	Outside	CI 95%
1-11 months (44)	84.64-234.38	passes	2,3%	122.25-236.88	1-8 years (351)	6.16-10.32	no passes	14.2%	5.4-10.02
1-11 years (1025)	113.28-189.45	no passes	13.7%	104-204	9-11 years (317)	5.48-9.31	no passes	14.8%	4.8-9.52
12-14 years (506)	97.66-176.43	no passes	10.7%	86.67-182.3	12-13 years female (142)	5.08-8.34	no passes	15.5%	4.66-8.98
15-16 years female (254)	92.45-141.93	no passes	22.8%	78-181.25	12-13 years male (77)	5.01-8.28	no passes	11.7%	4.48-8.51
15-16 years male (95)	93.75-156.25	no passes	15.8%	81.6-154.6	14-18 years female (496)	5.46-12.99	no passes	14.3%	4.48-10.46
17-18 years (415)	89.84-167.97	no passes	18.6%	75.4-193.6	14-18 years male (228)	4.68-8.62	passes	5,7%	4.47-8.33
FT3 pg/mL Ages (n)					FT4 ng/dL Ages (n)				
1-11 years (253)	2.79-4.42	passes	8,7%	2.64-4.56	1-11 months (32)	0.89-1.70	passes	6,3%	*
12-14 years female (59)	2.5-3.95	no passes	15.3%	2.05-4.4	1-18 years (1849)	0.89-1.37	passes	7,8%	0.86-1.40
12-14 years male (56)	2.89-4.33	passes	8,9%	2.6-4.47					
15-18 years female (141)	2.31-3.71	passes	9,2%	2.2-3.75					
15-18 years male (56)	2.25-3.85	passes	8,9%	1.99-3.81					

A-099

Evaluation of Analytical Performance of the Beckman Coulter Total βhCG (5th IS) Immunoassay

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Background: Clinical uses of human chorionic gonadotropin (hCG) include determination of pregnancy, diagnosis and monitoring of germ cell tumors and gestational trophoblastic diseases, and prenatal screening for Down syndrome when used in combination with other markers. Recently, the first commercially available total βhCG reagent (Beckman Coulter, Inc) calibrated against the WHO 5th International Standard (IS 07/364) was introduced. This new reagent is expected to result in a change in assay analytical performance as well as in a systematic bias when compared to assays standardized against the hCG WHO 3rd International Standard. In this study we evaluated the analytical performance of the **total βhCG (5th IS)** immunoassay.

Methods: Imprecision studies were conducted using Liquichek® Immunoassay and Lyphochek® Fertility quality control (QC) materials (Bio-Rad, Inc). Analyte measurement range (AMR) studies were conducted by testing a lot of hCG calibrators, different than what was used for calibration of the assay, as unknowns. Limit of quantitation (LOQ) studies were conducted using a serum sample with low hCG concentration. Carryover was assessed by running a blank sample after samples containing elevated hCG concentrations. Susceptibility of the assay to heterophile antibodies interference was assessed by evaluating three samples with known interference in the total βhCG (3rd IS) assay. Method comparison studies with the total βhCG (3rd IS) was performed using de-identified serum samples. De-identified samples from 600 patients undergoing 1st or 2nd trimester Down syndrome screening were utilized to derive trimester specific medians.

Results: Intra- and inter-assay imprecision studies produced coefficient of variation (CV) of ≤ 6% (range 3-6%) at concentrations of 4.8, 345 and 16861 IU/L. The AMR of the assay was 0.6 to 1350 IU/L with Passing-Bablok regression fit of y = 1.03x +0.15 (r^2=0.999). Serial dilution (x1000) to expand the AMR produced an average recovery of 100% (range 86-107%). LOQ was determined to be 0.5 ng/mL (CV = 20%). Assay comparison with the total βhCG (3rd IS) assay (n=50, range 0.9-771 IU/L) showed good correlation (r^2=0.992) but a systematic bias with a slope of 1.26 and intercept of 0.26 by Passing-Bablok regression fit. Significant carryover was observed at hCG concentrations ≥120,000 IU/L. The assay was less susceptible to heterophile antibody interference than the total βhCG (3rd IS) assay. Due to the observed bias, adjustment of the coefficients A and B used on the maternal screening median calculation was as follow: 1st trimester, A= 675.292 and B = 0.977; and 2nd trimester, A= 18.758 and B = 41.153.

Conclusion: The analytical performance of the total βhCG (5th IS) was established. The amount of carryover observed might be problematic for laboratories using the assay for tumor marker purposes. Given the expected bias, rebaseline of patients undergoing serial monitoring will be necessary. Laboratories using the assay for maternal screening purposes will need to compute new medians to prevent an adverse impact in the Down syndrome detection rate and false positive rate.

A-100

Prevalence of subclinical hypothyroidism in diabetic patients over 60 years using specific diagnostic criteria for elderly patients, and the impact of treatment on the diagnosis

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Background: Several studies suggested that hypothyroidism is more frequent in patients with diabetes mellitus (DM), and this would be particularly relevant in elderly. However, there is an adaptation of TSH levels to age, and it is not totally clear how much the medications used for DM treatment or for associated co-morbidities are capable of interfering in TSH levels. The authors tested the occurrence of subclinical hypothyroidism in elderly patients with DM, analyzed the interference of some medication regularly used for DM and for co-morbidities treatment on a group of patients, and if they could change the screening for subclinical hypothyroidism.

Methods:TSH, T4L, and TPOAb were measured prospectively, in 590 patients with DM, older than 60 years (71% women), mean duration of DM of 9.7 years, diagnosed over 2 years before, according to criteria of American Thyroid Association. Groups of patients with treatment without use of medications for co-morbidities (n=378) were patients in only diet regimen, metformin, DPP4 inhibitor, insulin, pioglitazone, and on two or more of these medications. Groups of patients with co-morbidities treatments that could exert potential interference with the TSH level (n=212) were patients in use of propranolol, atenolol, acetil salicylic acid (antiagregant dose), amlodipin, carbamazepine, hydantoin, and of two or more than one drug that could interfere on TSH.

Results: Prevalence of subclinical hypothyroidism in diabetics was 4.1% from 60 to 79 years and 2.6% in ≥ 80 years.There was no significant difference of TSH among groups of treatment for DM (Median TSH- diet only: 1.8 mU/L, metformin:1.7 mU/L, sulfonylurea:1.9 mU/L, DPP4 inhibitor: 1.7 mU/L, insulin: 1.8 mU/L, pioglitazone: 1.7 mU/L, more than one drug: 1.8 mU/L. TSH was significatively higher in patients treated with more than one drug that could potentialy increase its levels, like

carbamazepine plus hydantoine or beta blockers plus one of this medications: 2.3 to 2.9 mU/L. TSH was lower in patients in use of amlodipine: 1.2 mU/L. Levels of FT4 were not different in all groups studied

Conclusion: The prevalence of subclinical hypothyroidism was slightly higher in the diabetic group compared to a group of non-diabetic elderly evaluated under similar conditions. Some medications only in associaiton increased TSH. Amlodipine decreased significantly TSH. None of them, as some drugs usually used for the treatment of diabetes mellitus, interfered with the screening of hypothyroidism. This is the first report of the effect of Amlodipine decreasing TSH levels.

A-101

LCMSMS quantitative analysis of Methylmalonic Acid in urine and serum and evaluation and comparison of sample preparation techniques

R. M. Doyle. *Agilent Technologies, Wilmington, DE*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. The analysis of Methylmalonic acid (MMA) has become ubiquitous in the measurement of Vitamin B12 deficiency. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of Methylmalonic acid by QQQ. Various sample preparation techniques that includes dilute and shoot in urine, ultracentrifugation (UF), protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D) and two (2D) dimensional chromatographic configurations were evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness. The deriviatization of the compound was also evaluated as well as different mass spectrometer platforms. The described method achieves the required sensitivity and is capable of quantitating the analyte over a dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure MMA in urine and serum.**Methods:** An Agilent 6460 tandem mass spectrometer with Jet Stream technology in negative Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 ul of urine and serum was used for the analysis of MMA. Various columns were evaluated and an Agilent Poroshell 120 Bonus-RP 100 x 3.0 mm, 2.7 um with a water:methanol mixture containing 0.1% formic acid gradient achieved baseline chromatographic separation in less than 4 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in negative mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and urine and serum samples.

Results: Good linearity and reproducibility were obtained with the initial concentration range of 1 ng/ml (8.5 nM) to 500 ng/ml (4250 nM) for MMA with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be initially 0.5 ng/ml and 1 ng/ml. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations.

Conclusion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of MMA in urine and serum. The sample preparation techniques are quick and easily applied for high throughput analysis.

A-102

LCMSMS quantitative analysis of Steroids in serum and urine and evaluation and comparison of sample preparation techniques

R. M. Doyle. *Agilent Technologies, Wilmington, DE*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. Steroids control metabolism, inflammation, immune functions, salt and water balance, development of sexual characteristics, and help in the body's ability to withstand illness and injury. The Steroids analyzed include Androstenedione, DHEA-S, Testosterone, Cortisol, DHEA, 11-Deoxycortisol, Corticosterone, 17-OH-Pregnenolone, Progesterone, 18-OH-Corticosterone, Deoxycorticosterone, Androstanddiol, etc. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of Steroids by QQQ. Various sample preparation techniques that includes dilute and shoot for urine, protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D) and two (2D) dimensional chromatographic configurations are evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness. The deriviatization of these compounds was also evaluated as well as different mass spectrometer platforms. The described method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure Steroids in serum and urine.

Methods: An Agilent 6495 tandem mass spectrometer with Jet Stream technology in positive and negative Electrospray mode and an Agilent Infinity 1290 HPLC system were utilized for this analysis. 200 ul of serum and urine were used for the analysis of Steroids. Various columns were evaluated and an Agilent Poroshell 120 EC-C18 100 x 2.1 mm, 2.7 um with a water:acetonitrile mixture containing 0.1% formic acid and 5mM Ammonium Formate gradient achieved baseline chromatographic separation in less than 9 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive and negative mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and serum and urine samples.

Results: Good linearity and reproducibility were obtained with the concentration range from 5 pg/ml to 1000 ng/ml for the respective Steroids with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 1 to 5 pg/ml. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations.

Conclusion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of Steroids in serum and urine. The sample preparation techniques are quick and easily applied for high throughput analysis.

A-103

LCMSMS quantitative analysis of Bile Acids in serum and evaluation and comparison of sample preparation techniques

R. M. Doyle. *Agilent Technologies, Wilmington, DE*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. The main function of bile acids is to facilitate the formation of micelles, which promotes digestion and absorption of dietary fat, but they are increasingly being shown to have hormonal actions throughout the body. The bile acids analyzed include Chenodeoxycholic acid, Cholic Acid, Deoxycholic acid, Glycochenodeoxycholic acid, Glycocholic acid, Glycodeoxycholic acid, Glycolithocholic acid, Glycoursodeoxycholicacid, Lithocholic acid, Taurochenodeoxycholic acid, Taurocholic acid, Taurodeoxycholic acid, Taurolithocholic acid, Tauroursodeoxycholic acid, Ursodeoxycholic acid, etc. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of Bile acids by QQQ. Various sample preparation techniques that includes protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D) and two (2D) dimensional chromatographic configurations are evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness as well as different mass spectrometer platforms. The described method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure bile acids in serum.

Methods: An Agilent 6460 tandem mass spectrometer with Jet Stream technology in negative Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 ul of serum were used for the analysis of bile acids. Various columns were evaluated and an Agilent Poroshell 120 EC-C18 100 x 2.1 mm, 2.7 um with a water:acetonitrile mixture containing 0.1% formic acid and 5 mM Ammonium Acetate gradient achieved baseline chromatographic separation in less than 6 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in negative mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and serum samples.

Results: Good linearity and reproducibility were obtained with the concentration range from 1 nM to 1000 nM for the respective bile acids with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.1 to 0.5 nM. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations.

Conclusion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of bile acids in serum. The sample preparation techniques are quick and easily applied for high throughput analysis.

A-104

Development of a New High Sensitivity Biochip Based Direct Immunoassay for the-Measurement of Low Levels of Total Testosterone in Serum

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Background:

Testosterone is one of the most commonly measured serum hormones. Accurate and reliable measurement has important clinical implications as this hormone plays significant physiological roles both in men and women. Measurement of the low levels found in male hypogonadism, women and prepubertal subjects is analytically challenging. Immunoassays are used in many clinical laboratories for routine measurement, as they provide rapid and cost effective information regarding circulating testosterone levels; however, it has been reported that immunoassays lack sufficient sensitivity, accuracy and precision when low levels of testosterone are measured. In 2007 the Endocrine Society recommended to carry out the measurements for women and prepubertal subjects only by an extraction liquid chromatography tandem mass spectrometry (LCMS) method due to the inaccuracies of current immunoassays.The current study aimed at developing a new biochip based immunoassay standardized to the industry "Gold Standard" isotope-dilution liquid chromatography tandem mass spectrometry (ID-LCMS-MS) for the detection of low levels of testosterone in serum. This represents an advantageous new analytical tool for endocrinology research and clinical applications.

Methods:

A direct competitive chemiluminescent immunoassay on a biochip platform with the semi-automated Evidence Investigator biochip analyzer was utilized. Assay sensitivity was determined as limit of quantitation (LOQ) in accordance with Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A; 15 replicates of 4 individual patient samples were tested over 5 independent runs. These samples had predetermined target concentrations from ID-LCMS-MS. Method accuracy was assessed as a measure of bias using the results of LOQ testing. Intra assay precision was determined by the analysis of 20 replicates at 4 clinically relevant concentrations in the assay range. The correlation study was conducted by analyzing 36 serum samples and comparing with the ID-LCMS-MS method.

Results:

The assay was target specific presenting cross-reactivity <1% for estradiol, progesterone, DHEA-S, methyltestosterone, estrone and cortisol.

The LOQ was 0.118 ng/mL within the total allowable error of 25% (set byWestgard). This provided an assay range of 0.118 ng/mL to 15.264ng/mL. The accuracy of this new assay; calculated as a bias of the test result from the LCMS value was + 0.6 %. Intra assay precision expressed as CV (%) for 20 samples at the following concentrations: 0.743, 1.221, 3.393, and 7.036 ng/mL was 6.8%, 5.7%, 6.1%, 6.7%respectively. In the correlation study, linear regression on the resulting data generated r values of 0.9858 for samples with testosterone levels from LOQ 0.118-14.4 ng/mL and 0.915 for female samples in the concentration range 0.118-1.573 ng/mL.

Conclusion:

The results show that the reported new biochip based immunoassay can determine testosterone levels <1.573 ng/mL in serum samples and can be used for the measurement of the low levels found in male hypogonadism, women and prepubertal subjects. This immunoassay is therefore a valuable and reliable new analytical tool for the measurement of low levels of testosterone in serum, which is relevant for endocrinology research and clinical applications.

A-105

Concordance between GH peak and IGF-1 quartiles in 200 short children submitted to GH stimulations test.

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Background:

Although it has been recognized that clinical evaluation is the most valuable aspect of assessment in growth disorders, most clinicians will evaluate children with short stature on the ground of the results of growth hormone stimulating tests (GHST) and insulin-like growth factor-1 (IGF-1) measurement. The assessment of IGF-1 secretion is an important diagnostic tool, as its synthesis is growth hormone (GH) dependent and, on the contrary to GH levels, has relatively stable levels. The diagnosis of GH deficiency seems unquestionable when both GH peak on GHST and IGF-1 concentration are decreased. On the other hand, GH deficiency may be excluded in patients, in whom both GH and IGF-1 secretion are normal. However, in clinical practice, the discrepancies between the results of GHST and IGF-1 secretion are quite frequently observed.

Methods:

The aim of our study was to investigate the relationship of peak stimulated GH and IGF-1 in short stature children. We retrospectively reviewed IGF-1 and GHST levels from 200 consecutive short stature children submitted to simultaneous measurement of IGF-1 and GHST with Clonidine in a Brazilian reference laboratory. Peak GH above 5ng/mL was considered responsive. For comparison among children of different age and sex, IGF-1 concentrations were expressed as IGF-1 quartiles. Serum GH was measured by chemiluminescen immunometric assay and IGF1 by an immunometric chemiluminescence assay. Comparisons of GH peak response between groups were performed using Anova one way

test and correlation between tests was evaluated by Spearman test. $P < 0.05$ was considered statistically significant.

Results:

Our 200 subjects (146 boys and 54 girls) age 10,3 ± 2,9 (mean ± SD) were distributed in accordance to IGF-1 quartiles as follows: quartile 1: 113 (56,5%), quartile 2: 48 (24%), quartile 3: 25 (12,5%) and quartile 4: 14 (7%). Mean GH peak and percentage of responsive GHST did not differ between the quartiles. Albeit we observed a tendency to higher GH peak levels and higher percentage of responsive tests according to crescent IGF-1 quartiles, there was no statistical significance between groups (p respectively: 0,764 and 0,8). Forty seven (24,5%) children did not reach a GH peak above 5ng/mL on GHST. On the other hand, 24 (12%) demonstrated IGF-1 levels below the reference range for sex and age. The concordance between inadequate GH peak and low IGF-1 levels was only 17% and only one third of patients with IGF-1 below reference range had in fact inadequate GH response on GHST.

Conclusions:

Like other studies, we were not able to demonstrate concordance between GH peak at GHST and IGF-1 quartiles. This discrepancy may be explained by several limitations observed in GH stimulation tests and IGF-1 determination. Unfortunately, it is important to consider that we still cannot rely on one single test to confirm or exclude GH deficiency, and subjects with low IGF-1 levels may not be spared of more cumbersome

provocative tests. We remark that clinical judgment should remain the most important tool in the evaluation of short stature and in the interpretation of laboratory tests.

A-106

The Laboratory And Clinician Interaction: A Case Study Of A Challenging Differential Diagnosis

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Objective/Relevance/Case Description:

Diagnostic testing is a complex and continually evolving landscape of medicine, requiring clinicians to understand which tests to order, how to interpret the results, and how to integrate the findings into the clinical picture. This is of particular importance when the differential diagnosis is unclear or especially challenging. Laboratory clinical chemists are in a unique position to provide guidance throughout the analytical process. In the following case study, we describe a case of a 17 year-old Tanner stage 2 female admitted with altered mental status, lethargy, blurry vision, hyperglycemia, and polyuria and polydipsia with no ketones. A head CT scan performed at an outside hospital indicated a suprasellar mass of unknown significance. The laboratory was consulted regarding various aspects of diagnostic testing throughout the differential diagnosis as described below.

In-house Laboratory Result	Reference Interval	Laboratory-Clinician Discussion Points
Serum glucose: 128 mg/dL POC glucose: 433 mg/dL	61-199	Site collection Possible interferences (IV) Serum vs POC methodology
Urine ketones: Negative Urine specific gravity: 1.006	Negative 1.003-1.030	Utilized methodologies (nitroprusside reaction, reflectance spectrophotometry, refractometry). Recommend: beta-hydroxybutyrate
Urine toxicology: Negative Osmol gap: 22mOsm	Negative <10	Immunoassay methods Dilute urine specimens Osmol gap calculation Recommended: methanol, isopropanol, acetone, ethylene glycol
Prolactinoma?	Prolactin: <26ng/mL	Chemiluminescence vs RIA and limitations Hook effect and heterophile antibodies
Cortisol: 1.0ug/dL ACTH: 18pg/mL	5.0-25.0 <=46	Cosyntropin collection time and specimen handling

Conclusions:

The final diagnosis in this patient was a craniopharyngioma, resulting in panhypopituitarism and diabetes insipidus, with concurrent diabetes mellitus. The patient was treated with several rounds of IV chemotherapy. Consultation with the laboratory throughout the differential diagnosis aided in proper test selection and interpretation, preventing unnecessary blood draws, inappropriate test orders, and possible misinterpretation of results. The events described herein highlight the opportunities for increased interactions between ordering clinicians and clinical chemists.

A-107

Is free T3 useful to evaluate thyroid status?

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Background: Thyroid function is always assessed using TSH and free T4 (FT4). However FT4 measurement by immunoassay is affected in special conditions, such as pregnancy, heparin treatment and other unspecific interference. In a small subset of thyrotoxicosis (T3 toxicosis), only T3 is elevated. Usually, free T3 (FT3) measurement is not necessary since total T3 (TT3) suffices. However, FT3 levels may be required to evaluate clinically euthyroid patients who have an altered distribution of binding proteins, especially dysalbuminemia.

Objective: Comparison among total and free thyroid hormones using TSH to define thyroid status.

Methods: We analyzed 1,622 patients' samples from a large database of a private reference clinical laboratory, tested for FT3, FT4, TT3, total T4 (TT4) and TSH all by Chemiluminescence assays (Advia Centaur, Siemens Healthcare). Patients were ranked by TSH into normal range (0.5-5.0 mIU/L), mild elevated (5.0-10 mUI/mL), high TSH (>10 mIU/mL), mild suppressed (0.1-0.5 mIU/mL) and suppressed (<0.1 mIU/mL).

Results: 46.9% were between 18 to 39 years-old, being 1,124 Male: 498 Female. TSH was in the normal range in 57.6% (Table 1). All samples had no differences according to age and gender. We found good correlation between FT3 and TT3 ($r=0.701$, $p<0.001$) and also between FT3 and FT4 ($p=0.702$, $p<0.001$). In the other hand, FT3 and TT3 were not correlated to TSH as FT4 and TT4 were inversely correlated to TSH, as expected ($p=0.018$ and $p<0.001$, respectively). FT3 was correlated only with suppressed TSH ($p<0.001$).

Conclusion: Although some investigators recommend the FT3 assay for monitoring ideal thyroid replacement therapy, in our cohort FT3 levels were not related to TSH ranking, suggesting that its clinical role is not precisely defined. Both FT4 and FT3 determinations must have assay interferences, but only FT4 accurately reports thyroid status. We conclude that FT3 and TT3 levels are less influenced by normal and borderline TSH ranges.

Table 1: Mean (±SD) of FT3 and FT4 according to TSH ranking

		FT3(pg/mL)		FT4 (ng/dL)
TSH (mIU/L)		Mean (±SD)		Mean (±SD)
Normal range	935	3.46 (1.76)	810	1.34 (0.49)
Mild elevated	88	4.01 (1.68)	79	1.52 (0.63)
High	39	3.35 (1.59)	38	1.32 (0.66)
Mild suppressed	48	3.52 (1.67)	46	1.42 (0.61)
Suppressed	52	7.39 (8.33)	50	2.39 (1.63)
Total	1,162	3.67 (2.57)	1,023	1.40 (0.65)
Normal values		2.00-4.00		0.7-1.8

A-108

Vitamin D status in Rio de Janeiro: results from vitamin D levels observed in a large reference laboratory

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Background: Vitamin D is a lipophilic hormone involved in calcium homeostasis and bone metabolism. Although severe 25-hydroxyvitamin D (25[OH]D) deficiency is rare in Brazil, there is accumulating evidence of an increased prevalence of subclinical 25(OH)D deficiency in non-elderly people. The aim of this study was to evaluate 25(OH)D and parathyroid hormone (PTH) status in patients from Rio de Janeiro, Brazil, a city located on the tropics, referred to a large reference laboratory.

Methods: We conducted a retrospective evaluation of consecutive serum measurements of 25(OH)D (chemiluminescent immunoassay, ARCHITECT, Abbott Diagnostics) and PTH (chemiluminescent immunoenzymatic assay, Beckman Coulter), from january to june 2014, obtained from lab LIS database. Patients aged below 12 and above 60 years-old, and patients using glucocorticoids, bisphosphonates, calcium and/or vitamin D replacement were excluded. For comparative analysis, the population was stratified in 3 groups of age: 12-18 (adolescents), 19-30 (young adults) and 31-60 (adults). Levels of vitamin D sufficiency followed recommendations from Endocrine Society as follows: deficiency (<20.0ng/ml), insufficiency (21.1-29.9ng/mL) and adequate (>30.0ng/mL). Statistical significance was determined at $p<0.05$.

Results: A total of 5334 samples were evaluated (78.5% women). Population distributions and analyte results are displayed at Table 1. 25(OH)D inversely correlated with PTH levels ($p<0.001$). Although 25(OH)D result means decreased with increasing age, this difference was not significant ($p=0.243$). On the other hand, the increase in PTH level means significantly correlated with age increase ($p<0.001$).

Discussion: Our survey shows that adequate levels of 25(OH)D are present in 56.1% of the population studied, and deficiency levels are more prevalent among young adults and adults. Although the prevalence of insufficient levels is higher among adolescents, this seems not to be of clinical relevancy, since mean PTH level is lower in this age category. Therefore, a better definition on the optimal level of vitamin D is yet to be determined.

Table 1. Distribution of demographic and laboratory findings.

Age (n)	Mean Age (±SD)	25(OH)D (±SD)	PTH (±SD)	25(OH)D status		
				Deficiency	Insufficiency	Adequate
12-18 (119)	15.5 ± 1.8	36.32 ± 20.88	30.37 ± 26.32	1.7%	47.1%	51.3%
19-30 (611)	25.75 ± 3.26	35.59 ± 25.23	34.27 ± 37.01	8.2%	33.9%	57.9%
31-60 (4604)	47.44 ± 8.63	34.52 ± 26.30	47.88 ± 90.84	7.6%	36.5%	56.0%
Total (5334)	44.24 ± 11.48	34.69 ± 26.07	45.93 ± 85.55	7.5%	36.4%	56.1%

A-109

Thyroglobulin Measurements to Monitor Reoccurrence in Papillary Thyroid Cancer (PTC) Patients.

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Introduction: Serum Thyroglobulin (TG) measurements are frequently used as a post operative marker for the follow up of patients with thyroid cancers. Presence of TG antibody (TGAB) interference and analytical issues of various methodologies of TG measurements creates difficulty in the follow up of these patients. Physician's frequently request TG measurements by different methodologies to rule out possible recurrence. This study evaluates TG levels by Chemiluminescent Immunoassay (CIA) and Radioimmunoassay (RIA) methods in both TGAB positive and negative patients with PTC, post thyroidectomy.

Methods: TG and TGAB were measured by both CIA (Siemens Immulite 2000) and RIA (USC Endocrine Labs, Pasadena, CA) from 38 patients (10 TGAB positive and 28 TGAB negative) diagnosed with PTC.

Results: In TGAB positive patients all 15 samples showed measurable TG levels (0.9-10.8 ng/mL) by RIA method, where as only 2 samples showed measurable levels (3.0-3.2 ng/mL) by CIA method. In TGAB negative patients only 9 out of 41 samples showed measurable TG levels (0.2-1.7 ng/mL) by RIA vs 19 out of 38 samples showed levels ranging from 0.3 to 6.4 ng/mL) by CIA. All patients were followed by Ultrasound of Neck measurements and some patients had FNA biopsy to confirm no recurrence.

Thyroglobulin Methodology	Thyroglobulin Antibody	
	Positive	Negative
CIA	2*/15	19*/38
RIA	15*/15	9*/41
* Measurable TG levels		

Conclusion: TG levels by both CIA and RIA methods showed measurable levels in both TGAB positive and negative patients post thyroidectomy. In TGAB positive patients RIA method has measurable levels on all (100%) samples; whereas only 2 (13%) samples had measurable levels by CIA method. In TGAB negative patients RIA method has measurable levels in 22% of samples while 50% of samples had measurable levels by CIA. Given the clinical follow up of these patients with no reoccurrence, these small measurable levels have no significant clinical impact for monitoring recurrence.

A-110

Adrenal vein sampling (AVS) for hyperaldosteronism: concordance with computed tomography (CT) imaging

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BACKGROUND: Adrenal vein sampling (AVS) is recommended for patients with hyperaldosteronism to whom surgery would be offered if the results indicate unilateral hypersecretion. The objective of this study is to assess the performance of AVS against radiological findings. METHODS: This is a retrospective study of 18 patients with hyperaldosteronism who underwent both AVS and computed tomography (CT) imaging in our hospital since 2012. Catheterisation was considered successful when the ratio between the cortisol values in the adrenal vein and the peripheral vessel (Cv/Cp) > 3. As cosyntropin was infused during the AVS procedure, a cortisol-corrected aldosterone ratio from the high to the low side (lateralisation ratio) of > 4 was used to indicate unilateral disease and < 3 to indicate bilateral hypersecretion. RESULTS: The mean age was 60 and 56% were male. All patients were above 40 years old except 2 patients. Ten of the 18 patients underwent a unilateral laparoscopic adrenalectomy. Two patients had bilateral adrenal nodules on CT imaging, 9 had left adrenal nodules, and 7 had right adrenal nodules. Successful bilateral adrenal vein catheterisation was achieved in 11 patients. Of these, CT and AVS results were concordant in 63%. The 4 patients with discordant results were treated medically. CONCLUSION: AVS is important in establishing unilateral or bilateral adrenal secretion of aldosterone in patients with hyperaldosteronism.

Age	Sex	Left Cv/Cp	Right Cv/Cp	CT imaging	AVS	AVS Lateralization ratio	Concordance	Treatment	Histology
65	Male	12	**1**	Bilateral nodules	Left	20.1	No	Medical	
69	Male	5	**1**	Right adrenal nodule	Left	6.8	No	Medical	
65	Male	12	**1**	Right adrenal nodule	Bilateral	2.8	No	Surgical	adenoma
59	Male	9	**2**	Left adrenal nodule	Right	6.5	No	Medical	
50	Male	18	**3**	Left adrenal nodule	Bilateral	1.2	No	Surgical	adenoma
61	Male	**3**	52	Left adrenal nodule	Left	4.7	Yes	Surgical	adenoma
56	Female	**3**	24	Right adrenal nodule	Bilateral	2.2	No	Medical	
38	Male	16	23	Right adrenal nodule	Right	4.5	Yes	Surgical	adenoma
64	Female	17	11	Left adrenal nodule	Bilateral	1.8	No	Medical	
53	Female	8	23	Right adrenal nodule	Right	6.3	Yes	Surgical	adenoma
58	Female	6	28	Left adrenal nodule	Left	7.3	Yes	Surgical	adenoma
75	Female	5	14	Bilateral nodules	Left	8.1	No	Medical	
58	Female	9	22	Left adrenal nodule	Left	8.5	Yes	Surgical	adenoma
60	Male	13	20	Right adrenal nodule	Left	9.9	No	Medical	
64	Male	16	22	Left adrenal nodule	Right	11.0	No	Medical	
39	Male	11	25	Left adrenal nodule	Left	19.0	Yes	Surgical	adenoma
46	Female	8	10	Right adrenal nodule	Right	20.2	Yes	Surgical	adenoma
56	Female	9	26	Left adrenal nodule	Left	27.7	Yes	Surgical	adenoma

A-111

HbA1c Quality Control Material

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Background: The Bioresource Technology HbA1c Control is a frozen liquid quality control material used to assess the accuracy and precision of Immunoassay as well as HPLC laboratory test methods used for measurement of HbA1c in patient samples.

Methods: The HbA1c Control is supplied liquid in two levels and consists of a human red blood cell lysate in a preservative mix sourced from normal and diabetic whole blood. Donors are selected based on target ranges of 4.0-6.0% for Level 1 and 10.0-14.0% HbA1c for Level 2.

Results: The Control material was assayed on immunoassay (DCA 2000+) and HPLC (Tosoh G7/G8) platforms. The table compares %HbA1c results of three different lots of the Control by the alternate test methods.

	%HbA1c	
	DCA	**Tosoh**
	2000+	**G7 / G8**
Level 1	5.2	5.3
	5.3	5.4
	5.2	5.4
Level 2	11.9	12.1
	10.5	11.5
	11.5	12.5

Real-time stability studies indicate the HbA1c controls are stable for 90 days closed vial at 2-8°C, 30 days open vial at 2-8°C and 7 days closed vial at 25°C. Accelerated stability studies predict a shelf life of 24 months when stored frozen at -15 to -25°C.

Conclusion: The Bioresource Technology HbA1c Control is a suitable quality control material to monitor the precision and accuracy of immunoassay as well as HPLC HbA1c laboratory test procedures.

A-112

Mild vitamin D deficiency is related to PTH and calcium homoestasis?

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Background: Vitamin D deficiency occurs even in tropical country and is associated with osteoporosis and extra skeletal manifestations, including cardiovascular, immune system and glycemic control. Definition of vitamin D sufficiency is under debate and several Committees suggest that a minimum level of 20 or 30 ng/mL must be necessary related to bone health. Therefore 25(OH)D levels between 30 to 40 ng/mL is the optimal concentration range. Most patients with 25(OH)D levels between 15-to 20 ng/mL have normal serum calcium, phosphorus and PTH. Prolonged and severe vitamin D deficiency reduces calcium and phosphorus intestinal absorption, causing hypocalcemia and secondary hyperparathyroidism.

Objective Analyze serum calcium, phosphorus and PTH levels according to 25(OH)D levels in patients with normal renal function.

Methods: We analyzed 1,383 patients samples from a large database of a private reference clinical laboratory, tested for 25OHD (Chemiluminescence, Architect Abbott), intact PTH (Chemiluminescence, Immulite2000 Siemens), calcium and phosphorus (colorimetric CPC and phosphomolybdate/UV principle, Advia2400 Siemens, respectively) in the period from October till November 2014 (1,010 M: 373F) and grouped as young adults (18 to 35 years-old), adults (36 to 60 ys) and old adults (> 60 ys). Patients were also classified according to 25(OH)D levels in vitamin D insufficiency and deficiency groups, < 30 and < 20 ng/mL, respectively. All patients with primary hyperparathyroidism and kidney failure were excluded.

Results: We found 28% and 43% vitamin D insufficiency and deficiency, respectively in our cohort. PTH levels were inversely related to 25(OH)D status and phosphorus levels and directly related to calcium levels, as expected. Calcium levels correlated to vitamin D insufficiency and deficiency (p=0.010 and p=0.030). Phosphorus levels were not statistically significant related to vitamin D status and PTH levels.

Conclusions: 25(OH)D levels below 30 ng/mL were associated to higher PTH and lower calcium. Therefore vitamin D replacement must be considered, even if phosphorus levels were at normal range, independently of age as bone homeostasis might be affected.

A-113

Characteristics of the New Beckman Coulter Thyroid-Stimulating Hormone Assay (TSH(3rd IS))

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BACKGROUND: There is a clinical need for TSH assays to exhibit a high degree of sensitivity (LoB, LoD and LoQ) with the ability to accurately and precisely measure TSH concentrations in the hyperthyroid and subclinical hyperthyroid range.

METHODS: Beckman Coulter is redeveloping their TSH assay. The new TSH (3rd IS) assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroid stimulating hormone in human serum and plasma for use on the Access Family of Immunoassay Systems.

RESULTS: This new TSH (3rd IS) assay exhibited improved sensitivity in comparison to other devices currently available with an estimated LoB of <0.001 µIU/mL, an LoD of ≤0.002 µIU/mL, and a 10% total assay CV LoQ concentration of <0.005 µIU/mL. Total assay imprecision for each of four sample pools (concentrations range from 0.02 µIU/mL to 36 µIU/mL) was less than 6%.There was no detectable cross-reactivity to FSH (<0.0003%), LH (<0.00001%), or hCG (<0.00000004%). The assay was robust against common interferences including hemoglobin (500 mg/dL), bilirubin (40 mg/dL), unconjugated bilirubin (40 mg/dL), and triglyceride/intralipid (3,000 mg/dL). The correlation between serum and Li-Heparin plasma samples was y = 1.01x + 0.00. No hook effect was detected up to 1,500 µIU/mL TSH concentration. The correlation between the current Beckman Coulter Access HYPERsensitive hTSH assay and the new TSH (3rd IS) assay was determined by comparing 149 samples with concentrations ranging from 0.004 to 43 µIU/mL. The resulting slope was y = 1.01x + -0.01.

CONCLUSIONS: The new Beckman Coulter TSH (3rd IS) assay is sensitive and sufficiently accurate to precisely measure TSH concentrations in hyperthyroid patient samples down to 0.005 µIU/mL.

A-114

Performance of two fully automated anti-Müllerian hormone immunoassays compared to an established ELISA

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Background: Because of missing standardization, the results of AMH ELISA assays are sometimes hardly to compare. BCI AMH Gen II assay became the clinical reference assay in the last years. Two newly developed fully automated, highly sensitive AMH immunoassays, based on the AMH Gen II antibody composition are available since September 2014. The aim of the study was to evaluate how the new fully automated AMH assays released perform in the laboratory routine compared to the clinical standard assay, i.e. the BCI AMH Gen II ELISA.

Methods: Samples of 94 women with an unfilled desire for a child, 29 samples women with polycystic ovary syndrome and 32 women approaching menopause were included to the study. The precision and the linearity in dilution of the two new AMH assays were determined and the assay results were compared to the clinical reference, the BCI AMH Gen II assay and to the antral follicle counts of the study participants. Cut-off value for the discrimination between each of two predefined groups were calculated using receiver operating characteristic (ROC) analysis.<br Results: The performance evaluation of the AMH assays resulted in a within run and intermediate precision between 0.9-1.9% and 2.5-6.5% with the Elecsys® and 0.9-3.6% or 4.4-10.7% with the Access® immunoassay, respectively. Pearson´s coefficient of correlation was 0.991 for the method comparison between both assays with a bias of 0.003 ng/mL and a slope of 0.97. The discrimination of the new immunoassays between subfertile women and women approaching menopause was significantly better compared to the BCI Gen II assay (87.5% vs 68.8%, p < 0.0001).

Conclusion: Both assays perform nearly identical with a higher analytical sensitivity compared to the AMH Gen II assay. The findings of the study are in good agreement with studies that used high sensitive AMH ELISA assays. The application of AMH measurement onto an automated immunoassay platform was the major step forward to allow rapid access of an AMH result to the health care providers and adopting its measurement into daily clinical practice.

A-115

Anti - GAD: Important help in the early diagnosis of type I diabetes

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The Type I diabetes is characterized by insufficient production of insulin by the pancreas being called autoimmune destruction, affecting mainly children, adolescents and young adults. The measure of antibodies to glutamic acid decarboxylase (anti - GAD) has been used to estimate the risk of development of Type I diabetes and can be tested previously to the disease establishment, during the period called prediabetes. This study aimed to validate theantibodies to glutamic acid decarboxylase testing by ELISA manufactured by EUROIMMUN AG.

The validation process was performed according to the information contained in the manufacturer's manual (ANTI-GAD IgG - EUROIMMUN AG) through a comparative study between the diagnostic method carried out using Radioimmunoassay (RIA) and ELISA RADIOIMMUNOASSAY -RIE - (Autoantibodies Anti -Glutamic Acid Decarboxylase - RSR Limited). Assays were performed using a total of 60 samples

selected from the RIA patients, 30 positive and 30 negative. The samples were compared using the cut-off values: i) values equal to or higher than 10.0 U / mL are considered positive by ELISA; ii) values higher than 1.0 U / mL are considered positive by RIA. The results show that the absolute agreement was only 67%. Twenty pairs were discordant, with a clear prevalence of positive results in the RIA and negative in the ELISA. The kappa index was 0.333 (95% CI: 0.122 to 0.544). In both protocols, a proficiency study with analysis of samples of newly diagnosed patients with type 1 diabetes and serum from healthy controls was conducted. In this study, the ELISA test achieved a better area under the curve than the RIA test, and calibrated ELISA method with the first reference of the World Health Organization. Thus, the ELISA using anti-GAD IgG kit - EUROIMMUN AG test designed to detect antibodies to glutamic acid decarboxylase (anti - GAD) has been validated and approved for routine use in our laboratory.

A-116

Hypothyroidism Prevalence in Term Pregnant Women in Port Harcourt

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Background

Hypothyroidism in pregnancy is associated with an increased risk of adverse pregnancy and fetal outcomes. Its prevalence varies with different thyroid stimulating hormone (TSH) cut-off values as well as in different regions of the world, with higher prevalence in iodine-deficient regions.

Aim/Objective

To screen for hypothyroidism in pregnant women at term and to determine the association between iodine intake and TSH levels.

Subjects and Methods

The study was conducted in four health care facilities in Port Harcourt, a major city in Nigeria. Consenting pregnant women that came into the facilities for delivery were consecutively enrolled and their serum TSH and free thyroxine (FT4) were analysed using the Vitros ECi/ECiQ immunodiagnostic autoanalyser. Hypothyroidism was defined according to the 2012 joint American Association of Clinical Endocrinologists (AACE) and American Thyroid Association (ATA) recommendation of serum TSH concentration greater than 3.5 mIU/L in the third trimester. Overt hypothyroidism (OH) was defined as serum TSH >3.5 mIU/L with decreased serum FT4 and subclinical hypothyroidism (SCH) was defined as serum TSH > 3.5 mIU/L with normal serum FT4 levels.

Statistical analysis used: Statistical Package for Social Sciences (SPSS) software version 17.0 (SPSS Inc., Chicago, Illinois, USA)

Results

One hundred and seventy-eight pregnant women were included in this study. Mean (SD) TSH and FT4 were 1.9 (1.4) mIU/L and 12.0 (4.8) pmol/L respectively. Twenty-four (13.5%) women had TSH values greater than 3.5 mIU/L; 20 (11.2%) had SCH and 4 (2.3%) had OH. There was no significant difference between the TSH of those who used iodized salt and those who did not.

Conclusion

The prevalence of hypothyroidism (13.5%) is higher than that reported in some earlier studies. Most of the women with hypothyroidism had SCH. Further studies are required to investigate hypothyroidism prevalence in first and second trimesters and to determine etiology.

Keywords: Hypothyroidism, prevalence, term, pregnant women, Port Harcourt.

A-117

Vitamin D Population Study Through The Seasons in the Brazilian Biggest City

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Background: Vitamin D is an important hormone involved in bone health and calcium homeostasis. Recent studies have associated low vitamin D levels with higher risk for certain cancers, autoimmune diseases, and cardiovascular disease. Reports show that over 1 billion people worldwide are vitamin D deficient, but we have to take into account the difference between cities or countries concerning the type of food and time exposure to the sun. A specific study of assessment of vitamin D status (deficiency, insufficiency, sufficiency, or toxicity) focused on a country or region becomes more necessary nowadays. This study will evaluate differences between seasons, gender and age group in the multicultural city of São Paulo and surroundings (almost 18 MM inhabitants).**Objectives:** This study evaluates the characteristic levels of vitamin D in routine samples of a large clinical laboratory database in São Paulo, Brazil

Materials and Methods: Data was collected from one reference laboratory routine during the year of 2014, in the city of São Paulo. 33342 samples (85% of women and 15% of men) dosages were performed and evaluated on the ADVIA Centaur XP® Systems (Siemens Healthcare Diagnostics). **Results:** The average values of vitamin D was 29.67 ng/mL, clinically observed as 16.9% deficient, 43% insufficient, 39.6% sufficiency, and 0.5 % toxicity results. The tables below show the total average by season, gender and by age group.

	Summer	Autumn	Winter	Spring
Female	30.53	27.70	30.46	29.34
Male	31.83	28.89	31.32	30.71
All	30.73	27.88	30.59	29.57
N	7570	8458	8774	8540

Age (year)	Result (ng/mL)	N		
10 to 20	28.98	1377		
20 to 30	27.45	4500		
30 to 40	28.31	8052		
40 to 50	29.17	6489		
50 to 60	31.22	5573		
over 60	31.92	7351		

Conclusion: The results showed subtle differences between seasons, gender and age group. The highest difference was found between Autumn and Summer, 9.27%. The gender difference was 4% and considering the age group, age 20 to 30 shows the lowest level and age over 60 shows the highest level, it should be because the medical indication of supplementation of vitamin D. The results show 60% of the samples are vitamin D deficient or insufficient. More studies are necessary and the medication intake should be considered.

A-118

Contributory Antioxidant Role of Testosterone and Oxidative Stress Biomarkers in Males Exposed to Mixed Chemicals in an Automobile Repair Community

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Background: Testosterone is a known androgenic and anabolic steroid, primarily secreted in the testes. It plays an important role in the development of testes and prostate, and has a range of biological actions. There is evidence that exposure to mixed chemicals in the work place leads to generation of free radicals and inadequate antioxidants leading to oxidative stress, which may serve as an early indicator of a pathophysiologic state. Based on findings, testosterone show direct antioxidant effects by increasing the activities of antioxidant enzymes like glutathione peroxidase, thus indirectly contributing to antioxidant capacity.

Objective: To evaluate the antioxidant role of testosterone as well as the relationship between testosterone and oxidative stress biomarkers in males exposed to mixed chemicals in the automobile repair community.

Methods: The study included 43 participants aged 22- 60years exposed to mixed chemicals (EMC) from the automobile repair community. Forty (40) apparently healthy, unexposed, age matched controls were recruited after informed consent. Demographic, sexual and anthropometric characteristics were obtained from pre-test structured questionnaires using standard methods. Blood samples (10mls) were collected from each subject into plain bottles and sera obtained were used for biochemical analyses. Serum levels of testosterone and luteinizing hormone (LH) were determined by enzyme immunoassay method, EIA (Immunometrics UK.LTD). Levels of total antioxidant capacity (TAC), total plasma peroxide (TPP), Malondialdehyde (MDA), hydrogen peroxide (H2O2), glutathione peroxide (GPX), superoxide dismutase (SOD), glutathione-S-transferase (GST), and reduced glutathione (GSH) were determined using spectrophotometric methods respectively. Results obtained were analyzed using the Student's t-test and Chi-square test for quantitative variables and qualitative variables respectively. Multiple regression was used to find associations and relationships between the variables.

Results: Significant higher concentrations of TPP, MDA, OSI, H2O2 and GST were observed in EMC compared with controls ($p<0.001$). Within EMC, significantly higher levels of testosterone, LH and TAC were observed in eugonadic when compared with

hypogonadic participants (p<0.001). Diastolic blood pressure, waist circumference, waist height ratio and waist hip ratio were significantly higher in participants EMC compared with the controls. Sexual history and dietary intake showed that the controls had normal erection during sex and took more vegetables in their diet which may therefore be beneficial.

Conclusion: The significantly increased levels of total antioxidant capacity in males exposed to mixed chemicals despite their exposure may probably reflect the contributory antioxidant role of testosterone that prevents oxidative stress.

A-119

Prevalence of subclinical hypothyroidism in adults without known thyroid disease: An epidemiological study in Chengdu,China

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Background:

Subclinical hypothryrodism (SH), which has previously been associated with an increased risk for many serious diseases, is a serious threat to human health. However, due to the lack of typical clinical symptoms, SH is rarely to be noticed. As there is a paucity of data on the prevalence of SH in healthy adult population in Sichuan, this four years epidemiological study was conducted in Chengdu to estimate prevalence of SH among healthy adults.

Methods:

All participants answered a questionnaire that included demographic data, reproductive history, smoking history, previous thyroid disease, family history of thyroid disease, etc. and had a blood sample collected to assess levels of thyrotropin, free-thyroxine and free triiodothyronine when enrolled. SH were diagnosed on the basis of laboratory results.

Results:

(1) The prevalence of SH in the overall study population was 15.3% (16894/110484), and in 2011, 2012, 2013 and 2014, the prevalence of SH in Chengdu were 16.5% (365/2209), 15.9% (3017/19034), 15.8% (5362/34017) and 14.8% (8150/55224), respectively. (2)As shown in Table 1, prevalence of SH increased gradually with age strata both in males and females, and SH prevalence in females was higher than that in males (P<0.05)

Conclusion:

The prevalence of SH in Chengdu was high, affecting approximately 2 in 10 adults in the study population. Female gender and older age were found to have significant association with SH. Adults in Chengdu, especially female over 40 years old, should regularly check thyroid function and take timely corresponding intervention.

Table 1. Male and Female prevalence of subclinical hypothyroidism in different age strata in Chengdu

Age-strata(Years)	Male(%, n/N)	Female(%, n/N)	Total(%, n/N)
18-39	10.3, 2171/21095	16.6, 3087/18544	13.3, 5258/39639
40-59	10.8, 3899/36033	22.4, 4721/21067	15.1, 8620/57100
60-79	18.0, 1455/8101	27.6, 1124/4068	21.2, 2579/12169
>80	26.1, 325/1245	33.8, 112/331	27.7, 437/1576
Total	11.8, 7850/66474	20.5, 9044/44010	15.3, 16894/110484

A-120

A low alkaline phosphatase activity. Does it matter?

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Objective: Describe two incidental findings of low plasma alkaline phosphatase (ALP) activity in middle aged women and outline the differential diagnosis of such a finding while characterising the adult form of hypophosphatasia.

Methods: Clinical, biochemical, radiologic and molecular studies explore the differential diagnosis of hypophosphatasaemia. Phenotypic features, management and pitfalls of adult form of hypophosphatasia are reviewed.

Results:

Case 1: A 36-year old woman with short stature reported the early loss of 18 permanent teeth. Her plasma ALP activity was 16 IU/L (35-104) with no other routine biochemical abnormality. Bone mineral density was unremarkable. Raised urine phosphoetanolamine (PEA) of 17 umol/mmol of creatinine (< 5) was only confirmed on a repeat sample when the age adjusted reference range was applied. Sequencing of the gene encoding the tissue-nonspecific isoenzyme of ALP (TNSALP) demonstrated that the patient was heterozygous for a missense, non-conservative mutation c.881A>C in exon 9.

Case 2: A 34-year old asymptomatic woman. Plasma ALP activity was 28 IU/L and skeletal survey was abnormal. Urinary PEA was 7 umol/mmol of creatinine (4.3-17.5), but plasma pyridoxal phosphate (PLP) was raised at 583 nmol/L (15-73). Sequencing of the TNSALP revealed that the patient was heterozygous for the mutation c.571G>A; previously described in patients with moderate forms of hypophosphatasia.

Conclusion: Hypophosphatasia is a group of inherited disorders due to inactivating mutations of the gene encoding TNSALP characterized by accumulation of PLP, PEA and inorganic pyrophosphate. In the adult form, dental disease often precedes symptomatic osteomalacia, which can cause slowly healing stress fractures. PEA excretion, an age-related phenomenon must be corrected for age, to avoid erroneous phenotypic definition related to gene dosage. Hypophosphatasia patients may beerroneously diagnosed with osteoporosis. Clinicians should identify, among patients for whom bisphosphonates are being considered those with possible hypophosphatasia. The diagnosis should help avoid therapies such as bisphosphonates for osteoporosis or osteomalacia in adults since it could be potentially harmful.

A-121

Comparison of the Abbott Architect 2nd Generation Testosterone Assay with a Radioimmunoassay and LC-MS/MS

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Background: Accurate and precise measurement of testosterone is necessary to diagnose and manage patients with gonadotropin hormone imbalances. Testosterone levels are also measured to monitor disease progression in patients with prostate cancer who undergo antiandrogen therapy. The Abbott Architect 2nd generation testosterone assay (2G-TESTO) is a one step chemiluminescent immunoassay for the determination of total testosterone in human serum or plasma. The assay involves mixing of serum or plasma with diluents and anti-testosterone antibody-coated microparticles followed by incubation, washing and addition of chemiluminescent-labeled testosterone to form chemiluminescence.

Objectives: The objective of this study was to validate the precision, sensitivity, and linearity of Architect 2G-TESTO and to compare this assay with Siemens Coat-A-Count®RIA and LC-MS/MS performed at a national reference lab.

Method: The precision study was conducted using the Architect 2G-TESTO high and low control materials and pooled human serum. Within-day precision was determined by analyzing 10 samples sequentially and between-day precision was obtained over thirty days. The limit of quantitation (LoQ) and limit of blank (LoB) studies were conducted using diluted low QC material (assayed in triplicate) and a water blank (assayed 10 times). The linearity study was performed using the Architect 2G-TESTO calibration material over the range of 95.09 - 907.7 ng/dL. For additional concentrations diluted and undiluted quality control (QC) materials over the range of 0.00 - 1058 ng/dL were used. We compared Architect 2G-TESTO assay with Siemens RIA and LC-MS/MS testosterone assay using patient serum sample collected for routine testosterone determination. The LC-MS/MS method used in the national reference laboratory used online extraction followed by LC-MS/MS (Singh R (2008) Steroid 73:1339-1344). It has a functional sensitivity of 7ng/mL based on the inter-assay precision of 18.8% and an imprecision (CV) below 10.7% at various concentrations spanning the analytical measurement range.

Result: Within-day imprecision (CV) at concentrations of 10.5 and 195.5 ng/dL were 5.8% and 4.2%, respectively. The imprecision for the pooled serum at a concentration of 4.8 ng/dL displayed a CV of 3.6%. Between-day imprecision for low (10.7 ng/dL), medium (73.1 ng/dL) and high (236.3 ng/dL) quality controls were 10.7 ng/dL, 9.2%;73.1 ng/dL, 8.37%;236.3 ng/dl, 8.35%, respectively. The 2SD LoB (95% CI) was 1.435ng/dL and the LoD was verified to be 4.3ng/dL. The assay was linear over the analytical measurement range of 4.3- 1009.4 ng/dL.

The comparison between Siemens RIA and Architect 2G-TESTO (n=40, range 10.00 to 797.0 ng/dL) show spearman correlation coefficient of 0.994 with a slope of 1.39 and intercept of -6.872 by Deming regression analysis. Comparison with the LC-MS/MS method at the reference lab (n=76, range 16.1 to 1249.3 ng/dL) had spearman correlation coefficient of 0.988 with a slope of 1.22 and intercept of 2.96.

Conclusion: Our validation shows that Architect 2G-TESTO has acceptable precision and linearity over the analytical measurement range. Architect 2G-TESTO show excellent sensitivity. The correlation with RIA and LC-MS/MS at low and high

testosterone concentrations show that Architect 2G-TESTO were generally higher than the other two assays.

A-122

The analytical performances of two different HbA1c assays (capillary electrophoresis and boronate affinity by HPLC) against ion exchange HPLC.

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BACKGROUND: Along the years, HbA1c has been established as an important tool in diagnosis, control of diabetic patients and as a marker of prediabetes risk. Ion exchange HPLC assay has been consolidated as a gold standard to measure HbA1c, but other methodologies like capillary electrophoresis and boronate affinity HPLC have also been widely accepted due to their good performances. In this study, the analytical performance (precision, accuracy, linearity, and detection of hemoglobin variants) in two HbA1c assays (Sebia Capillarys Flex Piercing® -capillary electrophoresis and Trinity Biotech-Premier HB9210® - boronate affinity HPCL) versus Bio-Rad Variant II turbo® (ion exchange HPLC) platform was evaluated.

RESULTS: Samples with results near medical decision points measured in Bio-Rad Variant II Turbo were selected. All studies were evaluated using CLSI guidelines. Within-run (Capillarys Flex Piercing® values: 5.8 and 6.6%; Premier HB9210® values: 6.7 and 7.0%) and between-run (Capillarys Flex Piercing® values:5.8 and 6.7%; Premier HB9210® values: 5.8 and 6.6%) imprecision values (CV%) were less than 1.8 (laboratory allowable error for all range of results = 2.4%). Accuracy values were 94 and 90% for capillarys electrophoresis and boronate affinity HPCL, respectively. Comparison of both methods against Bio-Rad Variant Turbo II demonstrated significant correlation (Capillarys Flex Piercing®: r = 0.99.; slope = 1.0; intercept = 0.11; Premier HB9210®: r = 0.99; slope = 0.94; intercept = 0.3). Hemoglobin variants (HbS, HbC and HbF: 0,3 to 10,6%) as well as samples from patients with beta-thalassemia trait did not interfere in the tested assays as demonstrated by NGSDP (http://www.ngsp.org).

CONCLUSIONS: These results indicate that both assays present good precision and accuracy values besides no interference with the most common hemoglobin variants. Thus, both assays are alternative options for large volume testing HbA1c in clinical laboratories.

A-123

Evaluation of the Premier Hb9210 HbA1c analyser

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Background:

The American Diabetes Association (ADA) as well as other national Diabetes Organizations now recommend using HbA1c to diagnose diabetes using a National Glycohemoglobin Standardization Program (NGSP)-certified method and a cutoff of HbA1c ≥6.5%. The requirements for a device to be used as an aid in diagnosis of diabetes consequently became more rigorous, demanding very low level of imprecision at HbA1c from 5% to 12%, specifically CVs <2% (NGSP). In addition little to no interference from hemoglobin variants or substances is asked for. Here we report the evaluation of the Premier Hb9210 (Trinity Biotech USA Inc, Jamestown, NY), a boronate affinity high performance liquid chromatography (HPLC) system. The glycation specific binding of boronate affinity detects all of the glycated Hb species present in the sample and results in one glycated and one non-glycated peak in the chromatogram. The area of the glycated peak, correlating directly to the HbA1c, is then converted to a HbA1c value. Sample capacity is 210 in both batch or continuous loading mode with a sample analysis time of 66 seconds. Minimum sample requirements are 10 microliter whole blood (Dilution 1 : 150) or 5 microliter packed red blood cells (Dilution 1 : 300). Column temperature is 55°C and detection is done at413 +/- 2nm (LED).

Methods

Imprecision of the assay was evaluated according to CLSI protocol. Intra-assay imprecision was done with four patients pools and two quality controls, inter-assay imprecision with two quality controls. Whole blood (EDTA) samples (n= 197) from healthy controls, diabetic patients and patients on hemodialysis were analysed for HbA1c values on the Premier Hb9210, Tosoh HLC-723 G7, Bio-Rad D-10, Menarini HA 8160 and Roche Cobas Integra 800. Method comparison was done by Passing/Bablock and Bland/Altman. Interferences were tested for haemoglobin variants AS, AC, AD, AE and elevated F. Possible interferences from acetylated or carbamylated Hb were tested with in vitro acetylated (Acetaldehyde, acetylsalicylic acid) and carbamylated red blood cells (urea, potassium cyanate).

Results

Inter-assay imprecision (n=20) for the Premier Hb9210 ranged from 1.52 % (HbA1c 5.69%) to 1.58 % (HbA1c 11.47%) and from 1.79 % (HbA1c 38.67 mmol/mol) to 1.96 % (HbA1c 101.87 mmol/mol). Intra-assay imprecision (n=10) for the Premier Hb9210 ranged from 0.43 % (HbA1c 5.42%) to 0.86 % (HbA1c 13.22 %) and from 0.71 % (HbA1c 35.70 mmol/mol) to 1.04 % (HbA1c 121.02 mmol/mol). The imprecision data from the other HPLC systems were in the same range as the Premier Hb9210, but somewhat higher, whereas the Roche system showed CVs higher than 2.0 %. Acetylation caused higher HbA1c values for the Tosoh and Bio-Rad system and Carbamylation only for the Bio-Rad system. None of the haemoglobin variants showed significant interferences for the Premier Hb9210. Over all the analyser showed good correlations.

Conclusion

ThePremier Hb9210 performs well with a short analysis time, so far no detected interferences and is very well suitable for routine analysis of HbA1c.

A-124

Prevalence of Thyroid Antibodies and Thyroid Dysfunction in a Healthy South African Population

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Background:

Autoimmune thyroid disease is characterised by antibodies to antigenic elements of the thyroid gland such as thyroglobulin (TgAb) and thyroid peroxidase (TPOAb), in the presence of thyroid dysfunction. The subclinical and clinical forms of thyroid disorders have been associated with hyperlipidaemia, neuropsychiatric disorders and cardiovascular diseases. Little is known about prevalence rates of the above in the South African population. The study objectives were to determine the prevalence of thyroid dysfunction and antithyroid antibodies in a cohort of healthy South African adults; and to assess gender, age or ethnic-related differences in the prevalence rates of anti-thyroid antibodies.

Methods:

A total of 627 adults were recruited from the general public. Venous samples were analysed for free T3 (fT3), Free T4 (fT4), thyroid stimulating (TSH), and thyroid autoantibodies (TGAb and TPOAb). Thyroid dysfunction and antibody positivity were assessed using current reference intervals.

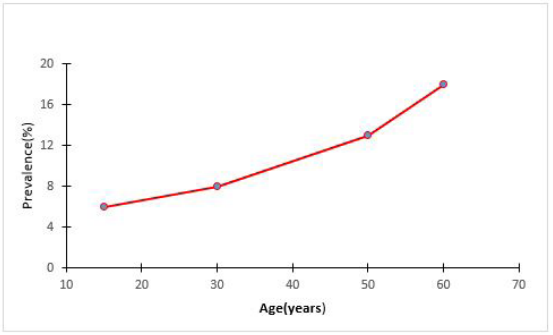
Results:

There were a total of 627 participants aged 18 - 76yrs, 420 (67.0%) of these were females. Participants were of Caucasian (54.0%), Mixed (34%) or African (12%) ethnicity. Subclinical hypothyroidism occurred in 21 (3.3%) adults, while subclinical hyperthyroidism was observed in 4 (0.6%) adults. Six (1.0%) participants were biochemically hyperthyroid while 4 (0.6%) were biochemically hypothyroid. Thyroid antibodies were positive in 55 (9.0%) of study participants. No gender differences in antibody positivity were observed. The highest prevalence of thyroid antibodies were observed in Caucasian subjects (10.0%) followed by subjects of Mixed ancestry (9.0%), with the lowest prevalence (3.0%) seen amongst African subjects. TSH levels ($p<0.01$) and age ($p<0.01$) were significantly higher in seropositive subjects when compared to seronegative individuals.

Conclusion:

Thyroid dysfunction was observed in 35 (5.6%) of participants while thyroid antibodies were present in 55 (9.0%) of participants. Age and ethnic differences were observed in seropositive vs. seronegative participants and a follow-up study is recommended to explore the clinical implications of seropositivity.

Figure 1. Prevalence of Thyroid Antibodies in the various age groups (There is a rise in the prevalence with increasing age.



A-125

Influence of high-normal serum TSH and metabolic syndrome presence in young patients with premature coronary artery disease (age < 45 years)

M. G. CASTELO[1], D. Gonçalves[1], R. Dias Filho[2], A. Pereira[2], G. Coelho[1], T. C. Sousa[1], C. F. Pereira[3], O. P. Denardim[3], T. Bandeira[1], F. Furtado[1]. *[1]DASA, FORTALEZA, Brazil, [2]USP, São Paulo, Brazil, [3]DASA, São Paulo, Brazil*

Background:

The existence of an association between thyrotropin (thyroid stimulating hormone, TSH) levels and cardiovascular risk factor in euthyroid subjects is controversial. We examined the TSH levels and the presence of metabolic syndrome in patients with premature coronary artery (PCAD) disease (age < 45 years).

Methods:

This was a cross-sectional study conducted from November 2010 to January 2015 which included 103 young patients under age 45 years, both sex, with acute myocardial infarction (AMI) diagnosis. We defined metabolic syndrome using the 2007 International Diabetes Federation criteria. The TSH parameter of these patients with PCAD and 267 age and sex matched euthyroid controls without family history were evaluated. In addition, the patients were classified according to the number of affected arteries into 2 groups: single vessel (greater than 50% involvement in a coronary artery) and multivessels (two or more coronary artery involvement). Comparisons were made between TSH levels of these two groups.

Results: Mean serum TSH was significantly higher in patients with PCAD than controls (TSH 2,6±2,61 *versus* **1,8±0,1 mUI/L;** ***p*****: 0,001). The comparison between single-vessel and multivessel groups showed higher TSH levels in PACD with multivessel affected (TSH 2,3±1,5** *versus* **2,8±1,3 mUI/L;** ***p:*** **0,01). The prevalence of metabolic syndrome was significantly higher in patients with PCAD than controls (45,65%** *vs* **21,78%;** ***p*****: 0,001).**

Conclusion:

Although observational studies indicate that serum TSH levels in the high normal range are related to cardiovascular risk factor, similar date are limited in young patients with PACD. In conclusion, the prevalence of metabolic syndrome and higher TSH level within the normal range were more frequent in patients with PACD. This pioneering study invites to discussion if TSH levels may be related to more aggressive coronary disease in euthyroid subjects.

A-126

Analysis of TSH, thyroid hormones and reverse T3 in elderly patients and its relation to the Index of Activity Daily Living

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Background: It is assumed that elderly subjects could have decreased T3 and increase of reverse T3 (rT3). The Index of Activity Daily Living developed by Katz (IADL Index) assess the functionality of the elderly in six functions called Daily Life Basic Activities - bathing, dressing, feeding, toileting, transferring, and continence. The zero index ranks the independent, and index 6, classifies the totally dependent elderly. The authors assessed the correlations of TSH and thyroid hormones in elderly subjects and tested if TSH and rT3 levels are different in those with different IADL Index.

Methods: We studied 129 patients 65 to 93 years (mean = 80 years), 104 women, who attended a geriatric outpatient clinic. Patients were submitted to a questionnaire that verified the functional status by observing the six daily life basic activities according to Katz index, which are classified in levels 1-6: index 0 indicates the independent individual to perform all activities, and index 6 indicates dependende to perform all of them. TSH, FT4, TT3 and FT3 were measured by quimiluminescence. rT3 was measured by radioimmunoassay.

Results: There was a negative correlation of TSH with fT4 (r = - 0.1736, p = 0.0417), with TT3 (r = - 0.2035, p = 0.0167), and especially with fT3 (r = - 0.2331, p = 0.0059). TSH increased with age, as well rT3, but a positive correlation among them was not quite significant (r = 0.06687, p= 0.0734). In the same way, correlations among rT3 and fT4 (r = -0.1143, p = 0.2006), TT3 (r = -0.08712, p = 0.3301), and fT3 (r = -0.1109, p = 0.2147) were not significant. Patients were categorized in Katz index 0, 1, and 3. In the segmentation of TSH levels among the groups, there was not significant difference. In respect to rT3, it was significantly higher in the group with index 3 (median rT3 = 0.2900) compared to those with index 0 and index 1 (median rT3= 0.2300 and 0.2100 respectively), p = 0.0047.

Conclusion: In this study, the hormone that showed the best correlation with TSH in elderly was fT3. This is in according with previous reports of decreased T3 in elderly subjects without significant decrease of T4. Despite a tendency to increase in rT3, we did not find a significant correlation with TSH.

In relation to IADL rT3 was higher in patients more dependent (Katz index 3), compared with those dependent in only one function ((Katz index 1), and with those completely independent (Katz index 0). Previous studies in literature showed worst physical performance in elderly with higher rT3 despite normal fT4.

Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Factors Affecting Test Results

A-127

Evaluation of Hemoglobin A1c Immunoassay and Capillary Electrophoresis Methods

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Background:

Hemoglobin A1c (HbA1c) results have a major impact on the diagnosis of diabetes, as well as patient management. Two methods reported to have minimal interferences from variant hemoglobins are the Vitros5600 HbA1c immunoassay (Ortho Clinical Diagnostics) and the CAPILLARYS 2 Flex Piercing HbA1c capillary electrophoresis (Sebia, Lisses, France.). Our laboratory uses both technologies with the 5600 configured in an open work-cell to facilitate testing across all shifts, while the capillary electrophoresis system is used within special chemistry on one shift. We sought to compare the analytical performance of these methods, as well as the impact of each on workflow within our Core Laboratory, and to characterize the incidence of Hb variants in the patient population undergoing HbA1c testing. Our laboratory receives ~100 samples for this analysis daily from across the hospital system.

Methods:

The study protocol (IRB# 14-2344) received exemption from IRB review. Over a 28-day period, all patient samples (n=943) submitted for HbA1c testing using the Vitros methodology were anonymized and reanalyzed using CAPILLARYS HbA1c. Discordant samples (>0.6% difference) were frozen (-80°C) and analyzed by an NGSP reference laboratory using ion-exchange HPLC (Tosoh G8). Discordant samples containing Hb variants were verified using boronate-affinity HPLC (Trinity Ultra 2). Additionally, known homozygous or double heterozygous hemoglobin variants (HbSS, HbEE, HbSC, HbDD, provided by Sebia, Inc.) were analyzed using each method. A 7-Cycle timing analysis was performed to determine the impact of each system on workflow. Data were analyzed using EP Evaluator software (Data Innovations, LLC).

Results:

Method comparison showed good correlation (y =1.03x-0.05, R=0.991) between the two methods. Seventeen discordant samples were sent to the NGSP laboratory. Four original Vitros results were >0.6% from the NGSP result of which 2 were found to have hemoglobin AS and AC variants. One Sebia result was >0.6% from the NGSP value; no Hb variant was detected. Prevalence of hemoglobin variants was 4.4% of the total population tested. One sample reported 4.6% HbA1c by the Vitros but showed an elevated hemoglobin F (27.5%) with no reportable HbA1c by CAPILLARYS HbA1c . NGSP laboratory reported 5.9% HbA1c for this sample. HbA1c values were not reported on known homozygous and double heterozygous samples using the CAPILLARYS HbA1c, but the Vitros method generated results (4.03-5.1%) for some glycated hemoglobin on several of these specimens.

The workflow study showed preparation time for the Vitros averaged 4.2 ±0.8 minutes compared to an average of 1.4 ±0.16 minutes for the CAPILLARYS2 Flex Piercing. The estimated run time for 40 samples was 60 minutes on the CAPILLARYS2 compared to 23 minutes using four Vitros5600's as the current configuration permits.

Conclusion:

This study reports high agreement for HbA1c measurement between the Vitros5600 immunoassay and the CAPILLARYS2 Flex Piercing electrophoresis methods in normal patient samples. The methodology of each system provides reliable results, with little interference from hemoglobin variants in the Vitros method. Thus, discrepant values observed are most likely due to the presence of a variant hemoglobin or random error. However, our current workflow design favors the use of the Vitros5600.

A-128

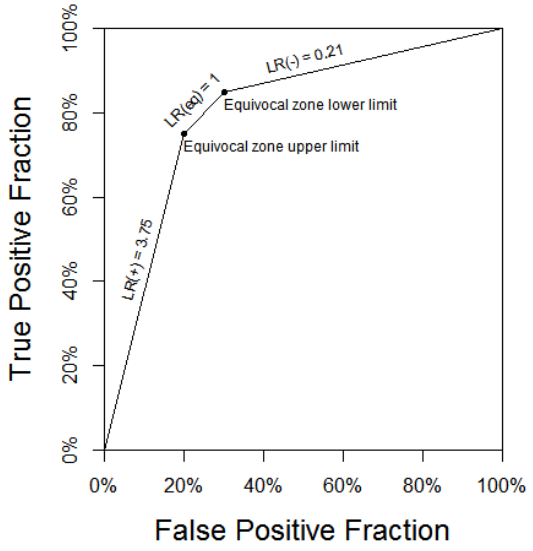
Assessing Performance for Assays Reporting Equivocal Results

V. H. Petrides[1], H. S. El Mubarak[2], M. V. Kondratovich[2], K. L. Meier[3], J. Ye[2], S. H. Gawel[1], S. Akselrod[2], A. Charnot-Katsikas[4], K. Simon[5]. *[1]Abbott Laboratories, Abbott Park, IL, [2]U.S. Food and Drug Administration, Silver Spring, MD, [3]Illumina, Inc., San Diego, CA, [4]The University of Chicago, Chicago, IL, [5]Biologics Consulting Group, Alexandria, VA*

Background: Assessing the performance of qualitative tests reporting one of two possible results (positive or negative) relative to a gold standard is typically straightforward. Common performance measures include positive and negative predictive values, sensitivity and specificity, and likelihood ratios. However, what performance measures should be reported when a test includes equivocal results? Equivocal results are often simply ignored. The objective of this investigation is to demonstrate how the perception of assay performance can vary depending on the how equivocal results are treated and to provide recommendations for assessing performance in these situations.

Methods: An equivocal result here is defined for *in vitro* diagnostic tests with a continuous underlying signal, where one of two results is preferred but not always possible. Equivocal results are non-missing, non-erroneous results which are neither positive nor negative. Typically, the percent of equivocal results in a study is relatively small. We consider hypothetical data with three possible outcomes to show how various approaches described in literature affect performance estimates.

Results: The true positive fraction results ranged from 75% to 85%, which corresponds to the same values when calculating sensitivity for a dichotomous test. The false positive fraction results ranged from 20% to 30%, which corresponds to specificity values ranging from 70% to 80% for a dichotomous test. The likelihood ratio positive, equivocal, and negative values were 3.75, 1.00, and 0.21; the percentages of positive, equivocal, and negative results were 38%, 10%, and 52%, respectively; and the pre-test risk of the target condition was 33%.



Conclusion: Sensitivity and specificity calculations alone can be misleading in understanding the performance of assays with equivocal results. Likelihood ratios along with the corresponding percent for each outcome and the pre-test risk provide useful information about performance of tests with equivocal results.

A-129

Serum concentrations of macro-AST relative to normal AST: limits consistent with IgG or IgA complexes and with normal rates of serum AST production

A. S. Rubin, L. J. McCloskey, B. M. Goldsmith, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: Our laboratory is occasionally asked to evaluate isolated enzyme elevations for the presence of macroenzymes. Evaluation is based on lability to polyethyleneglycol (PEG) precipitation. One recent case was consistent with macro-AST. The physician asked whether, despite this finding, one could still rule out a

circumstance of overproduction of AST. Our objective was to demonstrate our approach to answering this question for this specific case and for other instances of macro-AST reported in the literature.

Methods: Elevation of macroenzymes is assumed to be explained by the extended lifetime of enzyme in circulation. Normal concentration of enzyme (E) in serum/plasma is assumed to represent a steady-state (d[E]/dt = 0) balancing the rate of appearance in plasma (ki) and the rate of elimination (ke). The rate of elimination is assumed to be first order with respect to enzyme concentration, ke = k[E]. Thus, in steady-state, ki - k [E] = 0, or ki = k [E]. For macroenzyme E', altered ke is assumed to reflect a substantial change in the rate constant k to a lesser value, k'. If ki is unaltered, then [E'] should reflect an otherwise normal enzyme concentration [E] according to the equality k [E] = k' [E']. Our answer to the physician's question is that [E'] is consistent with altered circulation lifetime, rather than to an alteration in ki, if a calculated [E] = k'[E']/k (Eqn.1) is within the reference range for AST. For macro-AST, k' was assumed to be consistent with that of IgG (t1/2 = 20 days, k' = 0.035/day) or IgA (t1/2 = 6 days, k' = 0.116/day), where k' = -ln(0.5)/(t1/2). For normal AST, t1/2 = 17 hours (0.71 days); k = 0.98/day. Given patient values for [E'], we compared calculated values for [E] (according to Eqn.1) to the reference range for AST ([E] = 7-42 U/L).

Results: For our physician's macro-AST patient, [E'] = 428 U/L. By calculations per Methods, this value was consistent with normal AST [E] of 15 U/L, assuming IgG complexes, which was at the 14th percentile of the reference range for [E]. From literature, 49 examples of [E'] formed a bimodal distribution (a. 68% of total, 60-300 U/L, median 124 U/L; b. 32% of total, 300-1150 U/L, median 434 U/L). Among all [E'], 71% were compatible with normal [E] based on IgA (100% from (a)); 41% were compatible based on IgG (100% from (b)); 10% were compatible based on either IgA or IgG; 0% had elevated [E] based on IgG. Based on IgG, [E'] = 1180 U/L is the calculated upper limit for [E'] compatible with normal [E].

Conclusions: Calculations described can be used to assess whether values for macro-AST are consistent with normal rates of appearance of AST into circulation, assuming the lifetime of macro-AST is consistent with the lifetime of IgG or IgA. Values for macro-AST reported in literature were all found to be consistent with otherwise normal AST. Caveats to the analysis include selection bias and potential effects of comorbidities on AST lifetimes in cases where macro-AST was an incidental finding.

A-130

Monoclonal Protein Interference with Chemistry Measurements Performed on the Siemens Advia® 1800 Chemistry Analyzer

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BACKGROUND: Previous articles have reported monoclonal protein (MP) interference with routine automated chemistry and immunoassay tests on a number of testing platforms. Assays affected included inorganic phosphorus, glucose, and HDL, possibly due to precipitate formed by MP during reactions. We are unaware of any previous study that has examined MP interferences on Siemens Advia® 1800 chemistry analyzers.

OBJECTIVE: With a large elderly and oncology population, we were interested in determining what routine chemistries performed on the Siemens Advia® 1800 are impacted by the presence of MP. In addition, we wanted to investigate how the type and quantity of the MP affect the amount of interference observed.

STUDY DESIGN: Remnant sera from 15 patients with serum protein electrophoresis showing a MP greater than 1.5 g/dL were run on the Siemens Advia 1800 for albumin, alkaline phosphatase, ALT, AST, total bilirubin, blood urea nitrogen (BUN), total calcium, CO2, creatinine, glucose, magnesium, uric acid, total cholesterol, HDL, triglycerides, total protein, and inorganic phosphorus (IP). Reaction curves were analyzed to determine presence or absence of interference. Where quantity allowed, two-fold dilutions (5 samples) and protein-free filtrates (5 samples, using CentriFree® Ultrafiltration Devices, Millipore) were prepared and re-tested for inorganic phosphorus to determine the magnitude of the interference.

RESULTS: Patient characteristics were as follows: average age: 63 years (36-80, 9 male, 6 female); diagnoses: 3 B-cell lymphoma, 2 lymphoplasmacytic lymphoma, 9 multiple myeloma, 1 unknown; average total protein: 9.0 g/dL (7.2-14.7 g/dL); and average MP concentration: 3.08 g/dL (1.84-8.66 g/dL; 9 IgG kappa, 2 IgG lambda, 4 IgM kappa).

The majority of samples (12/15) showed a positive interference for IP. The average value obtained for IP in specimens with interference was 5.71 mg/dL (3.62-15.78 mg/dL), versus 3.38 mg/dL (3.13-3.55 mg/dL) in specimens without interference. Two-fold dilutions were not sufficient to abrogate the interference. Protein-free filtrate testing on 5 samples with IP interference (average IP 4.08 mg/dL, 3.62-4.63) showed an average reduction in IP of 0.66 mg/dL (0.37-0.77 mg/dL). Preparation of a protein-free filtrate did not significantly alter IP values in patients without MP. The lowest level of monoclonal protein at which interference was observed was 1.93 g/dL; however, one of the specimens not showing interference had a monoclonal protein level of 3.6 g/dL; both samples were IgG kappa.

CONCLUSIONS:

No interference due to MP was observed for albumin, alkaline phosphatase, ALT, AST, bilirubin, BUN, calcium, CO2, creatinine, glucose, magnesium, uric acid, cholesterol, HDL, triglycerides, or total protein.

The presence of MP >1.5 g/dL is very likely to cause positive interference with IP measurement on the Siemens Advia 1800. Preparation of a protein-free filtrate is a simple way to obtain an accurate IP value for these patients, and in studies of patients without a monoclonal protein does not significantly alter the IP values obtained.

A-131

Potassium vs. hematocrit in filter paper bloodspots: effect of large differences in potassium per unit area between center circular punch samples vs. outer annular remainder samples

M. L. Rufail, L. J. McCloskey, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: Certain quantitative assays using filter paper bloodspots (FPB) are adversely affected by hematocrit (HCT) as an unknown preanalytical variable. Capiau et al. [Anal Chem 2013;85:404] recently demonstrated use of potassium (K) from FPB punches to estimate HCT. Because the majority of K in blood is from red blood cells (RBCs), and because there is significant accretion of RBCs at the perimeter of FPB, our objective was to compare correlation of K with HCT for center punch specimens vs. remainder (outer annular) specimens. For our study, a remainder specimen was defined as the outer, annular specimen (a specimen with a middle hole) that remains after a single center punch has been removed from an intact bloodspot.

Methods: Primary samples were Li-heparin whole blood submitted to the lab for ionized calcium, on patients for whom simultaneously-drawn K-EDTA samples were submitted for complete blood count including HCT (Sysmex). FPB were formed by bolus addition of 40 µL whole blood to Whatman 903 cards (Perkin-Elmer). After drying (24 h), total bloodspot area was determined by image analysis. A standard office hole punch (approximately 6 mm diameter) was then used to produce center punch (P) and remainder (R) samples. Punch area measured by image analysis was treated as constant (0.314 cm^2). R sample areas were taken as the difference between total area and punch area. P and R samples were extracted in 400 µL 2.5 mM K-EDTA solution (>1 hour) with periodic vortexing. The resulting solution was measured for K (Roche Cobas c501). After correction for extraction solution K, values of K/area (α, µmol K/cm^2), and correlations of α to HCT, were compared between results for P and R samples.

Results: A total of 43 patient samples were utilized. Bloodspot areas (A, cm^2) were normally distributed (A = 0.91±0.046 cm^2) and were in aggregate negatively correlated with HCT (A = -0.0042 HCT(%) + 1.06 cm^2; r = -0.687). Combined P+R recovery of K from 40 µL whole blood was 88±4.5%. K/area (α) was normally distributed: α(P) = 1.23±0.26 µmol/cm^2; α(R) = 1.86±0.41 µmol/cm^2. Note that α(R)>>α(P); the overall ratio α(R)/α(P) was 1.51 ±0.15. For both P and R, α was highly correlated with HCT: α(P) = 0.030 HCT(%) + 0.015 µmol/cm^2 (r^2 = 0.795); α(R) = 0.052 HCT(%) + 0.010 (r^2 = 0.912). Slopes of correlations showed higher resolution for α(R) vs. HCT. Errors (E, calculated HCT - measured HCT) for calculated HCT were normally distributed: E(P) = -0.042 ±3.8%; E(R) = 0.059 ±2.4%. The standard deviation for E(R) was reduced by 37% compared to that for E(P).

Conclusions: K per area (α) was significantly higher in R samples compared to P samples, with approximately a 60:40 ratio in representation of total K per total FPB area (α(R)/α(P)=1.5). Correspondingly, whereas α for both punch (P) and remainder (R) samples were reasonably well correlated with HCT, there was higher resolution for α(R) vs. HCT. Both the correlation coefficient for α(R) vs. HCT, and standard deviation of errors E(R) in predicted HCT, were numerically superior compared to those for P samples.

A-132

Impact of Sample Handling on Intact Parathyroid Hormone (PTH) Concentrations in Specimens

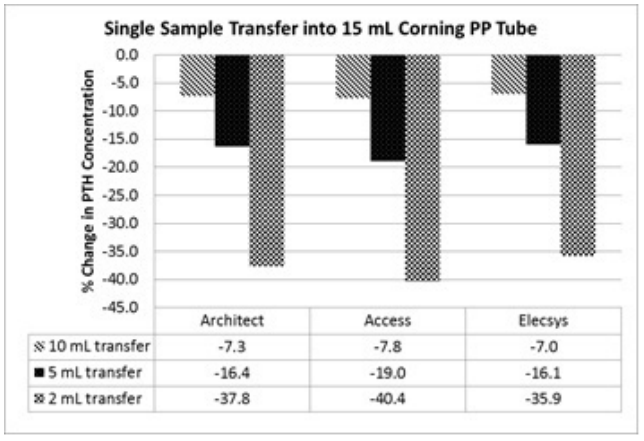
G. S. Beligere[1], E. M. Brate[1], D. Elliott[2], K. M. Gignac[1], F. C. Grenier[1], J. W. Jaraczewski[1], M. S. Matias[1], L. Sokoll[2]. *[1]Abbott Laboratories, North Chicago, IL, [2]Johns Hopkins Medical Institutions, Baltimore, MD*

Background: Parathyroid Hormone (PTH) is an 84 amino acid peptide hormone important in the regulation of calcium homeostasis. During recent studies with Intact PTH assays, we observed the PTH concentration in fresh plasma samples decreased approximately 4-8% after each transfer into a new tube. Because it is a common practice to aliquot samples for bio-banking, clinical studies and routine lab work, it is critical to be aware of the effect of pre-analytical factors on the PTH concentration. The loss of PTH due to sample handling was investigated using various tube types.

Method: Normal samples were freshly drawn into EDTA tubes and centrifuged to collect plasma. A portion of each specimen was spiked with PTH and the neat and spiked specimens were transferred sequentially to different tubes. All the specimens were tested on the Abbott Architect PTH STAT, Beckman Access 2 Intraoperative PTH and Roche Elecsys 2010 PTH STAT assays.

Results: Analysis of different tube types showed the PTH concentration decreased after each transfer by 6-15%. Nalgene cryovials and standard Eppendorf polypropylene tubes showed 10% and 50% decreases after one and three transfers, respectively. Eppendorf Protein LoBind tubes showed the smallest decrease. A similar effect was observed irrespective of the assays used with both endogenous PTH as well as spiked PTH specimens and in both fresh and frozen specimens. Increasing the surface area relative to volume also increased the magnitude of the effect (see Figure).

Conclusion: Pre-analytical factors can have a significant effect on PTH concentrations. In order to minimize changes in PTH concentration, it is necessary to select the appropriate tube type and size and avoid multiple tube-to-tube transfers.



	Architect	Access	Elecsys
10 mL transfer	-7.3	-7.8	-7.0
5 mL transfer	-16.4	-19.0	-16.1
2 mL transfer	-37.8	-40.4	-35.9

A-133

Gel Separator in Blood Collection Tubes may Cause Interference with 25-Hydroxyvitamin D Measurement by Liquid Chromatography-Tandem Mass Spectrometry Methods

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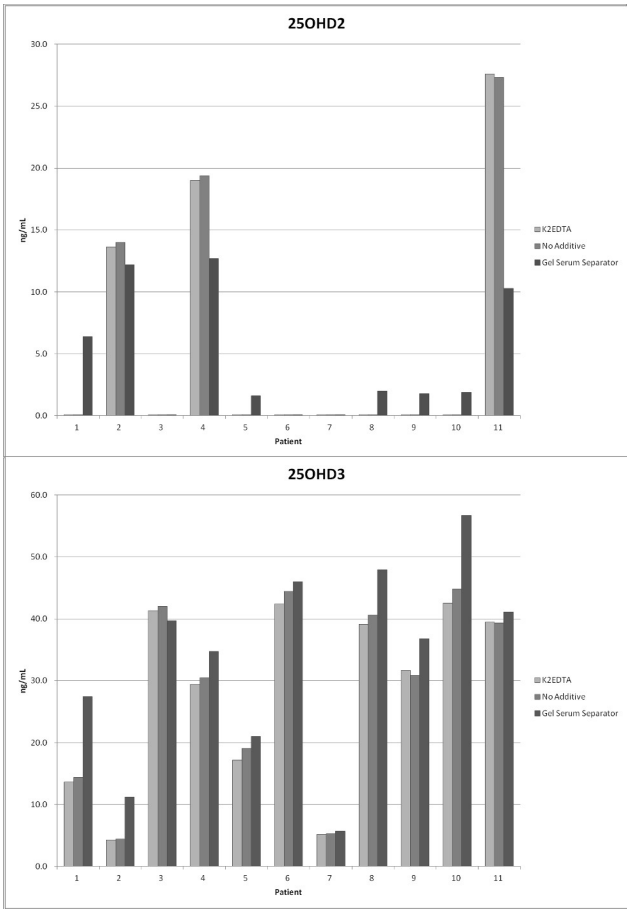
Background: Vitamin D nutritional status indicated by blood levels of 25-hydroxyvitamin D (25OHD) is important to bone health and is also linked to other aspects of human health. In 2009 we reported a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for quantitation of serum 25OHD2 and 25OHD3. Later on, unusual chromatography – especially for 25OHD2 – was noted for some patient samples. An investigation prompted the hypothesis that gel separator in the blood collection tubes may cause interference. Objective: To determine if gel separator tubes cause chromatographic interference with 25OHD2 and 25OHD3 by the LC-MS/MS method. Method: Three BD tube types were compared, gel separator serum tubes, K_2EDTA plasma tubes, and serum tubes with no gel separator. Specimens from each tube type, all from the same phlebotomy, were obtained from 11 patients. Each matched patient set was analyzed by the published LC-MS/MS method. Results: Patient samples collected in BD gel separator serum tubes exhibited different chromatography than the same patient samples collected in K_2EDTA or serum tubes with no gel separator. The altered chromatography from the gel separator serum tubes significantly affected the quantitation of both 25OHD2 and 25OHD3 for some patients. The degree of the interference varied across the patients. Conclusion: Gel separator serum collection tubes caused chromatographic interference with 25OHD2 and 25OHD3 quantitation by the LC-MS/MS method compared to K_2EDTA plasma tubes or serum collection tubes with no gel separator.



A-134

Using Urine Results Produced By Flow Cytometry On The Sysmex UF-1000i To Rationalise Urine Culture Orders, Increase Clinical Efficiency And Conserve Healthcare Spending.

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Background: The objective of this study is to see if the number of urine cultures could be reduced by using results from routine urinary analysis performed on the Sysmex UF1000i (Sysmex Corporation, Kobe, Japan) fluorescence flow cytometer. If this routine screening process is able to reliably predict the outcome of urine cultures, the laboratory will move away from physician based ordering of urine cultures to a reflex algorithm based on flow cytometry parameters. In this way, we intend to decrease the number of unnecessary cultures which do not manifest significant bacteria or urinary tract infection. By instituting the appropriate algorithm, we hope to automate this process, with an aim to increase analytical productivity and ensure consistency in our reporting.

Methodology: We reviewed 322 anonymised random urine results that had been analysed on the \ UF1000 and had subsequent urine cultures performed. Outcomes were analysed at five cutoffs of 30, 40, 50, 125 and 200 bacteria cells per microliter. Additionally, the presence of white blood cells (WBC) was used as an indicator of pyuria to see if the decision making could be made more sensitive or specific.

Results were analyzed using frequency analysis, quantitative and semi-quantitative comparisons.

Results: We found that a cutoff of 50 bacteria cells/uL provided the best specificity and sensitivity of 85.1% and 81.5% respectively. The negative and positive predictive values were 0.8086 and 0.8563. When a WBC count above 12 cells/uL was included as an additional indicator of infection, the specificity and sensitivity were 42.2% and 92.9% respectively. For the range of cutoffs from 30 to 200 bacteria cells/uL, the sensitivities ranged from 86.3% to 62.5% while the specificities ranged from 79.2% to 92.2% respectively.

Conclusion: Forty percent of urine cultures were eliminated by instituting an algorithm utilizing a single variable of a bacterial cell count cutoff of 50 cells/uL. This equated to a monetary saving of US$1400 per day of urine testing. Thirty-one out of 322 (9.6%) of patients tested would have been incorrectly diagnosed as having no urinary tract infection. Of these 31 patients, 17 of them were reported as mixed bacterial growth or having no significant growth and were therefore not clinically significant.

A-135

Blood Donation Temporarily Improves Glycated Hemoglobin (HbA1c) Status In Healthy Men

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Background: A significant change in glycemic status has previously been observed in patients with type 2 diabetes who underwent blood donation when compared to a control group. Our aim was to study the effect of whole blood donation on glycemic and metabolic markers in normal individuals at different time intervals.

Methods: 42 subjects with normal glucose tolerance (NGT) were recruited to the study. Glucose tolerance was assessed by oral glucose tolerance test before (visit A) and after the blood donation (1 day, visit B; 1 week, visit C; 3 weeks, visit D; and 3 months, visit E) on each subject. Fasting glucose, glycated hemoglobin (HbA1c), insulin, lipids, uric acid, C-reactive protein, homeostasis model assessment for insulin resistance (HOMA-IR) and Complete Blood Count were measured. A repeated measure ANOVA was used for comparisons of quantitative variables between different visits.

Results: After the blood donation, at visit C, both RBC count and total Hb concentration were decreased by ~ 9% from the baseline levels (visit A). At visit D, HbA1c ± SD (5.3 ±0.4%) was significantly lower compared to visit A (HbA1c: 5.5 ±0.4%, $p<0.05$) (Figure). Cholesterol at visit A (5.3 ±1.2 mmol/L) decreased significantly following donation and remained decreased except in visit E. HOMA-IR did not change significantly following donation.

Conclusions: After blood donation, the reduction in RBC count and total Hb contribute to a temporary improvement in glycated hemoglobin but with no significant change in insulin resistance.

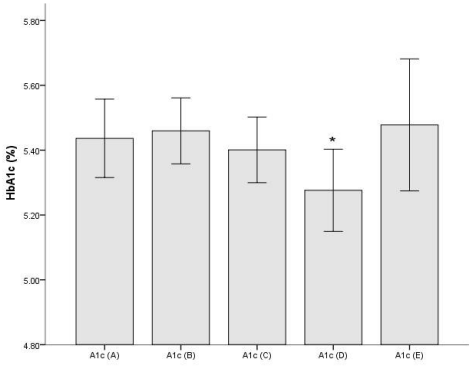

Figure: Levels of HbA1c before and at different time-points after blood donation (Error bars 95%CI). *p<0.05

A-136

Estimated Uncertainty using Routine Internal Quality Control Results for the Validation of Delta Check Method

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Background: Laboratory test results are valuable medical information used for management of patients. Among the utilities of laboratory tests, patient monitoring is one of the main purposes of laboratory testing. Therefore, the criteria for significant change in test results are of practical importance in the interpretation of test results. Also, the use of these criteria could be expanded to delta check. In metrological community, measurement uncertainty has been regarded as a key principle in the robustness of measurement results and the concept has been recently introduced in the clinical laboratory area. We assessed the criteria for determining significant difference in clinical laboratory testing using the estimated uncertainty with the routine internal quality control (QC) data from one laboratory.

Methods: The uncertainty was estimated directly by the CLSI EP29-A guideline and Guide to the Expression of Uncertainty in Measurement (GUM) using the top-down approach. We used one-year's internal QC data to estimate uncertainty of 6 analytes including albumin, total cholesterol, triglyceride, glucose, protein and creatinine. We identified the sources of uncertainty, including pipetting, calibration errors, and lot variations as the components of type B evaluation. We collected inpatient data for six test items, and then current results and results upto 60 days prior were collected as paired test results. We calculated the relative combined uncertainty including biological variation and reference change value (RCV) for each analyte and determined the criteria for significant difference. And RCV was compared with delta check method.

Results: Combined uncertainty were 5.83% and 5.11% for albumin, 7.90% and 7.43% for total cholesterol, 21.06% and 20.49% for triglyceride, 6.78% and 6.78% for glucose, 5.66% and 4.93% for protein, 13.84% and 11.08% for creatinine at level 1 and level 2, respectively. With the uncertainty, we could estimate the minimal difference to claim the difference between test results. And the results of comparison between RCV and delta check method showed that detection rate of significant difference in RCV was higher about 2 times to 12 times than delta check method for all test items. Delta check method underestimated the differences in albumin, glucose and protein. In case of total cholesterol, triglyceride and creatinine, over-detections by current delta criteria were showed.

Conclusions: Uncertainty based change assessment can reflect laboratory performance characteristics without collecting the large volume of patient data. And straightforward estimation of significant change was possible. Based on our findings, current delta criteria needs to be more or less stringent. We conclude that our assessment model can be the efficient tool for verifying delta check method with the biological variations considered and applicable.

A-137

Estimation of biological variation and quality specifications for plasma ammonia concentrations in healthy subjects

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Background: Most of the factors causing preanalytical and analytical variations in ammonia measurement are known. However, data on the biological variability of ammonia is still uncertain. The present study for the first time investigated the biological variation of ammonia in a group of healthy individuals by applying a recommended and strictly designed study protocol using fresh and frozen samples.

Methods: Twenty voluntary healthy individuals [12 female, 8 male; 21-55 years (min-max)] were recruited for this study. Blood samples were collected daily over a period of four consecutive days from each subject. Blood collections were performed in standardized conditions in order to minimize sources of pre-analytical variation. Blood samples for ammonia measurement were collected in K2EDTA (2.0 mL, K2EDTA 3.6 mg, BD Vacutainer®, UK) tubes. Immediately after sampling, blood samples were placed on ice bath, separated within 15 minutes of collection. All samples were immediately centrifuged (4°C, 10 min, at 2000xg). Also, after centrifugation each plasma sample was split into two aliquots; one was immediately analyzed as the samples were collected and the other was stored -80°C and analyzed at once in one analytical run at the end of the fourth day. All samples were assayed in

duplicate for both fresh and frozen samples. Data were analyzed by SPSS 15.0 and estimations were calculated according to the formulas described by Fraser and Harris.

Results: All of the estimations were performed for fresh and frozen samples. The intra-individual or within-subject (CVI) and inter-individual or between-subject (CVG) biological variation were 13.78% and 16.91% for fresh samples, respectively. The calculated CVI and CVG were 18.91% and 18.43% for frozen samples, respectively. Reference change values (RCV) for fresh and frozen samples were 43.37% and 56.85%, respectively and individuality indexes (II) of ammonia were 0.92 and 1.11, respectively. Minimum, desirable and optimal analytical goals for imprecision, bias, total error were also estimated by using obtained data for fresh and frozen samples. Derived desirable analytical goals for imprecision, bias, and total error resulted 6.89%, 4.61%, and 15.98%, by using obtained data for fresh samples, respectively. Derived desirable analytical goals for imprecision, bias, and total error resulted 9.45%, 5.01%, and 20.61%, by using obtained data for frozen samples, respectively.

Conclusion: This study for the first time described the components of biological variation for ammonia in healthy individuals. These data regarding biological variation of ammonia could be useful for a better evaluation of ammonia test results in clinical interpretation and for determining quality specifications based on biological variation.

A-138

The influence of analytical interference introduced by separator gel blood collection tubes on steroid molecules analysis

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Background: The ubiquitous use of serum gel separator tubes within clinical laboratories is a result of the numerous advantages they provide; including rapid serum separation, enhanced sample stability and reduced aerosolization. These advantages, however, can be offset by the release of molecules from the tube's gel element that can interfere with the qualitative and quantitative analysis of various analytes. Steroid molecules are one analyte class that is susceptible to analytical interference from molecules that are found within the gel element of serum separator tubes because of their low biological concentration and non-polar nature. Investigating the source of ion suppression and interference that results from the use of serum gel separator tubes and their influence on the quantification of steroid molecules, specifically 17-hydroxyprogesterone and aldosterone.

Methods: Five different blood collection tubes from BD Biosciences (Mississauga, ON, Canada) were utilized for this study and included gold top tubes (gel separator + clot activator [367977]), light green (mint) top tubes (gel separator + lithium heparin [367962]), red top tubes (clot activator [367812]), dark green top tubes (sodium heparin [367884]) and lavender top tubes (potassium EDTA [367861]). The blood samples collected within these tubes subsequently underwent serum separation, followed by analysis of the serum for 17-hydroxyprogesterone and aldosterone. Sample preparation for both analytes relied upon a methyl tert-butyl ether liquid-liquid extraction, while chromatographic separation of analytes was carried out using an Agilent 1290 liquid chromatographic separations module (Santa Clara, CA, USA) and Agilent Poroshell C18 column (2.1 x 100 mm, 2.7 µ) with a Methanol and 5 mM ammonium formate gradient. Identification of both analytes relied upon an Agilent 6460 triple quadrupole mass spectrometer using multiple reaction monitoring for quantification; 17-hydroxyprogesterone (331.0 ◊ 97.1/109.1 [H-085, Cerilliant, Round Rock, Texas, USA]) and 17-hydroxyprogesterone-d8 (339.2 ◊ 100.0/112.1 [H-096, Cerilliant]), aldosterone (359.4 ◊ 331.4 [A-096, Cerilliant]) and aldosterone-d4 (363.4 ◊ 335.4 [Isosciences, Trevose, PA, USA).

Results: An inability to correlate the serum concentrations of 17-hydroxyprogesterone and aldosterone between LC-MS/MS and immunoassay led to the hypothesis that tubes containing separator gel interfere with the quantification of these analytes by LC-MS/MS. Subsequent analysis of 17-hydroxyprogsterone concentrations within three patients using the five different blood collection tubes (identified above) resulted in a 1.48 (±0.25) times increase in its observed concentration within tubes containing separator gel; a decrease in concentration was attained for aldsoterone measured in tubes containing separator gel. These changes in the measured concentrations of both analytes is a result of the interference produced by separator gel that modifies the chromatographic baseline and peak area, resulting in changes in measured concentrations of each analyte as well as the ratio of their monitored product ions.

Conclusion: Blood collection tubes that contain separator gel (Gold top and light green [mint] top tubes) interfered with the quantification of steroid molecules 17-hydroxyprogesterone and aldosterone by introducing extraneous molecules that interfered with LC-MS/MS analysis.

A-139

The effect of microcentrifuge tube plastic leachates on laboratory sample analysis and the identification of leachates by LC-MS/MS and GC-MS

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Background: The prevalence of disposable plastic items within clinical laboratories is a result of their easy disposal and inexpensive nature. These products, however, are produced at varying levels of purity and then often sold as universally applicable to sample preparation, with some being advertised as compatible with liquid-liquid extraction protocols using organic solvents. Difference in the manufacturing and/or cleaning process of each tube can lead to sample contamination from materials that leached from the tubes and jeopardize patient management through their impact on the identification and quantification of certain molecules. Investigate the influence of various solvents on the leaching of materials that comprise microcentrifuge plastic tubes used for the preparation and storage of samples undergoing LC-MS/MS drug and steroid analysis.

Methods: Four different plastic microcentrifuge tubes were evaluated for leachates and included tubes from Bioplastics (Cat# 4036, Landgraaf, The Netherlands), Eppendorf (Cat# 022364111, Hauppauge, NY, USA) Rainin (LTT-170-N, Mississauga, ON, Canada) and VWR (Cat# 20170-026, Radnor, PA, USA). The effect of agitation and incubation time (0-90 minute) were evaluated on each plastic tube using; Millipore water, verified blank urine, verified blank serum, saline solution, methanol, ethanol, acetonitrile, methyl-tert-butyl-ether, isopropylalcohol and chloroform. LC separation of analytes was carried out using an Agilent 1290 liquid chromatographic separations module (Santa Clara, CA, USA) and Restek ultra biphenyl column (2.1 x 100 mm, 5 µ) and gradient of Acetonitrile and 5 mM ammonium formate. Identification of analytes relied upon an Agilent 6460 MS/MS. GC separation of analytes was carried out using an Agilent 6890 GC separations module (Santa Clara, CA, USA) and DB-5MS low bleed 5%-Diphenyl-95%-Dimethylsiloxane Copolymer capillary column (thickness 0.25µm, 0.25 x 15000 mm) (Chromatographic Specialties, Brockville, ON, Canada) with nitrogen as the carrier gas. Identification of analytes relied upon an Agilent 5973 MSD using electron impact full scan analysis.

Results: Incubation of aqueous solutions within each tube type did not lead to a significant level of leaching occurring from any of the tested tubes. Incubation or organic solvent within the tubes did lead to significant levels of leaching by the 1.5 mL VWR and bioplastic tubes for all tested organic solvents; with peak areas greater than 1.0e6 or 100 times greater than blank solvents. Conversely, the 1.5 mL Rainin and Eppendorf tubes reported peak areas comparable to the blank solvents; leaching was not found to be time dependent. GC-MS/MS analysis of the VWR and Bioplastic tubes as well as the correlation of discovered peaks with the NIST library allowed for the identification of some of the contaminants as hexadecanal, pentadecanal, methyl stearate, octadecanoic acid and assorted fatty acids. These identified products are common by-products of poor quality or recycled plastics and were found to interfere with the LC-MS/MS analysis of 17-hydroxyprogesterone in serum samples; a molecule of identical m/z was discovered in the VWR and bioplastic microcentrifuge tubes.

Conclusion: Microcentrifuge tubes from VWR and Bioplastics were not suitable for use with all 6 organic solvents evaluated because they leached molecules that interfered with LC-MS/MS and GC-MS analysis.

A-140

Establishing Evidence-Based Thresholds for Pseudohyperkalemia Alerts

P. C. Mathias, P. Ranjitkar, A. N. Hoofnagle, G. S. Baird, D. N. Greene. *University of Washington, Seattle, WA*

Background: Pseudohyperkalemia is a common pre-analytical error that can lead to inappropriate patient treatment and true hypokalemia. Previous work has suggested that pseudohyperkalemia is most pronounced at platelet counts above 500 x 10^9/L for serum samples and white blood cell (WBC) counts greater than 100 x 10^3/microL for plasma samples, but the generalizability of these thresholds is unclear. In 2010, our institution created laboratory information system (LIS) rules to append a result comment indicating the risk of falsely elevated potassium for patients with platelet counts above 500 x 10^9/L or WBC counts greater than 150 x 10^3/microL, irrespective of sample type. To evaluate the efficacy of these thresholds we analyzed 4 years of historical results.

Methods: Potassium results from 2011-2014 for two hospital laboratories were extracted from the LIS in conjunction with the most recent platelet count, WBC count, and whole blood potassium. The difference between serum/plasma potassium and whole blood potassium values collected within a 2 hour span was plotted as a function of platelet and WBC count and fit to a linear model. The number of results and distinct patients with relevant result comments was tallied, and the impact of changing platelet and WBC count thresholds was evaluated.

Results: Of the approximately 2 million potassium results from more than 300,000 distinct patients, 10,869 plasma and 1,676 serum potassiums were coupled with a whole blood potassium collected within 2 hours. Analysis of this data showed that plasma potassium concentrations were unaffected by platelet count but serum potassium concentrations increased by 0.05 mEq/L per 100 x 10^9/L increment in platelet count. Over 4 years, 45,430 results included a comment warning of the risk of falsely elevated potassium due to high platelet counts. However, over 90% were plasma samples, for which the comment is not relevant. In serum samples meeting platelet count criteria, 20% demonstrated a >1 mEq/L increase in serum potassium relative to whole blood. In contrast, high WBC counts had a minor impact on serum potassium but significantly impacted plasma potassium, with increases of 0.35 mEq/L per 50 x 10^3/microL increment in WBC count. The comment for interference due to high WBC count was appended to 443 results in 115 patients. Specimens with corresponding whole blood potassiums (n=83) showed a >1 mEq/L increase in over 70%, suggesting that the WBC count threshold should be lower. Lowering the threshold to a WBC count of 50 x 10^3/microL would have flagged 924 patients during this period, and 23% of samples above this threshold had a >1 mEq/L increase in plasma potassium relative to whole blood.

Conclusion: Samples with extreme platelet and WBC counts are rare, but analysis of a large number of results provides data-driven thresholds at which high platelet and WBC counts are clinically significant. The suggestion of a 500 x 10^9/L psuedohyperkalemia alert threshold for platelet count in serum samples is likely appropriate, but our data suggests the WBC counts greater than 50 x 10^3/microL in plasma samples may lead to clinically significant false elevations.

A-141

Therapeutic Concentrations of Hydroxocobalamin Interferes with Several Spectrophotometric Assays on Two Commonly Used Chemistry Analyzers

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Background: A 28 year-old female with cystic fibrosis underwent a bilateral orthotopic lung transplant. Her sample, drawn for a metabolic panel, had a distinct red discoloration, but a heme index of 0. A sample collected 6-h earlier appeared normal. Pharmacology review indicated that between specimen draws the patient had received a dose of hydroxocobalamin (5g IV infusion; OHCob). High-doses of OHCob are typically used to treat cyanide poisoning and more recently, cardiac complications (as in this patient). Since OHCob absorbs at multiple wavelegnths (274, 351, 500 and 526 nm) that are often used in colorimetric assays, spurious laboratory results are likely to occur. Furthermore, while these samples may appear hemolyzed, they are not flagged by automated analyzers due to differences in the absorbance spectrums of hemoglobin and OHCob. The extent of OHCob interference varies between platforms and had not been assessed using the specific instrumentation/assays used in our laboratory.

Objective: We aimed to examine interference caused by OHCob in colorimetric assays measured using the Beckman Coulter DxC800 and AU680.

Methods: OHCob concentrations were chosen based on observed therapeutic levels (0.4-1.3 mg/mL) after treatment for cyanide poisoning. OHCob (Sigma Aldrich, USA) was dissolved in water and spiked into pooled "healthy" and "unhealthy" patient samples at two different concentrations (0.15 and 1.5 mg/mL). For those analytes that showed a significant bias at 0.15 and 1.5 mg/mL, OHCob was titrated to the following concentrations: 0.2, 0.4, 0.8, 1, 1.2 and 1.5 mg/mL. Samples were run in duplicate or triplicate on the DxC and AU680 and compared to the unspiked pool. The following analytes were selected because their quantification relies on wavelengths near the wavelengths at which OHCob absorbs: Albumin, ALP, ALT, AST, DBil, TBil, Chol, CK, creatinine, Direct LDL, GGT, haptoglobin, HDL cholesterol, Fe, LDH, lipase, Mg, phosphate, total protein, transferrin, triglycerides and uric acid.

Results: Substantial interference was observed with low concentrations of OHCob (0.15 mg/mL) in TBil (0.6 mg/dL, 171% bias), Fe (5µg/dL, 31% bias) and triglyceride (77.3 µg/dL, 15% bias) results measured using the DxC; no interference was observed with the AU680. At high OHCob concentrations (1.5 mg/mL), significant bias was observed with a number of analytes on both the DxC and AU680: ALT, amylase, AST, TBil, Chol, creatinine, Mg, phosphate, and uric acid. DBil, Fe and triglyceride measurements were effected only on the DxC; direct LDL measurement was effected only on the AU680. Titration of OHCob revealed a >10% bias in TBil, Dbil, cholesterol, triglycerides and iron even at the low end of the OHCob therapeutic range (0.4 mg/mL) using the DxC.

Conclusions: OHCob is a colored compound that is known to unpredictably interfere with several colorimetric chemistry measurements. Between the DxC and AU680, we found several analytes that were falsely increased or decreased at therapeutic OHCob concentrations. We recommend that laboratories understand how OHCob influences results on their platforms and that there is a workflow in place to notify the lab when these samples are expected.

A-142

Assessing the impact of Enzymatic Creatinine on eGFR in an elderly outpatient population

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Background: The majority of laboratories in New Zealand use a rate blanked compensated Jaffe method for Creatinine measurement in serum. However it is widely accepted that enzymatic creatinine methods should be the preferred assay, especially in paediatric samples, due to limitations of the Jaffe method at low levels and the impact of various interfering substances. Literature search shows that enzymatic creatinine analysis in elderly outpatients does not appear to have been studied, particularly with regard to calculations of estimated glomerular filtration rate. The aim of this study was to establish the impact of enzymatic creatinine analysis versus Jaffe, particularly with regards to estimations of eGFR by the CKD-EPI equation, in a relatively consistently distributed for age and gender, outpatient population of patients greater than 65 years of age.

Methods: Cross-sectional study conducted in 100 elderly New Zealand outpatients, aged between 65 and 95 years of age. Mean age was 80 ± 9 years, with equal distribution of men and women (50% each). Creatinine was measured by a traceable Jaffe method (Cobas 702, Roche Diagnostics NZ) and by an enzymatic method (CREA plus, Roche Cobas 502). The Jaffe results were compensated to Roche global Cfas at a value of -20, as per regional protocol, as well as to the compensation value of -26 quoted by Roche. eGFR was calculated using CKD-EPI GFR calculator by Stephen Z. Fadem (http://touchcalc.com/calculators/epi). Creatinine values were assessed for trueness using CLSI EP15-A2 on StatusPro, and for clinical comparability based on the RCPA Allowable limits of performance for Creatinine (±8 up to 100; ±8% >100 µmol/L). The clinical impact of eGFR values was assessed based on the trigger point of <60 mL/min/1.73m2 indicating additional testing required. Change in CKD stage was also noted.

Results: The difference in creatinine values between the -20 Jaffe and enzymatic methods across all subjects met the bias claim of 0.5% at a significance level of 20%. In women, all results fell within the RCPA ALP, except for 1 patient with a very low creatinine level, however this difference did not alter the CKD stage of the patient. 5 of the CKD stages in women altered values around the trigger point of 60 mL/min/1.73m2, 2 removing the indication for further testing and 3 indicating further testing was required. There was more variation between the -20 Jaffe and enzymatic methods in men, with 4 patients values falling outside the RCPA ALP, and while there were resulting changes in CKD stage in 3 of these patients, none of were at the trigger point of 60 mL/min/1.73m2. Use of the -26 Jaffe gave 22 % of values outside the RCPA ALP in men and 6% in women.

Conclusion: Based on this study, the use of enzymatic creatinine in the elderly appears comparable to that of the in-use -20 Jaffe method, and the impact of the change of technique for creatinine measurement is unlikely to have a major impact on referral rates based on CKD stage changes. A compensation value of -20 appears to be the most suitable in this population.

A-143

Establishment Of Reference Interval for Liver Function Tests For Adult Apparently Healthy Nepalese Population: Nationwide Multicenter Study

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Background: During the last decades, the number of routine clinical chemistry tests has increased dramatically in Nepal, especially in its capital, Kathmandu. Reference intervals (RIs) are indispensable for the diagnosis of disease, its treatment and management. A reference range is usually defined as the set of values 95 percent of the normal population falls within (that is, 95% prediction interval).RIs are conceptually different for different countries, even for different region in the same countries, at different times, attitude and in same individuals at different ages and condition (eg.preganacy, lactation etc).It is therefore a relative and not an absolute condition. That's why the each laboratory should have its own RIs values for the interpretation of individual laboratory data there is no valid nationwide reference value for any biochemical parameter for Nepalese yet. There is a trend to use reference values supplied in the reagents kit which is mainly for either American or European population. Incidence of liver disease is increasing day by day in Nepal. This situation insisted us to design this study to establish the reference values for the frequently used liver function test for the diagnosis of liver disease diseases for Nepalese population.

Method: This study was conducted on 630 apparently healthy individual from five different region of the country. Blood samples were collected and sera were separated and transported to department of laboratory medicine, Grandi International Hospital ,Kathmandu where analysis were done using Vitros reagents on the Vitros 250 plateform.Reference material and panel sera were used for the standardization of test results. By using secondary exclusion criteria- the latent abnormal values exclusion, reference intervals (RIs) were derived by both parametric and non-parametric method by using reference master and statFlex software.

Results:

The reference intervals (nonparametric with LAVE) values (lower limit-median-upper limit) obtained for serum total bilirubin, total protein, albumin, alanine transaminase(ALT), aspartate transaminase (AST),alkaline phosphatase(ALP) ,gamma glutamyl transferase (γGT) were 0.2—0.5-1.3mg/dl, 6.2-7.5-8.5mg/dl, 3.5-4.5-5.4 mg/dl, 8-18-47 IU/L, 15-29-63 IU/L, 44-85-154 IU/L and 10-19-70 U/L, respectively. Furthermore, gender wise evaluation demonstrated higher range for males than females. These variations may be attributed due to dietary pattern, smoking, alcoholism and over all life styles.

Conclusion: This study is the first to assess clinical reference ranges for liver function tests for Nepalese adult population and it cab ne suggested that these reference values can be used not only for the interpretation of laboratory values obtained for individuals but also care and treatment of liver disease.

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A Comparison Of Two Formulae Calculating Estimated Glomerula Filtration Rates In A Healthy South East Asian Population

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Background: It is common clinical practice to include an estimated glomerula filtration rate (eGFR) based on a serum Creatinine determination when a renal panel assessment is done. There are two western centric formulae, namely the Modified Diet in Renal Disease (MDRD) and the more contemporary Chronic Kidney Disease Epidemiology (CKD-Epi). Both utilize gender and American/African-American coefficient adjustments with the latter including consideration for the concentration of serum Creatinine.

In this study, we looked at healthy male and female individuals in an anonymised data set. They comprised of the four main races in Singapore, namely Chinese, Malays, Indians and Others (Caucasians, Eurasians and Sikhs) in a ratio of 7:1:1:1.

Methods: We reviewed 5470 serum Creatinine results over a period of 8 months from individuals attending health screening and non-disease related hospital visits. We applied both the MDRD and CKD-Epi formulae applying the American ethnic coefficient. We analysed the data using Statistical Package for the Social Sciences (IBM, United States) according to formula, gender and ethnicity.

Results: Overall, there was a shift in the eGFR mean from 88.15 [95% confidence interval (CI) 87.5-88.8] to 94.1 (CI 93.4-94.8) when we compared MDRD to CKD-Epi. The mean eGFR of males was 83.4 (CI 82.6-84.3) using MDRD compared to 93.2 (CI 92.1-94.3) using CKD-Epi. The difference for females was less significant at 93.2 (CI 92.1-94.2) for MDRD versus 95.0 (CI 94.1-95.9) for CKD-Epi. The standard deviation for males expanded from 23.46 to 28.90 while the calculation for females was reduced from 26.89 to 23.01 for MDRD and CKD-Epi respectively.

The eGFR means for the four races, Chinese, Malay, Indian and Others were 89.2, 82.2, 85.7, and 89.1 respectively using MDRD and 94.8, 88.0, 92.8, and 96.0 respectively using CKD-Epi. Additionally, the standard deviations (SD) were 25.00, 30.46, 24.32 and 25.38 for MDRD and 25.38, 31.76, 26.15 and 25.49 for CKD-Epi for the four races.

Conclusion: Our laboratory uses the cut-off of 90 mL/min/1.73 m2 to demonstrate absence of kidney dysfunction when previously using MDRD and currently the CKD-Epi in assessing glomerula filtration rate. When we moved from an MDRD based calculation to a CKD-Epi based analysis, there was a clear reclassification of 14.3% of the population. This shift was predominantly in the male subjects where 18.1% were reclassified as having "normal" eGFR.

All races except Malays saw eGFR means being reclassified as "normal". The mean for Malays improved from 82.2 to 88.0 using the CKD-Epi formula. The Malays also had the largest standard deviation of 30.5 (MDRD) and 31.7 (CKD-Epi) compared to the all-race SD of 25.9. This may imply that this particular race may require an ethnic specific correction coefficient to be defined.

Our findings are consistent with current reports that there is clear improvement especially for eGFR values greater than 60 mL/min/1.73 m2, despite using a Caucasian derived correction coefficient on a predominantly Asian ethnic population. It is yet unclear if an Asian specific coefficient is necessary to improve eGFR specificity although an intra-ethnic coefficient for the Malay population may be indicated.

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Interference analysis of PCT testing in bacterial infection diagnosis

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Background: Procalcitonin (PCT) is a biomarker for the clinical diagnosis of bacteria infection that is more specific and earlier than fever, changes in white blood cell count, and blood cultures. The performance of instrument, conditions of sample processing, and underlying diseases in patients can affect PCT testing results that make clinical doctors confused. These factors should be confirmed, and the role of PCT in bacterial infection diagnosis should be re-evaluated when necessary.

Methods: In order to evaluate analytical performance, the precision and accuracy of detection, which was conducted using a Roche Cobas E601 Electrochemiluminescence Immunoassay Analyzer (Roche, Basel, Switzerland), were verified by following Document EP15-A2 from the Clinical and Laboratory Standards Institute(CLSI) . In order to determine the impacts of sample processing, the PCT levels between heparin plasma, EDTA plasma, serum in separation gel coagulation promoting tubes, and serum in tubes without additives, were compared at various intervals (0h,12h,1d, 3d and 7d) and storage temperatures(room temperature, 4℃,-30℃ and -70℃). Moreover, the interference of common endogenous substances, such as hemoglobin, direct bilirubin and chyle, were also analyzed using CLSI EP7-A2 Documents. In order to prove congestive heart failure could interfere with PCT levels in patients with or without bacterial infection, a total of 4698 cases, including those with different classes of congestive heart failure, bacteria infection, and bacteria infection complicated by congestive heart failure, and healthy individuals, were chosen for the diagnostic value analysis of PCT.

Results: The precision and accuracy were good for PCT detection using Cobas e601. The total coefficient of variation (CV) was below 3.59% and the deviation from definite value was below 3.38%, which were consistent with clinical requests. In all kinds of sample collection tube, PCT would degrade under room temperature after 24h. EDTA plasma was the most suitable sample type with lowest degradation rate, and could be stored at 4℃(or lower temperature) for more than 7d with at least twice freezing and thawing. The interference of common endogenous substances could not be observed when the levels up to Hb(2g/L), DB(428μmol/L), chyle(2000FIU). Patients with simple heart failure had significantly higher PCT levels than normal controls ($P < 0.01$), whereas patients with bacteria infection complicated by congestive heart failure had significantly higher PCT levels than those with simple infection ($P < 0.01$). Although it was useful for the diagnosis of infection (area under the ROC curve

>80%), the positive predictive value of PCT decreased significantly with increasing severity of heart failure ($P < 0.05$), and the cutoff value of PCT concentrations for infection complicated by class-II, -III, and -IV heart failure were up to 0.086, 0.192 and 0.657 μg/L, respectively.

Conclusions: The analytical performance of PCT assay on Roche Cobas e601 meets clinical request, and it has strong anti-interference capacity against endogenous substances. However, sample type selection and patients underlying factors should be given consideration in PCT diagnostic value analysis.

A-146

HbG-Coushatta: An unexpected discovery during HbA1c measurement

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Abstract

Background: Hemoglobin (Hb) A_{1c} is widely used as the gold standard for blood glucose level monitoring in diabetic patients. Various methods are being used in clinical laboratories for HbA_{1c} measurement. However, the presence of Hb variants may interfere with HbA_{1c} measurement.

Methods: The HbA1c assay was performed routinely in a 55-year old woman with type2 diabetes and hyperlipidemia that was prescribed with a therapy of oral anti-diabetic medication. The test results of CE-HPLC showed that there was an Hb variant which interfered with the quantitation of HbA1c. The patient Hb was profiled through capillary electrophoresis and DNA sequencing of the Hb β-chain gene (HBB) was performed to define its genotype.

Other assay methods like capillary electrophoresis and tandem HPLC-capillary electrophoresis (LC/CE), one of the two IFCC reference methods were used to determine the HbA1c of this sample.

Results: Hemoglobin electrophoresis showed that proportions of HbA2 and HbA was 2.6% and 54.1%, respectively. An Hb variant was confirmed by the presence of an abnormal peak in the HbD zone, with a proportion of 43.3%. Further DNA sequencing of the Hb β-chain gene (HBB) revealed that the variant was HbG Coushatta. The measurement of HbA1c through capillary electrophoresis yielded a result of 7.7% (60.6mmol/mol). Tandem HPLC-Capillary electrophoresis (LC/CE),

reported an HbA1c value of 6.7% (49.7 mmol/mol). Integrals from capillary electrophoresis suggested that glycation rate of HbG Coushatta may be less than HbA. This difference could affect the IFCC reference method determination of HbA1c.

Conclusion: Elimination of Hb variant interference is critical to achieve an accurate result of HbA1c. High resolution or low interference methods might be preferred in the regions with high prevalence of Hb variants. And staffs in clinical laboratory should pay more attention to analyze raw data of the test and find problems of the analysis.

Figure 1: CE-HPLC chromatogram for HbA1c analysis. The retention time of unknown peak was 1.75 min. It was not completely separated from HbA0. The proportion of HbA1c analyzed by CE-HPLC was 4.4% (24.6mmol/mol).

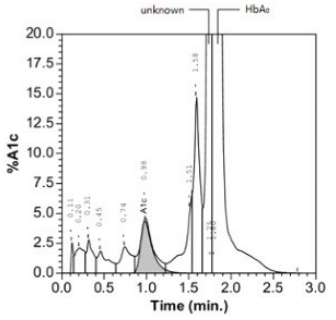


Figure2. The hemoglobin electrophoresis profile. An Hb variant was present in the HbD zone, with a proportion of 43.3%. The corresponding mutation of this variant (HbG Coushatta) was determined by DNA sequence analysis.

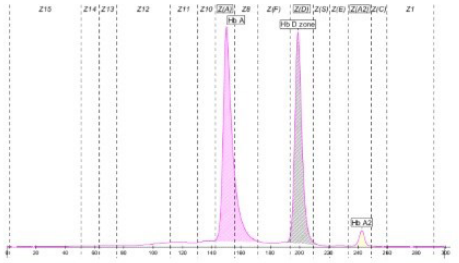


Figure 3: HbA1c assay by capillary electrophoresis. An unknown peak appears between HbA0 and HbA2, which corresponds to the non-glycated form of the variant (HbG0). HbG0 was completely separated from HbA0; while the glycated form of the variant (HbG1c) is also separated as it appears between HbA1c and HbA0. The proportion of HbA1c analyzed by capillary electrophoresis was 7.7% (60.6mmol/mol).

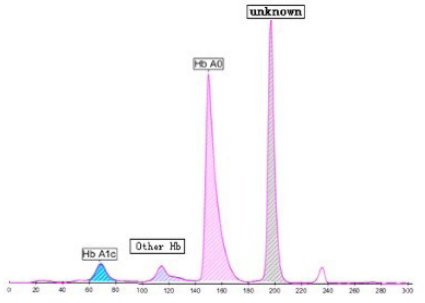
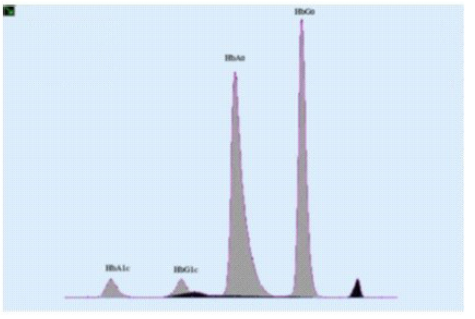


Figure 4: Electrophoregram of HbA1c assay by capillary electrophoresis.



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Effect of specimen types and storage on the activity of Lactate dehydrogenase in Serum and Plasma

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Objective: The study objective was to evaluate the effect of pre-analytical variables due to use and storage with various specimen tube types. For this purpose we measured Lactate dehydrogenase (LDH) activity in the serum and plasma samples stored separately in primary and aliquots tubes at 2-8 °C across 24 hours at various time points.

Method: The serum and plasma samples were collected from 15 apparently healthy volunteers using standard phlebotom techniques. Each sample was collected into Serum tubes, Serum separator tubes (SST), Lithium heparin tubes (Li-heparin) and Lithium heparin tubes with gel (PST) and aliquoted. LDH (Abbott, LN 2P56) activity was measured on Abbott ARCHITECT c8000 systems. The % recovery of LDH measured using different sample types at various times points were compared with results obtained using SST aliquot samples at 2 hours post collection.

Results: The data demonstrate that the LDH activity measured using samples stored in either SST primary tubes or SSTaliquots for various time points show very minimal or no change in the activity. In contrast, the plasma samples stored for 24 hours in the Li-heparin and PST primary collection tubes showed increased recovery of 20.8% and 33.0% respectively when compared to the SST aliquot tubes at 2 hours post collection. In addition, compared to SST aliquot tubes, the samples stored in the serum primary tubes recovered approximately 7%lower at 2 hours but demonstrated comparable recovery overtime.

Conclusion: The extended contact between blood cells and serum/plasma stored at 2-8 °C may produce spuriously high results for Lactate dehydrogenase. The increased LDH activity is likely related to the change in the cell permeability and to the fragility of erythrocyte membrane during prolonged storage. Therefore, LDH activity measured in patient samples stored for extended period of time should be interpreted cautiously.

Comparison of % Recovery of LDH using different specimen types at various time points with results obtained using SST aliquot samples at 2 hours post phlebotomy.

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8
	Serum	Serum Aliquot	SST	SST Aliquot	Li-Heparin	Li-Heparin Aliquot	PST	PST Aliquot
2 hrs	92.9	93.1	99.1	100.0	109.6	104.3	111.3	103.5
6 hrs	97.9	94.0	101.5	100.1	116.0	104.2	120.0	103.6
12 hrs	98.3	92.8	101.7	99.4	117.9	103.5	122.3	101.8
24 hrs	101.8	93.6	103.2	101.1	120.8	104.1	133.0	103.0

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Data mining of serial blood gas data reveals that use of safePICO syringes significantly reduces preanalytic variation

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Background:

In late 2010, Alberta Health Services replaced the Portex Pro Vent with *safe*PICO syringes for virtually all arterial blood gas testing. Presumably, the electrolyte-balanced heparin in these syringes would reduce the variation in calcium measurement. We discovered the new syringes reduced the preanalytic variation of most of other analytes.

Methods

The Portex Pro-Vent arterial blood sample kits (Keene, NH, USA), contain 23.5 to 25 IU/mL of dry lithium heparin. The *safe*PICO (Copenhagen, Denmark) uses 60 IU/mL of uniformly distributed dry electrolyte-balanced heparin. In 2010 we derived biologic variation (s_b) of various blood gas parameters using the methodology described in *Clin Chem Lab Med*. 48: 1447–1454. We applied the same methodology to the last 2 years of *safe*PICO arterial blood gas measurements from the identical general systems intensive care unit (approximately 40,000 Radiometer ABL800 FLEX pH, blood gas, electrolyte, and glucose measurements from 3079 patients). After exclusion of physiologic outliers we calculated the standard deviations of duplicates (SDD) of paired intra-patient results across intervals of 2 hours: 0-2h, 2-4h, 4-6h … 20-22h, and 22-24h. Linear regression was applied to data points derived from more than 1000 paired results. s_b was calculated from the formula where s_a represents the analytic variation at the patient mean.

Results:

The Table compares the calculated s_b, the corresponding patient means for both blood gas syringes and the reduction in the calculated s_b.

Conclusions:

This decrement in biologic variation can be classified as a decrease in preanalytic variation and results from the decreased variation in the sample milieu induced by the balanced heparin. Use of *safe*PICO reduces the preanalytic variation of at least sodium, bicarbonate, calcium, chloride and glucose measurements, findings that have been intimated in a handful of small syringe evaluations.

Test	s_b (mean) for non **safe**PICO	s_b (mean) for **safe**PICO	Reduction in s_b
Na^+	0.77 (140.3)	0.51(140.0)	-39.1%
HCO_3^-	0.84(24.1)	0.66(24.5)	-26.2%
iCa^{++}	0.026 (1.09)	0.020(1.10)	-25.4%
Cl^-	0.81(107.8)	0.65(107.0)	-20.6%
Glucose	0.70(6.93)	0.65(7.40)	-13.4%
K^+	0.19(3.89)	0.17(3.96)	-10.5%
pCO_2	2.26(39.0)	2.08(39.0)	-8.3%
pH	0.022(7.41)	0.021(7.41)	-6.9%
pO_2	11.48(85.0)	11.61 (85.1)	+1.0%

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Anomalous amylase activity on a diluted patient sample

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Background: Amylase is a digestive enzyme produced in the exocrine pancreas and salivary glands. The reference range in human serum is 30 to 110 U/L. Elevated amylase can be indicative of pancreatitis, pancreatic cancer, cholecystitis, and mumps. Amylase is also monitored in surgical wound drainage as a predictor for postoperative pancreatic fistula. Hemoglobin is known to interfere in the Vitros amylase method and under circumstances where a sample is markedly hemolyzed (≥ 1 g/dL), standard procedure calls for dilution of the sample using a commercial diluent in order to reduce or eliminate the interferent. Here, we report a case where amylase activity was measured on a markedly hemolyzed surgical drainage sample (75 U/L). Due to high hemolysis, the sample was diluted 1:10 but unexpectedly had an activity of 50 U/L, which was ~7-fold higher activity over the undiluted sample after taking the dilution factor into account. We hypothesized that the commercial diluent contained amylase which contributed to this falsely elevated activity. **Method:** The surgical drain sample was centrifuged (1,100 x g for 3 min) to separate blood cells from the supernatant before analysis. Hemoglobin concentration was determined using the Sysmex XE-2100. Amylase activity was analyzed on the Vitros 5,1 FS and the diluent amylase activity was confirmed on a Beckman DxC. Both analyzers measure the volumetric activity of amylase based on a colorimetric assay, but use different substrates. **Results:** The surgical drain sample contained a hemoglobin concentration of 2.8 g/dL post centrifugation. Serial dilution of patient sample gave increasing amylase activity: 1:10 dilution, 500 U/L (10 x 50); 1:100 dilution, 5100 U/L (100 x 51). Analysis of the commercial diluent gave an amylase activity of 50 U/L (Vitros) and 55 U/L (Beckman). This was confirmed on a previously unopened vial of diluent. **Conclusion:** We conclude that the true amylase activity in our patient sample was <75 U/L and that the diluent contributed to the anomalous results. We recommend that if amylase is present in the diluent, then the amylase activity in the diluent should be analyzed along with patient samples. Results on the diluted sample need to be corrected for the amylase activity in the diluent. We also recommend that manufacturers of diluents that contain amylase should clearly state the concentration and activity on their package insert.

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Serum Iron assays during inflammation - What do they measure? An investigation triggered from the results of a proficiency testing scheme.

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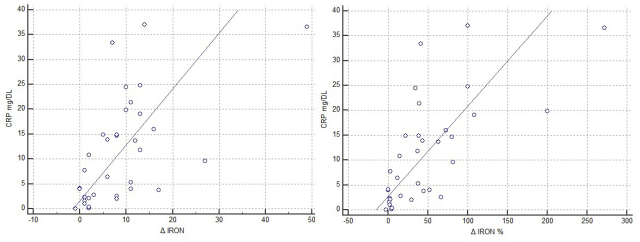
The role of Proficiency Testing schemes (ex. External Quality Control programs), with their interlaboratory comparisons, is of primary importance to the harmonization of laboratory results. Measurement of iron is considered an unreliable indicator of iron deficiency during inflammation and/or infection since low iron levels are a common finding, probably because iron is bound to the acute phase proteins (ferritin, NGAL etc.) and thus, cannot be reliably measured.

During last year's audit of the results from the Greek Proficiency Testing scheme (ESEAP), we observed that some iron measuring assays showed a persistent, but not constant, positive bias compared to the mean of the other methods. We tried to correlate the above bias with the value of CRP (as an inflammation marker) in the control samples, but the range of CRP in these samples was limited, inside the reference range, thus this study was inconclusive. To further investigate the influence of inflammation and/or infection on iron measurement, we measured iron levels in 34 patients with inflammation, infection and sepsis using two assays (Thermo-Scientific, Vantaa, Finland and Abbott Architect, Abbott Park Il as reference) and their CRP levels, using an immunoturbidimetric assay (Sentinel Milano, Italy). We observed differences between the two assays, increasing with the increase of the inflammatory status. We found a strong positive correlation between the levels of CRP and the absolute as well as the percent difference between the two methods (Spearman's rank correlation coefficient: r=0.619, p<0.0001 and r=0.702, p<0.0001 respectively - Figure 1). These differences indicate that the degree of inflammation has an impact of the levels of measured iron depending on the used assay.



As normally assays are specified to measure the total serum iron, we suggest that an effort from IFCC and other competent authorities has to prepare guidelines for the iron measuring assays in order to eliminate the interference from inflammation.

A-151

Level of cystatin C in patients with liver cirrhosis

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Introduction: Cirrhosis of the liver is often accompanied by functional renal failure particularly in advanced stages of liver disease. For the evaluation of renal failure, commonly used in clinical practice, creatinine, which depend on gender, age and race, and is therefore unreliable for accurate assessment of GFR, particularly in the initial renal function impairment. (CysC) has been proposed as a specific marker of glomerular filtration rate (GFR) and an early indicator of impaired renal function. **Objective** The aim of the study was to evaluate the level of CysC and its importance for the assessment of renal function, i.e. GFR in patients with liver cirrhosis. **Methods**: The study included 63 patients (aged 50.8±13.5 years), 47 males and 16 females with alcoholic 41 (65.1%) and viral cirrhosis 22 (34.9%) treated at the Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade. A healthy control group comprised of 30 age and gender-matched subjects. The study was conducted in accordance with Guidelines for Good Clinical Practice, the Declaration of Helsinki. The degree of liver insufficiency was assessed according to the Child-Pugh classification divided into three stages: A in 23 (36.5%), B in 21 (33.3%) and C in 19 (30.2%) patients. CysC serum concentration was determined by the PENIA method using commercial kits (Marburg, Germany), on a laser nephelometer (BN II SIEMENS). CysC referent value was 0.59-1.04 mg/L. Cr was determined according to the kinetic Jaffe's method, using commercial kits on analyzer Olympus AU 400 (*Hamburg, Germany*). Estimated GFR was calculated from serum Cr using the Modification of Diet in Renal Disease (MDRD) equation and from serum CysC using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation. Statistical analysis was performed using the *t* test, Mann-Whitney, ANOVA, Pearson's (*r*) or Spearman's and post-hoc multiple comparison procedures with the Statistical Package for Social Sciences version 15 (SPSS Inc., Chicago, IL, United States). **Results:** The average value of CysC measured in patients with liver cirrhosis was 1.09 ± 0.42 mg/L, significantly higher ($P = 0.036$) than in the control group (0.88 ± 0.12 mg/L). Increased values of CysC observed in 23 (40%) patients. Increased Cr values detected in 7 (11.1%) patients. Post-hoc comparisons showed statistically significant differences in values of CysC between Child-Pugh A and B ($P = 0.014$) and between A and C ($P = 0.007$) stages, while there was no difference between B and C stages ($P > 0.05$). The mean GFR estimated using CysC (GFR_{Cys}) and Cr (GFR_{Cr}) was 77.6 mL/min/1.73 m^2, respectively, 113.5 mL/min/1.73 m^2. GFR < 90 mL/min/1.73 m^2 was obtained in 40 (63.5%) patients using the GFR_{Cys} formula and in 13 (20.6%) patients using GFR_{Cr} formula GFR < 60 mL/min/1.73 m^2 observed in 16 (25.4%) patients using the GFR_{Cys} formula and in 7 (11.1%) patients using GFR_{Cr} formula. **Conclusion**: CysC significantly higher in patients with cirrhosis than in the control group. More patients with decreased glomerular filtration rate (GFR) were identified based on CysC GFR than on creatinine GFR. Serum cystatin C represent sensitive indicators of renal dysfunction in liver cirrhosis.

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Strong Negative Interference by Calcium Dobesilate in 8 Sarcosine Oxidase Assays of Serum Creatinine Involving the Trinder Reaction

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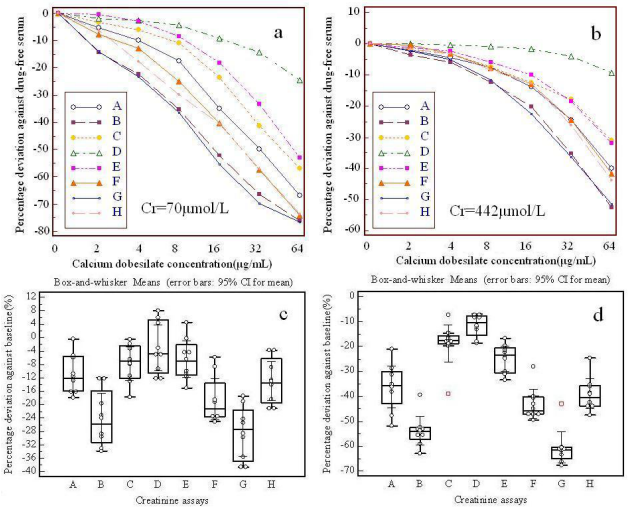
Background: Calcium dobesilate has been observed to interfere with creatinine (Cr) measurement using sarcosine oxidase assays. The aim of this study was to evaluate the interference in 8 sarcosine oxidase Cr assays (Roche, Beckman, Siemens, Ortho Clinical, Maker, Merit Choice, Leadman, Biosino) and to determine its clinical significance.

Methods: In the in vitro experiments, we measured Cr in pooled serum with final concentrations of calcium dobesilate additions (0, 2, 4, 8, 16, 32, and 64 μg/mL) using 8 enzymatic assays. Bias (%) was calculated relative to the drug-free specimen. In the in vivo experiments, 8 participants were recruited and baseline serum were collected, then calcium dobesilate was given 3 times per day (each dose, 500 mg orally) for 3 days. Blood samples were collected at 0 hour and 2 hours after another 500-mg dose administration on the fourth morning. The Cr concentration quantified using different assays at 0 hour and 2 hours were compared with the level at baseline. Cr levels of 10 specimens from those who have taken calcium dobesilate were measured by Roche, Beckman, Maker, Merit Choice assays and the LC-IDMS/MS method.

Results: The exogenous addition of calcium dobesilate negatively interfered with the Cr concentration in all 8 enzymatic Cr assays, which was highly dependent upon the calcium dobesilate concentration (Figure 1a,b). The observed Cr concentrations for 8 participants measured by enzymatic assays were inhibited by -28.5% to -3.1% at 0 hour (Figure 1c) and by -60.5% to -11.6% at 2 hours (Figure 1d) relative to the level at baseline. The Cr values of 10 patients measured by Roche, Beckman, Maker, Merit Choice assays showed an average deviation of -20.0%,-22.4%,-14.2%, and -29.6%, respectively, compared with the LC-IDMS/MS method.

Conclusion: Calcium dobesilate produced significantly negative interference with Cr in all Cr assays based on the sarcosine oxidase method. Significant differences in the degree of interference were observed among assays.



Note: Panels a and b showed the effects of the exogenous addition of calcium dobesilate on Cr quantification in 8 enzymatic assays when basal Cr concentrations was 70 and 442 μmol/L, respectively. Panels c and d showed the percentage deviation plots for the creatinine concentrations at 0 hour, 2 hours after drug administration from 8 participants against baseline by Box-and-whisker plot, respectively. Creatinine concentrations were measured by 8 enzymatic assays. A, Roche; B, Beckman; C, Siemens; D, Ortho Clinical; E, Maker; F, Merit Choice; G, Leadman; H, Biosino

A-153

ELF Panel range in a large healthy population and the influence of gender, age and BM.

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Relevance: Enhanced Liver Fibrosis (ELF) panel has its application as an indirect marker of liver fibrosis and comprises the serum concentration of hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinases-1(TIMP-1), and aminoterminal propeptide of type III procollagen (PIIINP). Its accuracy is well established in chronic liver diseases. There are few studies, so far, evaluating the performance of the test regarding intra- and inter-assay variation, stability of the samples and influence of host variables, such as age, gender and BMI.

Objective: to evaluate the laboratorial performance of ELF panel and its range of values in a large healthy population considering gender, age and BMI influence.

Material and Methods: Healthy blood donors were recruited. Exclusion criteria: age under 16 years or over 69 years, weight < 50kg and infection with HIV, hepatitis B or C. Blood samples were collected in the morning, clotted for 30 minutes at room temperature, centrifuged at 1600g for 15 min at 4^{0}C and sera was frozen at - 80^{0}C within 2 hours after collection. The analytes (PIIINP, HA, and TIMP-1) stability was verified in three fresh samples that were subsequently aliquoted and kept at -80^{0}C. These aliquots were submitted to 1 to 9 cycles of freezing/thawing and analytes were measured again. Two samples were kept at room temperature for 24 hours before ELF determination. Samples provided by the manufacturer were also examined. PIIINP, HA, and TIMP-1 were measured by magnetic separation enzyme immunoassay (ADVIA Centaur™, Siemens Healthcare Diagnostics, Tarrytown, NY, USA). ELF was calculated by the formula= 2.278 + 0.851 ln(HA) + 0.751 ln(PIIINP) + 0.394 ln(TIMP-1). IBM SPSS 18.0 was applied for due statistical analysis. All patients signed an informed consent.

Results: Among 2000 blood donors, in the period of one month, 949 were included. Mean ELF value was 8.65±0.76, ranging from 6.25 to 10.85. There was 53% of males and the mean age was 42.5±13.7 (range 16-68), with mean BMI 27±4kg/m^2. Mean ELF values were significantly different between female (8.53±0.75) and male groups (8.76±0.76), (p <0.001). In the whole group mean ELF values were significantly different throughout ages. (p <0.001) Among women ELF varied significantly throughout all ages (p <0.001) but for man only for individuals older than 51 years (p <0.001). Concerning BMI, only for individuals with BMI under 25 mean ELF values significantly varied (p <0.001). The stability study showed that the analytes remained unchanged after up to nine frozing/thawing cycles or if maintained at room temperature for 24h. The coefficients of variation (CV) of intra-assay varied from 3.39% to 4.38% for HA; from 2.86% to 3.43% for PIIIMP and from 1.53% to 2.34% for TIMP-1. The CV inter-assay varied from 3,61% to 5.162 for HA; from 3.34% to 5.35% for PIIIMP and from 2.38% to 3.25% for TIMP-1.

Conclusion: ELF panel is a test highly stable and reproducible, permitting the use of fresh or frozen samples. Gender, age and BMI may influence its results and should be properly taken into account when interpreting the method.

A-154

Status of Harmonization of Serum Albumin Measurement Procedures: Comparison of 21 BCG and BCP Methods to an Immunochemical Method

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Background:

Measurements of serum and plasma albumin are widely used in medicine, including their use as quality indicators for the care of patients in renal dialysis centers. We examined the current status of harmonization of measurement procedures for albumin.

Methods:

Pools (n=152) were prepared from non-frozen residual serum or plasma samples submitted for routine albumin testing at two academic medical centers during 3 weeks in the spring of 2014. Among these, 53 pools were predialysis serum samples from patients undergoing renal dialysis. Samples were distributed non-frozen on cold packs to manufacturers to perform quadruplicate albumin measurements by 21 measurement procedures: 9 used bromcresol green (BCG), 12 used bromcresol purple (BCP). The Roche Tina Quant immunochemical procedure recovered 101.4% of the IRMM ERM DA470k/IFCC reference material value and was used for comparison.

Results:

Table 1 shows that, for each type of sample (renal serum, nonrenal serum, and plasma), the mean of all results of the 9 BCG methods was higher than the corresponding mean of results from the 12 BCP methods. In general, BCG methods had a positive bias and BCP methods had a negative bias when compared to the Tina Quant immunochemical procedure. Biases varied among BCP methods and among BCG methods. For all but one method, biases were statistically significantly different (P<0.02) for serum vs. plasma samples and for renal dialysis vs. non-renal patient sera.

Conclusion:

BCG methods had higher values than did BCP methods, and both BCG and BCP methods were biased when compared with an immunochemical method. Moreover, bias of both BCG and BCP methods varied among manufacturers' methods and depending on sample types (serum vs. plasma) and patient types (dialysis patient vs. non dialysis patient). Results from albumin measurement procedures are not harmonized and fixed decision values for clinical decisions or for quality indicators are not appropriate.

Recovery of reference material and mean values for pooled samples measured by the indicated methods

Parameter	Immuno-chemical Method	12 BCP Methods	9 BCG Methods
Recovery of IRMM material (%), Grand mean (Min, Max Recovery within method group)	101.4	98.4 (95.5,102.3)	95.3 (80.5,100.7)
Mean Albumin Concentrations of Pools (g/dL)			
Renal Serum, Grand Mean (53 pools) (Min, Max Mean Concentrations)	3.42	3.29 (3.17, 3.40)	3.53 (3.34, 3.72)
Nonrenal serum, Grand Mean (50 pools) (Min, Max Mean Concentrations)	3.35	3.29 (3.19, 3.40)	3.53 (3.39, 3.76)
Nonrenal plasma, Grand mean (49 pools) (Min, Max Mean Concentrations)	3.05	3.11 (2.98, 3.21)	3.40 (3.21, 3.70)
Mean Bias vs Immunochemical Method (g/dL)			
Renal serum , Grand mean (Min, Max Mean Bias)	---	−0.13 (−0.26, −0.02)	0.10 (−0.08, 0.30)
Nonrenal serum, Grand mean (Min, Max Mean Bias)	---	-0.06 (−0.16, 0.05)	0.18 (0.04, 0.41)
Nonrenal plasma, Grand Mean (Min, Max Mean Bias)	---	0.06 (−0.08, 0.16)	0.35 (0.16, 0.64)

A-155

Evaluation of Sweat Collection Material for Sweat Chloride Testing

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Background: The Cystic Fibrosis Foundation guidelines on diagnostic sweat testing for cystic fibrosis specify that gauze or filter paper used for sample collection should have an area of 4 square inches. The aim of this study was to investigate various materials that could be used for sweat collection. **Methods:** Grades 1, 2, and 3MM filter paper squares (2 inch X 2 inch), as well as 4-ply, 2-ply, and 1-ply gauze squares (2 inch X 2 inch), were evaluated. Five chloride solutions with concentrations covering the analytical measurement range (10-160 mM) were added to vials containing the collection materials and were measured by the Schales and Schales chloride titration method. **Results:** Gauze squares were easier to manipulate with forceps and place into glass collection vials than filter paper squares. While 4-ply and 2-ply gauzes absorbed all of the titration volume making it hard to titrate, 1-ply gauze did not. All gauze tested contained chloride contamination, which was resolved by performing a blank on each lot of gauze. Chloride was linear from 9.7 to 161.0 mM, with recoveries of 97-103%. Simple Precision, performed at low, mid, and high levels, showed a %CV of <2.5%. **Conclusion:** 1-ply Gauze was determined to be the best material for our titration method, but was found to be contaminated with chloride that needed to be subtracted from the measured chloride concentrations. 1-ply gauze has been validated for patient testing.

Table 1. Recovery of chloride spiked on gauze.

Spiked Cl Conc. (mM)	Mean Yield without a Gauze Blank (mM)	Mean Yield with a Gauze Blank (mM)	Analytical Recovery without a Gauze Blank (%)	Analytical Recovery with a Gauze Blank (%)
160	148.0	161.0	93	101
80	80.7	82.0	101	103
40	40.7	40.0	102	100
20	25.0	20.0	125	100
10	15.0	9.7	150	97

A-156

Establishment of Analyte- and Concentration-Specific Hemolysis Index Thresholds for Aspartate Aminotransferase (AST) and Direct Bilirubin (DBIL) Reduces Specimen Rejection and Recollection Rates

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Background: Interference due to hemolysis is a common reason for specimen rejection in clinical laboratories. Assay manufacturers provide instrument- and test-specific hemolysis index (HI) limits for interference in their instructions for use, however laboratories may also perform interference studies to verify or establish hemolysis tolerance limits. Automated hemolysis detection using serum indices and rule-based algorithms based on analyte concentration and HI may increase laboratory efficiency and reduce specimen rejection rates. **Objectives**: The aims of the study were to (i) determine concentration-specific HI thresholds for aspartate aminotransferase (AST) and direct bilirubin (DBIL) on the Cobas® 6000/8000 chemistry platforms (Roche Diagnostics) and (ii) compare rule-based concentration-dependent algorithms to manufacturer recommended HI limits and measure the impact on specimen rejection and recollection rates. **Methods:** Residual specimens from physician-ordered AST or DBIL (serum) and hematology (EDTA whole blood) testing were used to prepare serum and hemolysate pools, respectively. Twelve serum pools with AST concentrations between 27-770 U/L and 19 serum pools with DBIL concentrations between 0.1-9.7 mg/dL were created. Hemolysate was prepared from washed red cells and lysed by freeze-thaw (-70°C). Varying hemoglobin concentrations were obtained by diluting hemolysate with water. Hemolysate (10% by volume) was then added to serum pools with known analyte concentrations to obtain HI ranging from 6 to 526. AST, DBIL and HI were measured using the Cobas 6000/8000 c501/c701 chemistry analyzers. The absolute and percent bias due to increasing HI was calculated. Recovery within ±5 U/L or ±10% of initial AST value and recovery within ±0.1 mg/dL (initial DBIL ≤0.3mg/dL) and ±0.2 mg/dL or 20% (initial DBIL >0.3 mg/dL) for DBIL were considered acceptable. Specimen rejection and recollection rates were calculated by applying manufacturer HI

limits and the established concentration-specific HI limits to results from physician-ordered AST and DBIL tests performed in the Central Clinical Laboratory at Mayo Clinic, Rochester, MN (January-February 2015). **Results:** Concentration-specific HI thresholds were established for AST <100 U/L, 100-200 U/L, 200-300 U/L and >300 U/L at HIs of 50, 100, 200 and 500, respectively. There were 10,605 orders for AST and 862 (8.1%) specimens would have been rejected and recollected based on manufacturer's recommendations (HI >20 for all AST concentrations). By applying concentration-specific hemolysis limits for AST, 154 (1.5%) specimens were rejected due to hemolysis, thus eliminating recollection of 708 specimens (82% reduction). For DBIL, HI tolerance limits of 70 and 50 were established for 0.0-0.3 mg/dL and >0.3 mg/dL, respectively. Out of 7062 orders for DBIL, 494 (7.0%) specimens would have been rejected based on manufacturer's recommendations (HI >30 for all DBIL concentrations). By applying the concentration-specific hemolysis thresholds for DBIL, 188 (2.7%) specimens were rejected due to hemolysis, thus eliminating recollection of 306 specimens (62% reduction). **Conclusions:** Analyte- and concentration-specific hemolysis index thresholds were established for AST and DBIL. Automated hemolysis detection using HI and rule-based algorithms based on analyte concentration significantly reduces specimen rejection and recollection rates due to hemolysis. Additional downstream benefits include improved turn around time and cost savings associated with not having to recollect patient specimens.

A-157

Protein Interference with Common Laboratory Tests

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Objective: Protein as an interferent is under appreciated. Therefore we evaluated interference by proteins on clinical laboratory tests. Methods: Base Pool was prepared using a diluted serum pool with low total protein. A High Pool was prepared by spiking proteins into the base pool (Concentrated human albumin and gamma-globulins 1:1; ASSURANCE™ Interference Test Kit, Sun Diagnostics, New Gloucester, ME). The base pool and high pool were intermixed to create 5 levels of total protein (3.5, 6.9, 9.4, 12.4, and 15.3 g/dL). Multiple analytes were measured on the Beckman AU5800. Results: Minimal effects were seen with ALP, AMY, DBIL, TBIL, CK, GGT, GLU, LDH, LIP, PHOS, BUN, UA, Lp(a), sdLDL-C, hsCRP, ferritin, LDL-C, HDL-C, K, CL, TRIG, and CHOL. Positive bias with increasing protein concentration was seen with ALT, AST, CA, CRE, FE, MG, homocysteine (HCY), fructosamine, and non-esterified fatty acids (NEFA). Because proteins bind analytes such as calcium, magnesium, iron, homocysteine, and NEFAs, we believe these increases were artifactual. Negative bias was seen with CO2, apo AI, and apo B. Conclusion: Manufacturers and laboratorians need to pay more attention to proteins as a potential interferent. The mechanism may be photometric or a volume depletion effect, whereby a very high protein concentration will reduce the available water so that the analyte concentration is artifactually low.

Analytes Affected by Protein						
Protein, g/dL	ALT, U/L	AST, U/L	CO2, mmol/L	CA, mg/dL	CRE, mg/dL	FE, µg/dL
3.5	8.3	8.4	8.5	4.7	0.44	35.8
6.9	9.2 (11.6%)	9.7 (15.1%)	8.0 (-5.8%)	6.0 (25.5%)	0.60 (35.4%)	55.3 (54.2%)
9.4	9.1 (10.1%)	10.1 (19.2%)	6.6 (-22.9%)	6.5 (36.3%)	0.67 (51.1%)	69.0 (92.5%)
12.4	9.8 (18.8%)	11.0 (30.5%)	5.7 (-32.7%)	7.4 (56.5%)	0.80 (79.7%)	87.5 (144%)
15.3	10.8 (30.4%)	11.7 (39.2%)	5.9 (-30.4%)	8.3 (75.5%)	0.93 (110%)	105.2 (194%)
Protein, g/dL	Apo AI, mg/dL	Apo B, mg/dL	MG, mg/dL	HCY, µmol/L	NEFA, mg/dL	NA, mmol/L
3.5	77.8	38.1	1.0	5.9	0.44	151
6.9	80.9 (3.9%)	38.4 (0.8%)	1.3 (23.3%)	10.9 (84.4%)	1.73 (293%)	148 (-1.8%)
9.4	68.6 (-11.9%)	27.6 (-27.5%)	1.4 (35.0%)	15.1 (156%)	2.89 (557%)	147 (-2.3%)
12.4	63.2 (-18.9%)	18.2 (-52.3%)	1.6 (53.4%)	19.8 (236%)	4.12 (836%)	146 (-3.2%)
15.3	55.4 (-28.8%)	11.1 (-70.8%)	1.8 (73.8%)	24.5 (316%)	5.29 (1102%)	146 (-3.4%)

A-158

Effects of Urine Specimen Containers with Metal Caps on Urine Test Results

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Background: Specimen collection containers must be carefully evaluated to ensure that the analytes being tested are not affected by the materials present in the containers. Due to the better sturdiness our institution intended to switch from plastic urine collection containers with plastic screw-caps to plastic containers with metal screw-caps. The aim of this study was to determine if this switch would cause any adverse impact on our current tests. **Methods:** Urine from each of 5 leftover plastic collection jugs was aliquotted to one plastic container with metal cap and another with plastic cap, for a total of ten aliquots. The urine samples were spiked at levels spanning the analytical measurement ranges if the original concentrations were low. The cups with metal caps were placed refrigerated upside-down for a minimum of 18 hours to allow for urine contact with the caps, while the corresponding cups with plastic caps were stored at the upright position side by side with those with metal caps. These samples were analyzed for citrate, oxalate, metanephrine, normetanephrine, 5-hydroxyindole-3-acetic acid, vanillylmandelic acid, copper, magnesium, zinc, porphyrins, porphobilinogen, morphine, codeine, 6-acetyl-morphine, hydromorphone, hydrocodone, dihydrocodeine, oxycodone, oxymorphone, O-desmethyl tramadol, tramadol, fentanyl, norfentanyl, buprenorphine, norbuprenorphine, methadone, EDDP, amphetamine, methamphetamine, benzoylecgonine, 11-nor-9-carboxy-delta-9-THC. **Results:** No significant difference (<20%) was observed between urine refrigerated in all-plastic containers and those in plastic containers with metal caps for all analytes tested except for 11-nor-9-carboxy-delta-9-THC (THC) which yielded significantly lower results for the samples in the containers with metal screw-caps (Table 1). **Conclusions:** The plastic containers with metal screw-caps offered similar results as the ones with plastic screw-caps for all analytes tested except for THC, which warrants further investigation.

Table 1. Difference in THC results.

Samples Refrigerated in Plastic Tubes (ng/mL)	Samples Refrigerated in Plastic Containers with Metal Screw Caps (ng/mL)	Difference (%)
1479	1207	-18
728	511	-30
312	151	-52
65	51	-22
24	12	-50

A-159

Impact of light exposure on serum vitamin B12 measurements

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Background: Environmental conditions such as light exposure can directly affect sample results for some analytes. However, there are discordant information about light-sensitive analytes and sample handling available in literature. Laboratory specimens submitted for assessment of vitamin B12 have traditionally been light-protected, although this practice can cause extra costs for the laboratory. This study evaluates if light exposure can have an impact on vitamin B12 measurements.

Methods: 75 individuals serum samples were collected into 2 tubes, one transparent tube (group 1) and one amber tube (group 2). Vitamin B12 measurements were performed on ADVIA Centaur XP (Siemens Healthcare Diagnostics) after sample collection (T0) and also, after 24 hours from the initial time (T24). The percent changes in Vitamin B12 concentration and the agreement between measurements on light-protected tubes to those exposed to light were evaluated.

Results: Through linear regression, a good agreement was observed (R2>0,95) between vitamin B12 measurements from amber and transparent plastic tubes on both T0 and T24. Moreover, the analysis of bias reveals no significant differences (maximum accepted bias was 17,62). The results are within allowable total error (33%). The data are summarized in the table below.

Conclusion: This study suggests that there is no need to protect samples from light prior to vitamin B12 assessment. Furthermore, the analyte remains stable under light exposure for at least 24 hours. No extra amber tubes can represent a cost benefit for the laboratory and optimize the workflow.

Table 1. Comparison between vitamin B12 levels on group 1 and group 2 on T0 and T24.

COMPARISON	R2 (LINEAR REGRESSION)	BIAS	TOTAL ERROR
T0 vs T24	0,9649	10	22
Light protected tube vs Light exposed tube on T0	0,9672	7,85	20
Light protected tube vs Light exposed tube on T24	0,95	6,74	19,11

A-160

Long-Term Stability of Novel Urinary Acute Kidney Injury Biomarkers

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Background

Acute kidney injury (AKI) is an abrupt loss of kidney function induced by multi-organ failure, sepsis, and nephrotoxicity caused by drugs in development. Several novel urinary biomarkers outperform creatinine as early and more sensitive indicators of AKI; however, their long-term stability (LTS) is unknown and is extremely crucial for consistent reliable results when evaluating drug treatment effects in clinical trials.

Objective

Standardizing LTS criteria across assays is complicated by factors including assay precision, robustness, matrix variation between subjects and protein post-modifications. This study compared two LTS methods and established stability for the following biomarkers: Cystatin C (Cys-C), KIM-1, NGAL, clusterin, and n-acetyl-beta-D-glucosaminidase (NAG).

Methods

Three pools, each containing urine from three individuals, was collected and aliquots were left at ambient temperature for ≤2h (baseline), then stored at -70°C until assayed at 1, 3, 15, 18 and 28 months. The between-run precision (BRP) of each biomarker assay was determined from QCs used over the 28-month LTS study. Stability was estimated using two methods; 1) Standard Method rendered a sample unstable if it exceeded the acceptance criteria (2*CV% of BRP from baseline) for two consecutive time points, and 2) Regression Method generated a linear regression through each time point with the y-intercept set as baseline; instability occurred when the rate of degradation (slope) intersected the acceptance criteria (1.5*CV% of BRP from baseline).

Results

The BRP for the urinary assays measuring Cys-C, KIM-1, NGAL, clusterin and NAG ranged from 6-25%CV. The assigned acceptance criteria ranged from 15-50% for the Standard Method and 10-30% for the Regression Method. The stability for Cys-C and KIM-1 in three separate pooled samples was variable when estimated by the Standard Method, ranging from 3-28 months and 1-28 months, respectively. The Regression Method narrowed the estimated range of Cys-C stability down to 12-28 months and 12-21 months for KIM-1. Clusterin's stability ranged from 1-3 months (Standard Method) and 8-11 months (Regression Method). Clusterin stability rapidly decreased within the first month (up to -28%), resulting in an overestimation of stability by the Regression Method due to a shift in baseline (y-intercept). NAG and NGAL were stable for all three samples during the entire 28 months regardless of the estimation method (up to 15% and 7% difference from baseline).

Conclusion

The Regression Method more precisely estimated stability when large gaps between time points were observed, such as with Cys-C and KIM-1. In addition, this method applied the assay imprecision to the initial baseline time point (y-intercept). The Standard Method may be more appropriate when early time points are lacking or if a linear rate of degradation cannot be established, such as with clusterin. Applying both methods provides a more precise estimation of stability. In this study, NGAL and NAG were the most stable AKI biomarkers, Cys-C and KIM-1 displayed mid-range stability and clusterin was the least stable biomarker.

A-161

Achieving Reduction of Lot-to-Lot Variation of the κ- and λ-Freelite Assay (Binding Site) through Standardization

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Background: Maintaining consistent results over multiple batches of immunoassay reagents can be a challenge for the clinical laboratory but is important for providing the best patient care. The complexity of generating antibodies and manufacturing kits for the Freelite assays can introduce more variation than observed with traditional chemistry assays due to the highly variable nature of monoclonal Free Light Chains (FLC). In a multi-site reference laboratory, it is critical to evaluate each new lot of a reagent to determine if the reagent stays within the total allowable error (TE_a) and to maintain standardization across testing locations. Lot-to-lot validation entails measuring QC material and random patient serum samples before and after each Freelite lot change. The use of patient samples, however, can introduce bias if they contain monoclonal proteins that do not dilute linearly or if they contain free kappa or lambda concentrations that fall outside of the standard measurement range of the assay. The goal of this project was to develop a standardized lot evaluation scheme that minimized the impact of instrument imprecision and sample selection bias for a multi-site reference laboratory.

Methods & Results: Quest Diagnostics partnered with The Binding Site (TBS) to achieve standardizations, including instrumentation and patient sample panels to limit introduction of imprecision and bias. In addition, 2 Quest Diagnostics laboratories (Chantilly, VA and Lenexa, KS) were used as pre-validation sites before the implementation of Freelite kits across all subsidiaries. Qualifications of approval for the new FLC reagent (percent bias determined by fraction of TEa) were also agreed upon by Quest Diagnostics and TBS. Patient panels of serum samples containing FLC concentrations spanning the analytical measuring range (κ 1/10: 2.7-127 mg/L, λ 1/8: 5.2-139 mg/L, Integra 800) were exchanged between TBS and Quest Diagnostics to standardize testing at all locations. Lot-to-lot analysis by TBS was conducted using Passing and Bablock regression analysis and Spearman's Rank Correlation Coefficient; Quest Diagnostics then utilized the bias with acceptability criteria based on a ¼TE_a, or 7.5% or 4 mg/L.

Prior to this work, differences between TBS and Quest Diagnostics evaluations could be as high as a 30% bias. Standardization of instrumentation to minimize instrument differences when evaluating new reagent lots and the exchange of patient panels reduced the differences between TBS and Quest Diagnostics kit evaluations to a maximum observed 11% bias; on average, the bias has only been ~6%. In addition, use of this protocol has allowed problems to be identified prior to widespread implementation across laboratories. Finally, standard patient panels are in development to be used for monitoring long-term bias across multiple lots. The use of these panels will prevent sampling bias from introducing error into the evaluation process.

Conclusions: The partnership between Quest Diagnostics and TBS helped reduce error by standardizing equipment and exchanging patient samples for lot evaluations. Reducing error can help streamline the evaluation of a new reagent product, improve efficiencies of testing of new reagent lots, and allow for clear observation of true analytical and clinical variation introduced by use of new Freelite lots.

A-162

The effects of interfering substances on Free Plasma Hemoglobin values measured using Hemocue Plasma/ Low Hemoglobin spectrophotometer

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Background:

Red blood cell hemolysis can be the result of a mechanical destruction with a ventricular device or extracorporeal membrane oxygenation (ECMO) system. The resulting free plasma hemoglobin is monitored using Hemocue™ Plasma/Low hemoglobin spectrophotometer. According to the manufacturer listed limitations for analysis of free plasma hemoglobin, the presence of lipids and bilirubin up to 30mg/dl may interfere with measurement of the free plasma hemoglobin and give erroneous results. Objective:The purpose of this study is to evaluate to what extent the assay is affected by the presence of bilirubin and lipids, and how each specimen received should be handled by the technologist prior to testing.

Methods:

Bilirubin interference was determined using 6 patient samples with known plasma hemoglobin results spiked with conjugated bilirubin. Samples were created to contain 0mg/dL, 10mg/dL, 20mg/dL and 30mg/dL additional conjugated bilirubin. Similarly another 6 samples were spiked for the unconjugated bilirubin study. Total and direct bilirubins were confirmed using a Roche Cobas analyzer. Each sample was measured again for free plasma hemoglobin concentration. Lipemia interference was determined using 12 patient samples. 6 samples with known values of lipemia, hemolysis and plasma hemoglobin were recorded at baseline. These samples were then centrifuged using the Vivaspin™ method at 1000k Daltons cut-off and results recorded post centrifugation. The next 6 samples were tested for plasma hemoglobin at baseline notating the levels of lipemia, hemolysis and triglyceride prior to spiking a small amount of harvested lipids (10,000mg/dL triglycerides and 1000mg/dL cholesterol) to yield 100, 250, 500, and 750mg/dL triglycerides respectively. Each sample was tested again for free plasma hemoglobin.

Results:

There was no significant interference associated with direct bilirubin up to 30mg/dl. However, there is a positive interference when indirect bilirubin is greater than 20 mg/dl. The interference led to falsely elevated results. Samples containing harvested lipids showed a constant upward trend of falsely elevated results at concentrations of triglycerides as low as 100mg/dL. Results after processing with the Vivaspin demonstrated significant decrease in measured plasma hemoglobin. However, we are not able to validate that all free plasma hemoglobin is recovered in the filtrate from lipemic sample post centrifugation.

Conclusion:

Samples tested for free plasma hemoglobin using the Hemocue Plasma low device should be checked for lipemia and icterus prior to analysis. Given the interference seen with triglycerides occurs at levels that may not be visually determined; the use of a spectrophotometric lipemia indice may be required. Result comments should include the information regarding icteric and lipemic interferences.

A-163

Sigma-metrics to Assess Analytical Quality of Certified HbA1c Methods

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Background:

The National Glycohemoglobin Standardization Program (NGSP) was launched in 1996 with the aim of harmonizing HbA1c testing to get more accurate and precise results for the care of diabetic patients. Most of the methods used by the clinical laboratories are NGSP certified and many of the laboratories that perform the test participate in the accuracy based GH2 PT program administrated by the CAP.

Methods:

This study was undertaken to calculate the Sigma-metrics for 26 manufacturers' HbA1c methods using imprecision and bias estimates derived from CAP surveys GH-2b 2013, GH-2a 2014, and GH-2b 2014. This approach was recently described by S. Westgard (http://www.westgard.com/six-hba1c-methods.htm). We used a total allowable error (TEa) of 6%, the quality requirement specified for HbA1c methods by the NGSP/CAP, and calculated Sigma-metrics as: Sigma = (TEa-bias)/CV (all expressed as %'s).

Results:

Table 1 shows the Sigma-metrics for a sample of the method groups that reported HbA1c results for the 3 samples from survey set GH-2b. The metrics for sample GH2-04, which had an expected value of 6.58 %, and is close to the clinical decision threshold for the diagnosis of diabetes, ranged from 0.74-3.27. The within-method average Sigma-metrics across the 3 survey specimens ranged from 0.10-9.91. Only 5.2 % of the 228 Sigma-metrics calculated for the 9 PT challenges over the 3 survey sets were > 3.

Conclusion:

Based on the metrics calculated from CAP survey data, the average defect rates for most of the HbA1c methods that are currently being used for the diagnosis and monitoring of diabetes were > 5 %, and exceeded the range of defect rates (1 to 5%) (Blumenthal D. Clin Chem 1997;43:1305) that are generally considered to be acceptable in healthcare.

Sigma-metrics for selected methods: CAP survey GH-2b 2014					
Method Group	N	GH2-04 [6.58 (6.51-6.66)]	GH2-05 [8.39 (8.32-8.47)]	GH2-06 [5.65 (5.58-5.72)]	Mean
Roche Cobas c500 series	350	1.87	2.14	1.59	1.87
Tosoh G8 Auto HPLC	330	2.10	1.66	2.08	1.95
Siemens DCA Vantage	307	1.37	0.98	0.73	1.02
Siemens Dimension Vista	269	0.85	2.35	1.59	1.60
Bio-Rad D-10	217	2.03	2.40	1.96	2.13
(Ortho Clin Diag) Vitros 5,1 FS, 4600, 5600	209	1.52	2.24	1.44	1.74
Beckman UniCel DxC Synchron	172	2.17	1.72	2.03	1.97
Bio-Rad Variant II Turbo	127	1.91	1.91	1.97	1.93
Bio-Rad Variant II Turbo 2.0	124	1.96	1.85	1.94	1.91
Beckman AU systems	67	0.74	0.78	0.88	0.80
Siemens Dimension RxL	67	1.06	2.16	1.10	1.44
Siemens Dimension Xpand	59	1.11	1.66	0.95	1.24
Abbott Architect c System	47	0.88	0.60	1.25	0.91
Siemens Advia Chemistry Systems	32	1.18	1.26	0.55	0.99

A-164

Evaluation of Technopath Controls on the ARCHITECT Family of Instruments

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Introduction: Quality controls are an important part of laboratory testing to ensure that released results meet the required quality in regards to accuracy and precision of patient results. Consolidation of controls is a current trend in laboratories to simplify QC testing. Multi-constituent control panels (MCCs) offered by Technopath Manufacturing Ltd. cover a wide range of clinical chemistry and immunoassay analytes for both serum and urine.

Objective: The goal of this study was to evaluate the performance of the Multichem S Plus, Multichem IA Plus and Multichem U control panels on the ARCHITECT family of instruments. Precision and accuracy compared to the target value were evaluated.

Methods: The three control panels were evaluated for a minimum of thirty days. Testing was performed on two ARCHITECT *c*8000 and three ARCHITECT i2000$_{SR}$ instruments. Data presented here are from the following serum clinical chemistry analytes: ALT, AST, total bilirubin, chloride, total cholesterol, creatinine (picrate), glucose, potassium, total protein, sodium, triglycerides and urea; the following immunoassay analytes: CEA, total PSA, free T3, free T4, TSH, troponin-I, total beta HCG, estradiol, ferritin, FSH, vitamin B12 and vitamin D; and the following clinical chemistry urine analytes: chloride, creatinine (picrate), glucose, potassium, sodium and urea. The Multichem S Plus and IA Plus panels are serum based with three control levels; the Multichem U panel is prepared from human urine with two control levels. All data were collected via

AbbottLink, allowing for automated data retrieval. Means, standard deviations and ranges were calculated for all controls. Sigma Metrics were also calculated for each analyte.

Results: The %CV for the 12 clinical chemistry analytes with the Multichem S Plus control ranged from 0.46 to 5.33%. The %CV for the 6 clinical chemistry urine analytes with the Multichem U control ranged from 0.51

to 3.2%. For both control panels, the majority of the CVs were less than 2%. The %CV for the 12 immunoassay analytes with the Multichem IA Plus control ranged from 1.34 to 18.87% (TnI, Level 1); however the majority of the CVs were less than 5%. Overall, little variation was seen from instrument to instrument.

Conclusions: The Technopath S Plus, IA Plus and U controls

performed well and demonstrated similar performance to the routine internal laboratory quality controls. The use of these MCCs reduced the number of controls required for the analytical quality control testing of both clinical chemistry and immunoassay analytes with no compromise to quality.

A-165

Glycosylated hemoglobin level in glucose-6-phosphate dehydrogenase-deficient patients in southern China

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Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common type of enzyme deficiency disease which showed a high prevalence rate in the word, especially in southern China. However, the activity of G6PD could impact glycosylated hemoglobin (HbA1c) level, which is widely used in diabetes diagnosis, through influencing the service life of the red blood cells. Whether HbA1c level could be impacted by G6PD deficiency remains unknown and needs to be confirmed.

Methods: In this study, commercially available reagent sets were used to measure HbA1c concentration (certificated by NGSP) and G6PD activity in 114 G6PD deficient patients (94 simple G6PD deficient patients, 20 cases complicated with diabetes) and 359 controls (189 diabetes patients without G6PD deficiency, 170 healthy adults).

Results: The HbA1c concentration was significantly lower in G6PD deficient patients than those without G6PD deficiency (P<0.05). However, the HbA1c level was significantly higher in G6PD deficiency complicated with diabetes than simple G6PD deficiency(P<0.01). HbA1c concentration showed positive correlation with G6PD activity in simple G6PD-deficient patients (r=0.215, P<0.01), but negative correlation in diabetes without G6PD deficiency (r=-0.191,P<0.05).

Conclusion: The study indicated that G6PD deficiency could downregulate the concentration of HbA1c in patients with or without diabetes, and the results of HbA1c in G6PD deficiency patients require special attention.

A-166

Evaluating the Effects of Preanalytical Factors on Common Biochemistry Parameters: Duration Before/After Centrifugation, Recentrifugation, and Storage Conditions

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Preanalytical factors should be considered for the samples conveyed from peripheral to central laboratories. Our aim was to evaluate the effects of duration before/after centrifugation, recentrifugation, storage conditions on the stability of 19 serum parameters.

Blood samples (n=10) were collected from healthy volunteers into vacutainer tubes with gel separator (Becton Dickinson, USA) which were allowed to clot for 30 min and allocated to 6 groups. First and second groups were centrifuged at 1300xg for 10 min immediately and stored at room temperature (RT) or 4°C until analyzed at 0, 2, 4, and 6 hrs. Third and fourth groups were stored at RT or 4°C for 2, 4, 6 hrs, then centrifuged and analyzed. Fifth and sixth groups were centrifuged after clotting, stored at RT or 4°C and recentrifuged at 2, 4, and 6 hrs before analysis. We measured below parameters (AU 680, Beckman Coulter, USA), calculated percentage relative bias from the baseline [(concentration of any hour-concentration of baseline)/ concentration of baseline]x100 and compared with desirable bias (Westgard QC database) to determine clinically significant variations.

ALP, phosphorus, total bilirubin, and uric acid levels were not affected. The desirable bias for direct billirubin was 12.2% and only 4th hour of fourth group exceeded this limit. Waiting at RT before and after centrifugation increased LDH concentrations when waiting time extended up to 6 hrs. Results for other parameters are given in Table 1.

Duration between centrifugation and analysis, storage before centrifugation, storage conditions, and recentrifugation may cause serious biases in analytes which should be taken into consideration for central laboratories receiving samples from peripheral units.

Table 1. Relative bias values for groups that exceeded desirable bias (DB)

Test **DB** (%)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
AST **6.54**			2h -8.70	4h 10.00	4h 13.33	4h 10.00
ALT **11.48**				4h 13.33	4h 13.33	4h 12.50
GGT **11.06**		6h 11.11	2h 16.67 6h 14.86	2,4,6h >11.1	2,4,6h >13.3	2,4,6h >11.6
Glucose **2.34**	6h -3.11	6h -2.97	2,4,6h <-9.52	2,4,6h <-5.71	4,6h <-5.38	4,6h <-3.23
BUN **5.6**		6h -6.67		4h -6.67	4h -6.67	
Creatinine **3.96**		4h -6.67	4h -6.67	4h -6.67	4h -6.67	4h -6.67
T. prot. **1.36**	2h -2.21 4.h -3.64	2h -1.59 6h 1.99	4h 2.75	2h -2.31 6h 3.15	2h -2.70 6h 2.01	2h -3.40 4,6h >1.69
Albumin **1.43**	4h -1.53 6h -3.31	4h -1.43 6h -1.46	4h 2.22	4h -2.45 6h -1.80	6h -3.23	6h -4.62
Na+ **0.23**	2,4,6h <-0.72	4h -0.72 6h -0.73	2h -0.72 4h -0.72	2,4,6h <-0.35	2h -0.72 4h -0.72	4h -0.73 6h -0.35
K+ **1.81**	4h -2.35 6h -2.38	2h 2.27	2h -2.44 4,6h >2.33	2,4,6h >7.14	6h 2.38	2,4,6h >2.38
Cl- **0.50**	2,4,6h <-1.01	4h -1.01 6h -1.99	4h -1.01 6h -1.98	2,4,6h <-0.98	4h -1.01 6h -1.01	4h -0.73 6h -0.35
Ca2+ **0.82**	2h 1.08	2h 0.98 6h 1.98	4h -0.93 6h -1.02	4h -1.05	2h 1.01	2h 1.01 4h -1.05
Mg2+ **1.80**	6h -3.04	2h 1.87 6h -1.84	2,4h >3.68 6h -1.99	6h -2.94	6h -3.67	6h -5.19

A-167

Stability of Extracted Samples for LC-MS/MS Analysis of Free Plasma Metanephrines

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Background: Plasma free metanephrines is the recommended first line laboratory testing for the diagnosis and follow-up of pheochromocytoma. The low physiological concentrations and potential interference make the quantification of these analytes challenging. Due to the labor intensive SPE extraction stability of the processed samples was investigated to determine how long the processed samples can be stored in case of instrument breakdown. **Method:** Stability was studied by running 3 levels (low, mid, and high), in triplicate, of spiked EDTA plasma using a previously validated LC-MS/MS method. All samples were extracted at time 0 in the final matrix of 1 mM ammonium formate + 0.1% formic acid. Set 1 was analyzed immediately after extraction and the remaining sets were left at 4 °C protected from light to mimic the conditions in a typical HPLC autosampler. Sets 2 and 3 were removed and analyzed immediately at 72 hours and 168 hours, respectively. **Results:** Processed plasma samples in 1 mM ammonium formate + 0.1% formic acid stored at 4 °C for 72 and 168 hours showed recoveries ranging from 85% to 113% compared to the baseline results for both free normetanephrine and metanephrine (Table 1). **Conclusion:** Processed plasma samples in 1 mM ammonium formate + 0.1% formic acid for plasma free metanephrine and normetanephrine are stable for up 168 hours when stored at 4 °C protected from light.

Table 1: Stability Data

Normetanephrine	Temperature	Timepoint (Hours)	n	Mean	%CV	%Difference
Low						
		0	3	310	3%	NA
	4°C	72	3	318	2%	3%
		168	3	345	4%	8%
Mid						
		0	3	1520	6%	NA
	4°C	72	3	1417	2%	-7%
		168	3	1507	4%	6%
High						
		0	3	3933	6%	NA
	4°C	72	3	3343	1%	-15%
		168	3	3793	2%	13%
Metanephrine	Temperature	Timepoint (Hours)	n	Mean	%CV	%Difference
Low						
		0	3	67	7%	NA
	4°C	72	3	64	3%	-4%
		168	3	66	2%	3%
Mid						
		0	3	576	5%	NA
	4°C	72	3	502	3%	-13%
		168	3	507	1%	1%
High						
		0	3	1038	10%	NA
	4°C	72	3	930	3%	-10%
		168	3	904	2%	-3%

A-168

Improved testing options through evaluation of specimen temperature stabilities.

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Objective: The specimen stability study was performed to determine how storage temperatures and length of storage affected result integrity.

Background: Specimen stability over time and varied storage temperatures is essential for a laboratory to understand in order to make process improvements for patient care, client service and laboratory operations. It allows the laboratory to improve patient care by offering the clinician an extended period to order add-on testing following the preliminary diagnosis without having to recollect from the patient. This will improve patient care delivery by accelerating time to result and improve the experience by limiting recollections. In addition, understanding specimen stability limits allows modification of shipping conditions, storage conditions and sample processing workflows. In order to fully understand potential areas of variability in qualitative changes of specimens over time, rigorous analysis is important to understand the potential areas of variability both within patient and between patients to determine acceptability of the new stability times and temperatures.

Materials and Methods: 10-15 specimens were selected based on assay methodologies, order volume and the frequency of Add-on testing. 39 analytes (albumin, alkaline phosphatase, ALT, AST, Bilirubin(conjugated and unconjugated), cholesterol, LDL, Ferritin, HDL, CRP, hsCRP, LD, phosphorous, PSA, TSH, sodium, potassium, chloride, calcium, parathyroid hormone, Lipoprotien (a), N-telopeptides type 1, hemoglobin A1C , total IgA, Gliadin antibodies (IgG, IgA), Endomysial IgA , Tissue transglutaminase antibodies (1gG, IgA), cardiolipin immunoglobulins (IgM, IgG, IgA) beta-2 glycoprotein immunoglobulins (IgM, IgG, IgA)) requiring frozen transport and specimens with less than one week refrigerated stability were selected for analysis. Residual patient samples were identified over low, normal and high result ranges for each analyte tested. Measurements were taken on sequential days for up to 14 days and results compared to the day 0 measurements. Stability acceptance criteria was based on the reading being within the accepted level of variance as published by the manufacturer and the CAP proficiency testing.

Results: In an analyte specific manner, room temperature stability was extended between 3 to 7 days allowing all 23 analytes to be shipped refrigerated. This has several benefits to both the client and the laboratory. Dry ice shipments require special

handling and the elimination of the "ship frozen" requirement improves logistics by simplifying shipments and reducing costs. In addition, extension of the stability in most cases will allow for add-on testing to be accommodated from an existing specimen thereby minimizing the need to redraw the patient which improves the time to result.

Conclusion: As methodologies and instrumentation change over time, laboratories should periodically evaluate testing sample requirements for stability as a means to improve the patient and client experience, logistics, work flows and to save on costs.

A-169

Enrichment of Nucleic Acid Purification via Process Optimization of Glass Fiber Manufacturing

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Boom chemistry is a solid phase extraction technology that has long been exploited as a common platform for nucleic acid purification. The mechanism of action has been established as being due to the chaotropic effect between the silica substrate and the nucleic acid. Glass fiber matrices are frequently utilized as a binding structure for the capture and purification of nucleic acids in a rapid and reliable manner. Disparity between glass fiber matrices of current manufacturers results in varying levels of recovery of nucleic acids from samples. A study has been undertaken between glass fiber manufacturers to compare performance of capture, purification and elution of nucleic acids from a spiked model sample. Results indicate significant improvement of Porex glass fiber over commonly utilized glass fiber matrixes with 91.2% recovery of 20ug of gDNA versus 74.7% and 77% by the competition respectively using the Qiagen DNeasy Blood and Tissue Kit as a standard protocol. Similarly, standard deviation in recovery rates decreased from 8.3% and 11.8% by the competition to 3.6% using a Porex glass fiber matrix. Recovery also improved using a plasmid DNA sample from 91.6% and 94% to 97.8% with Porex glass fiber. Standard deviation varied from 6.3% and 3.9% in the competitive samples to 2.9% in Porex glass fiber utilizing the same protocol. The results obtained by this study indicate significant differences in the performance of glass fiber membranes with similar properties. Factors effecting performance will be discussed as well as future areas of improvement of the glass fiber binding matrix.

A-170

N-linked Glycosylation in Human Corin, a Potential Factor in Diagnosis

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Background:

Corin is a type II transmembrane serine protease mainly expressed in heart. As the only known physiological activator of atrial natriuretic peptide, corin plays an important role in maintaining blood pressure. People with deficiency of corin activity may suffer high risks of hypertensive diseases.

Corin is synthesized in cardiac myocytes and anchored on the cell surface. The anchored corin may be cleaved from cardiac myocytes to generate three different kinds of soluble fragments. These soluble fragments will enter circulation system and be detected in blood. Previous studies showed plasma corin levels reduced significantly in patients with heart failure (HF) and may act as a biomarker for the diagnosis of HF.

Glycosidase digestion reveals that corin is abundantly N-glycosylated and one of the predicted N-linked glycosylation site (site 80) is located at the cleavage region for one soluble fragment. It is not clear whether the specific N-glycosylation affects the application of corin in the diagnosis. Here we evaluated the potential influence of the specific N-glycosylation to plasma corin levels.

Methods:

Plasmids expressing human corin mutants N80Q, F77N/K78G/N80Q, F77N/K78G/S79A/N80Q, N80Q/E83N/P84G/L85S and N80Q/E83N/P84G were made by PCR-based mutagenesis. Plasmids for WT corin and mutants were transfected into HEK293 cells, respectively. After two-day culture, soluble corin in conditioned medium was immunoprecipitated and analyzed by SDS-PAGE and Western blotting. HEK293 cells were collected and lysed. Corin in the lysate was also analyzed as an evaluation of plasmid expression.

Results:

Corin zymogen levels in the cell lysates were similar for WT corin and mutants. Compared with WT, F77N/K78G/N80Q and N80Q/E83N/P84G/L85S, levels of ~180-kDa soluble fragment, rather than the other two soluble fragments, in N80Q, F77N/K78G/S79A/N80Q and N80Q/E83N/P84G increased significantly. The total amounts of all the three soluble fragments increased in N80Q (270 ± 26% vs. WT), F77N/K78G/S79A/N80Q (281 ± 34% vs. WT) and N80Q/E83N/P84G (243 ± 10 % vs. WT) while those in WT, F77N/K78G/N80Q (93 ± 26% vs. WT) and N80Q/E83N/P84G/L85S (103 ± 22% vs. WT) were normal. These results indicate that N-glycosylation at or close to site 80 protects corin from ectodomain shedding.

Conclusions:

Lack of N-glycosylation at site 80 causes increase in the total amount of all the three soluble fragments, which may lead to an abnormally high plasma corin level. In HF patients, this abnormally high plasma corin level may give a false negative diagnosis result if corin is used as the biomarker. Till now, no human corin variant corresponding to N-glycosylation at site 80 has been found. However, disorder of N-linked glycosylation is a possible reason for abnormally high plasma corin level and, since N-linked glycosylation occurs to plenty of glycoprotein in most of human cells, N-linked glycosylation may affect other biomarkers containing N-glycans in diagnoses. Therefore, further studies about disorder of N-linked glycosylation and N-glycosylation related genetic polymorphism are urgent in diagnoses using N-glycosylated biomarkers.

A-171

Case Report: Delta bilirubin as an "interference" in direct bilirubin assays

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Background: Delta bilirubin (B_δ) forms slowly and non-enzymatically from conjugated bilirubin (Bc) and albumin in circulation, is normally undetectable in adults as Bc is excreted into the biliary tract and eliminiated through the gut. Traditional direct bilirubin (DBIL) assays, often assumed to be equivalent to Bc, includes Bc & B_δ. BuBc assays measures conjugated and unconjugated bilirubin (Bu) quantitatively.

Methods: BuBc was measured using the Vitros 5600 Chemistry system. TBIL and DBIL on are measured using 3,5-dichlorophenyldiazonium tetrafluoroborate (DPD) method on Beckman Coulter AU5800 Chemistry system. Repeat analyses were performed using a bilirubinometer and vanadate oxidase method on Siemens Advia Chemistry system.

Case Report: A 56 year old female patient undergoing cancer treatment with presented with a Total Bilirubin (TBIL) of 29 µmol/L, Bu of 13 µmol/L and Bc of 16 µmol/L. On the following day, her bilirubin results were 124 µmol/L, 61 µmol/L and 63 µmol/L for TBIL, Bu and Bc respectively, with "mild icterus" tagged to her results. 12 hours later, her bilirubin results were 22 µmol/L, 12 µmol/L and 10 µmol/L for TBIL, Bu and Bc respectively. An investigation started to determine if there had been any errorneous results. Visual inspection of the samples showed mild icterus. Audit trail records revealed that the first and third bilirubin measurements were performed on the Vitros while the second set of results were from the AU5800. A repeat sample from the patient was then analysed using various methodologies and compared:

Analyser	TBIL (µmol/L)	Bu (µmol/L)	Bc (µmol/L)	Icterus Index
Vitros 5600 Chemistry	78 (measured)	12	9	Normal
Bilirubinometer	76	-	-	-
Beckman Coulter AU5800 Chemistry	96	50	46	Mild icterus
Siemens Advia Chemistry	82	20	62	Mild icterus

Conclusion: This is a case of B_δ due to drug-induced hepatobiliary obstruction. B_δ acts as an "interference" in traditional direct bilirubin assays. TBIL (measured) should be reported for adults when using the Vitros method. B_δ has a maximum absorption at 440 nm which is not measured in the serum indices on Vitros. Visual inspection of sample condition is as valuable as automated serum indices measurements.

A-172

Validation of lithium heparin tube for Cardiac markers in a Clinical Laboratory and its benefits.

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Background:

Accurate laboratory testing requires an understanding of the complex interactions between collection devices and blood specimens. Clinical laboratories must consider the pre-analytical challenges in laboratory testing. Proper blood collection and timely

processing are critical pre-analytical steps required for the integrity of laboratory results. Although the influence of blood collection devices on laboratory tests is often overlooked, correct pre-analytical handling is essential. In this study, we discuss the use of plasma to chemistry analysis with an emphasis on heparin tube.

Objective:

Currently the clot activator with separator gel tube is the first option for biochemical analysis, the objective of the study is to evaluate the possibility and benefits of the introduction of lithium heparin tube for holding some biochemical studies.

Methods:

Twenty paired samples using the two tubes were carried out simultaneously. Both tubes were centrifuged before analysis with the difference that the clot activator tube with gel separator needs a time for the formation of clot before the centrifugation procedure. Samples were analyzed for CK mass and Troponin by two different methods: electrochemiluminescence (ECL), in the E411 Roche platform and fluorescence enzyme immunoassay (ELFA), in the Vidas 30 bioMerieux instrument.

Results:

For the ECL CK mass analysis, the Test F, T and the Pearson correlation showed up as expected. 5.14% of systematic constant was observed for level I and 0.82% for Level II that proved to be insignificant. Proportional systematic error was -3.37% with no impact on levels of clinical decision. The comparative test conducted with clot activator tube with gel separator and plasma from lithium heparin tube showed satisfactory results with total error obtained 7.39% and 8.17% for levels 1 and 2, respectively, less than the total allowable error of 30.6%. The troponin, F test and Pearson correlation showed results as expected. Constant systematic error was 4.06% for level 1 and 0.37% for level 2 which were considered not relevant. Proportional systematic error was -6.57% with no impact on levels of clinical decision. The comparative test conducted from gel x lytic plasma serum samples showed satisfactory results with total error obtained 7.63% and 11.32% for levels 1 and 2 respectively less than the total allowable error 48.9%

For ELFA , CK Mass showed a correlated and Kappa index within the references, F test and Pearson correlation as expected. We observed constant and proportional systematic error of zero for the two levels. Comparative tests conducted between serum samples x lytic gel showed satisfactory results with plasma total error of 8.39% obtained for the two lower levels of total allowable error 27.91%.

Conclusion:

We observed that the use of both tubes had good performance in the evaluation of cardiac markers, CK Mass and troponin. The final result for the doctor had a shorter TAT, with high quality and efficiency, as the tube with plasma showed the benefit to be processed before the tube with the clot activator and separator gel.

A-173

Storage stability of selected nutritional biomarkers at -20°C for up to 12 months

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Background: Storage stability is an important preanalytical variable that affects the quality of biospecimens and integrity of results. Little is known about how frozen storage at suboptimal temperature affects nutritional biomarkers. We used quality control (QC) pools to investigate the stability of selected nutritional biomarkers at -20°C for up to 12 months. Methods: Multiple sets of QC vials (low, medium, and high concentration) were stored at -20°C. Three sets of vials were removed from the freezer after 3, 6, 9, and 12 months and placed into a -70°C freezer until analysis. Three separate sets of vials were stored at -70°C for the entire study period and used as the optimally stored reference condition. The content of each vial was analyzed in duplicate for a total of six replicates per condition. All replicates from one condition were analyzed in the same analytical run. We measured four iron-status indicators, one indicator of inflammation, seven biomarkers of water-soluble B vitamins, vitamin C, and five biomarkers of fat-soluble vitamins. We calculated least squares geometric mean concentrations (to account for the right-skewed distributions and for the increasing variance with increasing concentration) using 2-way (time and pool) ANOVA. Since time by pool interactions were generally not significant, differences relative to the reference condition were expressed as mean percent change of geometric means across the three pools. Results: The majority of nutritional biomarkers showed no difference in concentrations after 12 months of storage at -20°C (Table 1). Ferritin (1.2%) and transferrin receptor (-1.7%) showed a small but significant change in concentration. Folate (-12%), pyridoxal-5'-phosphate (-33%), and vitamin C (-49%) showed large and significant decreases. Conclusions: Most nutritional biomarkers showed remarkable stability when stored at -20°C for up to 12 months. This information is important for the low-resource setting, where access to -70°C freezers may be limited.

Table 1: Change of geometric mean concentrations from baseline for selected nutritional biomarkers s

Biomarkers (Serum)	Change, %	P value
Iron-status indicators		
Ferritin, ng/mL	1.22	0.0468
Transferrin receptor, mg/L	-1.70	0.0233
C-reactive protein, mg/L	-0.27	0.80
Water-soluble vitamins		
Folate, nmol/L	-11.7	0.0156
Vit B6 (PLP), nmol/L	-33.3	<0.0001
Vit B12, pg/mL	1.97	0.09
Vit C, mg/dL	-49.3	<0.0001
Fat-soluble vitamins		
Vit A, ug/dL	-1.25	0.27
Vit E, ug/dL	-0.97	0.33
Vit D (25OHD), ng/mL	-0.75	0.65

A-174

The Performance of Chemically Modified Plastic Blood Collection Tubes To Achieve Interior Hydrophilic Surface on Clinical Chemistry Analytes

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Background: Blood collection tubes (BCTs) cannot be regarded as inert specimen carriers as evident by previous studies reporting significant differences in particular serum hormone concentrations collected in different types of BCTs. Surfactant(s) are added to plastic [poly(ethylene terephthalate); (PET)] BCTs to prevent adsorption of cells, platelets, and proteins onto its hydrophobic interior surface. Such interferences from BCT surfactant(s) may not be detected by current laboratory quality control and external proficiency testing programs. The assay interference issue remains unresolved despite reducing the surfactant(s) amounts inside the tubes. Recently, we developed chemically-modified BCTs via a base-catalyzed transesterification reaction that has an interior surface similar to glass which do not contain surfactant (ChemoPET). We demonstrated that the ChemoPET tubes when compared to glass tubes, produced significantly lower biases for cortisol, total triiodothyronine (TT3), and total thyroxine (TT4), compared to other BCT types. However, this study was performed with BCTs containing the same volume of blood but with different tube blood draw volumes. The objective of this study is to determine whether there would be any significant differences in analyte concentrations when BCTs are completely filled to their pre-determined volume by vacuum.

Materials and Methods: Seven types of evacuated BCTs were used in this study: (1) plastic Vacuette™ tube; (2) glass tube; (3) plastic SST™ tube; (4) plastic rapid serum tube (RST™); (5) plastic red-top tube; (6) unmodified PET tube; and (7) ChemoPET tube. Blood samples were drawn in a randomized order after written informed consent from 50 healthy volunteers (18 males, 32 females; age range: 25-70 years). BCTs were inverted eight times after blood draw and allowed to clot for 60 minutes. Following centrifugation, serum specimens were transferred to plastic tubes and stored at -70°C until analysis. Serum cortisol, TT3, and TT4, were measured in random order on a Siemens Immulite™ 1000 analyzer and routine chemistry analytes were measured on a Siemens Dimension RxL™ analyzer. The means of triplicate results for cortisol, TT3, and TT4 measurements were used for statistical analysis. All other analytes were analyzed in singleton. A Student t-test and ANOVA were used to analyze serum specimen test results among the different BCTs. All P values were adjusted for multiple comparisons using a Bonferroni correction.

Results: When BCTs are completely filled to their draw volume by vacuum, we found that some individual BCTs when compared to glass tubes showed statistically and clinically significant differences in analyte concentrations ($P<0.007$), which was not observed when analyte concentrations from different BCT types were averaged.

Conclusions: It is conceivable that the quality and/or quantity of surfactant(s) in each BCT may be different and that a threshold of surfactant concentration to blood volume must be exceeded before significant changes in test results may be observed. Because the clinical laboratory frequently receives BCTs that are partially-filled, the findings of the current and previous studies with these BCTs have important implications for alterations in hormone analyte concentrations. We anticipate that the use of ChemoPET BCTs that do not contain surfactant would produce more accurate and consistent test results regardless of blood draw volumes.

A-175

Accuracy of various Estimated Creatinine Clearance methods in estimating Glomerular Filtration Rate in Indians

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Background:: The aim of this study was to compare the efficacy of GFR derived from various estimated creatinine clearance methods like Jelliffe, Cockcroft and Gault, and 4MDRD equations as compared to measured glomerular filtration rate (GFR) with in Indians

>Methods:We enrolled 80 patients in the study. GFR was determined by technetium-99m diethyl triamine penta-acetic acid (Tc99mDTPA) clearance . Height, body weight and serum creatinine were measured, and GFR and creatinine clearance (CrCl) estimates calculated by various equations. Spearemans correlation was used to assess relationships between measured GFR (Tc99mDTPA clearance) and estimated clearances using the three formulae. Difference between the measured GFR and estimated clearances compared with measured GFR were examined to determine whether prediction error was independent from measurement magnitude. Analyses of differences were used to determine bias and precision. Bias was assessed by mean percentage error (MPE), calculated as the percentage difference between the estimated clearances for each formula and measured GFR. A positive bias indicates overestimation of GFR, and a negative bias indicates underestimation. Relationships were also assessed by gender and varying levels of renal function: GFR <60 ml / min, and GFR >60 ml/ min.

Results:The mean measured GFR was 77.2 ml / min (range 17 to 152 ml / min). The mean bias (mean percentage error) was -4.9, -10.3 and -1.57% respectively for the, Jelliffe , Cockcroft and Gault, and 4MDRD formulas, respectively. The 4 MDRD formula slightly overestimates the GFR in patients having GFR less than 60ml/ min, where as,it underestimates for GFR more than 60ml / min.

Conclusion:: In Indians 4 MDRD equation of estimated creatinine clearence seems to be most accuratet in estimating GFR .

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Reducing Adhesion of Proteins on Stainless Steel Components by the Application of a Carboxysilane Coating.

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Background:

Protein binding and carryover in analytical systems is a challenge that can reduce accuracy and throughput of analysis. This work presents data on the use of coatings over stainless steel surfaces to compare analytical accuracy, reduction of carryover and increase of throughput for a range of proteins. Comparisons are made against other substrates and surface coatings. The areas of use are broad from all sample-contacting components of medical diagnostic equipment to HPLC columns, transfer tubing, and more.

Methods:

The protein-resistant properties of a carboxysilane coating (deposited via chemical vapor deposition) was studied using quartz crystal microbalance with dissipation monitoring (QCM-D) and compared to that of bare stainless steel and a Teflon-like fluoropolymer coating (AF1600).

Results:

With the assistance of a nonionic surfactant-containing wash solution, the carboxysilane coating was found to facilitate 100% removal of adsorbed proteins (BSA, mouse IgG and NHP), whereas these proteins remain adsorbed on the bare stainless steel surface under the same conditions. Compared to the carboxysilane coating, AF1600 showed similar resistance to plasma protein adhesion at initial use, but the AF1600 performance degraded due to mechanical wear-induced surface delamination. The carboxysilane coating, on the other hand, maintained the same level of protein resistance through harsh chemical washes and multiple sonication cycles, demonstrating excellent chemical stability and physical durability of the CVD coating.

Conclusion:

A carboxysilane coating on stainless steel components will improve reliability, reduce carryover and cycle time, and increase the durability of analytical systems.

Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Hematology/Coagulation

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Evaluation of Multiplex Ligation-Dependent Probe Amplification as a method for detection of IKZF1 (Ikaros) deletions in B-cell precursor acute lymphoblastic leukemia

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The IKAROS (IKZF1) gene encodes a transcription factor that belongs to the family of zinc-finger DNA binding proteins associated with chromatin remodeling. IKZF1 transcription factor is crucial for many aspects of hematopoiesis. The expression of this protein is restricted to the fetal and adult hemo-lymphopoietic system and it functions as a regulator of lymphocyte differentiation. Total or partial deletions of IKZF1 are frequent in B-precursor acute lymphoblastic leukemia (pB-ALL), especially in adults positive for BCR-ABL. IKZF1 deletions have been associated with a poor prognosis in terms of overall survival and frequency of relapse. The aim of this study was to evaluate of IKZF1 deletions in B-ALL using different methodologies. A total of 34 non-consecutive pB-ALL patients, including 25 children and 9 adults, were enrolled in this study. Ten patients (3 adults) had been previously shown by RT-PCR to be BCR-ABL carriers. Bone marrow DNA was extracted with QIAamp DNA Blood Mini kit (Qiagen). IKZF1 deletions were identified using the SALSA P-202 B1 Multiplex Ligation-Dependent Probe Amplification (MLPA) assay (MRC-Holland). The analysis was performed using the GeneMarker v2.6.2 software. In 11 cases, DNA was also evaluated by Comparative Genomic Hybridization analysis (180K aCGH/SNPs; Agilent Technologies) and the data were analyzed with Agilent CytoGenomics Edition 2.9.2.4 software. MLPA analyses revealed that IKZF1 deletions occurred in 10 of pB-ALL cases. Deletions were present in 60% of the BCR-ABL positive cases, and in 17% of the BCR-ABL negative cases only. The extension of the deletions was variable, the most common ones comprised exons 4 to 7 (30%) or exons 1 to 7 (30%). Whole-gene deletions including all exons occurred in a single case, a finding consistent with the loss of a short arm of chromosome 7 and presence of a long arm isochromosome [i(7)(q10)] seen in the karyotype. Among IKZF1 deletion carriers, 4 had a normal karyotype, 4 presented complexes karyotypes including t(9;22) (q34;q11.2) and additional chromosome aberrations, and two had complexes karyotypes with aberrations other than t(9;22). aCGH confirmed the absence of IKZF1 deletions in 10 cases and presence of a deletion in one. In this latter case, the initial aCGH software

call indicated a deletion of exon 5 and its 5´intron, however a close analyses showed that the deletion extended to exon 7, as reported in MLPA results. Therefore in our study, IKZF1 deletions were associated with BCR-ABL fusion gene and comprised mainly exons 4 to 7, corroborating literature data. MLPA has the disadvantage of not detecting deletions affecting minor subclones (<20% of the cells), however their results were shown to be able to identify patients who subsequently relapsed better than gene expression-based assyas. In conclusion, the detection of IKZF1 alterations by MLPA commercial kit was relatively easy to perform, and allowed a good definition of partial gene deletions since the kit has two probes for each exon. Care should be taken during the technique standardization because some interpretation issues exist, such as low amplification of one probe only or amplification values close to the cut-off points.

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Increased Von Willebrand Factor and High Circulating Placental Growth Factor Correlate with Inflammation and Iron Overload in Patients with Compound Heterozygous Sickle Cell and Beta-Thalassemia

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Background: Sickle cell disease (SCD) is characterized by pathological polymerization of hemoglobin, increased red cell rigidity and poor microvascular blood flow. Thus, hemolytic anemia, vaso-occlusion and vasculopathy are the hallmarks of its clinical presentation. Several other factors contribute to the clinical variability, which is present in SCD, including leukocyte dysfunction, platelet interactions with endothelial cells, pro-inflammatory cytokines, oxidative stress, reduced nitric oxide (NO) availability and hemostatic activation. Placental growth factor (PlGF) plays an important role in both inflammation and neoangiogenesis. Recently it has been reported that markers of iron overload are associated with high plasma level of PlGF and early mortality. Furthermore, in SCD patients, hemolysis can lead to a prothrombotic state by increasing the activity of von Willebrand factor (vWF). ADAMTS-13 is a member of the ADAMTS (A-Disintegrin-Metalloprotease- ThromboSpondin-type-1-repeats) family that cleaves vWF. Recent observations suggest that SCD patients suffer from an acquired ADAMTS-13 deficiency primarily because Hb competitively binds and blocks the proteolysis of vWF, leading to the accumulation of ultra-large VWF multimers in circulation and on endothelium. The aim of this study was to evaluate inflammation, endothelial dysfunction and angiogenesis in patients with compound heterozygous SCD and beta-thalassemia (HbS/βthal) and explore possible association with iron overload and other disease features.

Patients and Methods: Eighty-nine adult caucasian patients with HbS/bthal were included in the study, while 20 apparently healthy individuals served as controls. Patients with HbS/βthal divided in two groups: group A included 49 patients under hydroxycarbamide (HC+) treatment and group B included 40 patients without hydroxycarbamide (HC-) treatment. Along with hematology and blood chemistry parameters determination, measurements of circulating high-sensitivity C-reactive protein (hs-CRP), vWF, D-Dimers, ADAMTS-13, hs-Troponin-T (hs-TnT) and PlGF were measured in patients with HbS/βthal and controls using immunoenzymatic techniques.

Results: Levels of hs-CRP, vWF and PlGF were elevated in patients with HbS/βthal compared to controls (6.9±5.3 vs. 0.4±0.4mg/L, 170.1±78.3 vs. 85.3±22.1IU/dL and 20.2±7.6 vs, 15.3±2.4pg/mL, respectively, $p<0.001$), while ADAMTS-13 levels were decreased in patients with HbS/βthal compared to controls (965.2±244.0 vs. 1144.2±187.0pg/mL, $p<0.001$). No significant differences were found for the above parameters between patients of groups A and B. Hs-CRP correlated positively with vWF, PlGF and ADAMTS-13 ($p<0.01$). PlGF levels in patients with HbS/βthal correlated positively with markers of hemolysis such as reticulocyte counts, LDH and with uric acid levels ($p<0.01$). Ferritin levels correlated positively with hs-CRP, vWF, PlGF, D-Dimers and with markers of hemolysis ($p<0.01$). Furthermore, only 6 patients had slightly increased hs-TnT levels and almost all patients had pathologic D-Dimers levels.

Conclusions: Our findings demonstrate that patients with HbS/βthal have a significant degree of endothelial dysfunction as assessed by increased vWF. The increased levels of the D-Dimers in almost all patients indicate the activation of coagulation and fibrinolytic systems even in the steady state of the disease. Iron overload and inflammation along with reduced circulating ADAMTS-13 contribute, at least partially, to the increased levels of vWF. Similarly, inflammation and iron overload enhance the production of angiogenesis markers, such as PlGF suggesting a possible pathogenetic role for iron load in SCD biology.

A-179

Preclinical validation of fluorescence in situ hybridization assay for detection of the AML1/ETO translocation

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Background:The Fluorescence in situ Hybridization (FISH) is a widely used tool to study hematologic diseases. FISH can be performed in metaphase or interphase cells, which is important when dealing with leukemia cell with low proliferation. Although the performance of most FISH probes has been evaluated by the manufacturer prior to marketing, they also must be validated prior to implementation of the assay for clinical use. Clinical laboratories must independently adopt protocols to verify the performance of the assay. Rearrangements involving the AML1 and ETO genes are generated by the t(8;21)(q21;q22) translocation and are present in 30-40% of acute myeloid leukemia (LMA) subtype M2 patients. The resulting chimeric fusion protein AML1/ETO inhibits a transcription factor that acts as a tumor suppressor and, therefore, has multiple effects on the proliferation, differentiation, and viability of the leukemic cells. The rapid identification of this rearrangement allows guiding prognosis and treatment.Objective: To validate FISH assay for detection of translocation AML1/ETO following recommendations from the American College of Medical Genetics (ACMG). Methods: We used the AML1/ETO Translocation, Dual fusion probe manufactured by Cytocell®. In the familiarization phase, the analysts should become familiar with the probe labeling, testing probe strategy and result reporting. Metaphase cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its analytical sensitivity and specificity. To establish a reference range (normal cutoff) we estimated the false positive rate from 10 uncultured bone marrow samples and 10 uncultured blood samples from non-hematological patients with normal karyotypes who would be unlikely to harbor theAML1/ETO translocation. Two analysts scored 500 interphase cells (250 per analyst). All AML1/ETO probe signal patterns were recorded. The cutoffs for each signal pattern were calculated using the beta inverse (BETAINV) function. Results: The AML1/ETO kit presents the AML1 (21q22) and ETO (8q21) probes labeled respectively with red and greenfluorophore. A normal result should show 2 green and 2 red signals (2G2R). Two fusion signals in addition to the one green and one red signals (2F1G1R) indicates the presence of the translocation.The probe demonstrated 100% specificity and analytical sensitivity.In the analysis of bone marrow and blood samples, we identified three and four atypical signal patterns, respectively. We did not observe change in the cutoffs with the increase in cell count. The signal patterns and their cutoffs for bone marrow samples were 2G1R (2,34%), 1G2R (3,1%) and 3G3R (2,34%). The signal patterns and their cutoffs for blood samples were1F1G1R (2,34%), 2G1R (3,1%), 1G2R (2,34%) and 3G1R (2,34%). These cutoffs were obtained from 200-cell count. The analyses of normal and abnormal samples by FISH were in agreement with the conventional cytogenetic. Conclusion: The FISH assay for detection of the AML1/ETO translocation showed excellent reproducibility and high quality in different hybridizations, and probe specificity higher than recommended by the ACMG.

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High sensitivity Hepcidin-25 bioactive ELISA: a sensitive, fast and straightforward competitive Elisa for the quantification of Hepcidin-25 in human serum and plasma

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Hepcidin is an iron homoeostasis regulator peptide. The bioactive peptide Hepcidin-25 is generated predominantly in the liver by proteolytic cleavage of the C-terminal 25 amino acids of prohepcidin. Subsequent N-terminal processing of Hepcidin-25 results in smaller peptides of 20-24 amino acids that show greatly reduced activity and accumulate in the urine.

Hepcidin exerts its regulatory function by inhibiting ferroportin, the major cellular iron exporter in the membrane of macrophages, hepatocytes, and the basolateral site of enterocytes. Hepcidin-25 induces the internalization and degradation of ferroportin, resulting in increased intracellular iron stores, decreased dietary iron absorption, and decreased circulating iron concentrations.

Hepatocellular hepcidin synthesis decreases under conditions of increased demand for circulating iron like iron deficiency, hypoxia, anemia, and erythropoiesis. In contrast, hepcidin synthesis is induced by inflammation and infection.

Serum Hepcidin-25 has been shown to add value to identify and differentiate specific disease conditions. Hepcidin deficiency causes hereditary hemochromatosis, characterized by body iron overload that may progress to liver cirrhosis. In addition, low Hepcidin-25 concentration can be induced by iron loading anemias and chronic hepatitis C. In contrast, high Hepcidin-25 levels have been found in iron-refractory iron-deficiency anemia, during infection, chronic kidney disease, and after intensive exercise, explaining the high iron deficiency among athletes.

The new high sensitive DRG Hepcidin-25 assay is a colorimetric solid phase enzyme-linked immunosorbent assay (ELISA) based on the competitive binding of Hepcidin of the sample and biotinylated Hepcidin to immobilized anti-Hepcidin antibody, followed by the detection with a Streptavidin-HRP conjugate.

The total assay time is 1.5 hours. The Elisa allows the quantitative determination of Hepcidin-25 covering a measuring range form 0.15-81.0 ng/mL. Serum and plasma (EDTA, heparin, Citrate) can be used for this assay. The analytical sensitivity of the assay is 0.153 ng/ml. The test shows good reproducibility with an intra-assay precision of 6.97% (mean of 20 repeated measurements of 3 different samples) and an inter-assay precision of 12.0% (average of 40 repeated measurements of 4 different samples by two observers on 20 days with 2 different lots). The recovery was determined with 97.3% (mean of 3 samples, each spiked with 4 concentrations of Hepcidin-25; range from 88.0-108.8%). Linear dilution gave an excellent overall recovery of 97.9% (mean of 3 samples, each diluted 4-fold with dilution buffer; range from 89.1-105.2%). We found no matrix interference with haemoglobin (up to 4 mg/ml), bilirubin (up to 0.5 mg/mL) and triglycerides (up to 7.5 mg/mL). Inter-Lot precision was 7.69% (mean of 3 samples measured with 3 lots in 6 determinations).

The new assay EIA-5782) shows a good correlation to the current manual Elisa (EIA-5258; y=0.991x+0.06; r = 0.992; n=72).

Benefits of the new assay are a very straight forward procedure with ready-to-use reagents, no shaking, high sensitivity, and a short total assay time of 1.5 hours.

A-181

Lipid effect on antithrombotic and prothrombotic activities of thrombomodulin (TM)

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Background:

Endothelial thrombomodulin (TM) plays central roles in haemostatic balance by facilitating both thrombin-mediated protein C (PC) activation (antithrombotic) and thrombin-activatable fibrinolysis inhibitor (TAFI) activation (prothrombotic). Activated PC (APC), an anticoagulant protease, inactivates coagulation factors Va and VIIIa, thus preventing excessive coagulation. Activated TAFI (TAFIα) cleaves C-terminal lysine and arginine residues from partially degraded fibrin, suppressing plasminogen activation and thereby delaying clot lysis. It has been known that lipid membrane serves as an essential cofactor for membrane protein functions. In this study, we proposed membrane mimetic systems of TM and different lipids for defining lipid effect on TM substrate specificity for PC and TAFI activation. The research provides a unique technique to understand the biological significance and predict the effect of the lipids on TM's capacity to alter APC and TAFIα levels thus modulating progression of thrombo-inflammatory pathologies such as sepsis.

Methods:

TM was incorporated into liposomes by a reaction with swelled lipid solutions of different composition. APC and TAFIα were generated by incubation with liposomal TM and thrombin in otherwise identical conditions. APC and TAFIα levels were measured by hydrolysis of spectrozyme PCa (spectrophotometrically) and hippuryl-arginine (HPLC), respectively. In addition, time scale profiles of TAFI activation were compared by densitometric analysis of SDS PAGE and activity in different lipids. All reactions were done in triplicate independent experiments, with controls and internal standards where appropriate.

Results:

Most significant increase in APC amount was observed with 5% phosphatidylethanolamine (PtEtn) liposomes (153 ± 5%) in comparison to free TM (69 ± 8%), where TM in phosphatidylcholine (PtCho) was set as a reference (100%). Incorporation of TM into 5% phosphatidylserine (PtSer) liposomes showed a decrease in APC amount (82 ± 18%). Further decrease in APC was observed in 10% PtEtn and PtSer liposomes (70 ± 21 % and 86 ± 26%, respectively). Competitive studies using co-incubation with TAFI showed significant decreases of APC in PtCho-TM liposomes (58 ± 14%) and in 10% PtSer as well as 10% PtEtn liposomes (24 ± 21 % and 34 ± 31 %, respectively). On the other hand, increase in TAFIα was observed in 5% PtEtn-TM (207 ± 16%) and 5% PtSer-TM (234 ± 21%) liposomes compared to PtCho-TM (100 %). Reduction of TAFIα generation in competitive conditions was observed (from 207 ± 16% to 83 ± 30 %) in 5% PtEtn liposomes.

Conclusion:

We determined that 5% PtSer and 5% PtEtn liposomes showed highest increases in TAFIα levels while only 5% PtEtn resulted in APC increase. PtSer had a reducing effect on APC generation. Higher concentrations of both PtSer and PtEtn resulted in reduction of TAFI and PC activation, both in regular and competitive scenarios. This demonstrates that PtEtn has a concentration-dependent effect on TAFI and PC activation while PtSer increases TAFI activation only. Given that PtEtn and PtSer both significantly increase in cell trauma, this study suggests a link between phospholipid exposure and reduced anticoagulant and anti-inflammatory potential of the endothelium.

A-182

The Activity of Recombinant Endothelial Nitric Oxide Synthase Oxygenase Domain on Human Apo AI Derived Discoidal Lipid Particles

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Background: Cardiovascular diseases (CVDs) are the number 1 cause of death worldwide. Nitric oxide (NO), an important signaling molecule in the cardiovascular system, was recognized with a Nobel Prize in medicine in 1998. Endothelial nitric oxide synthase (eNOS) is one member of the nitric oxide generating family, and is the dominant isoform in the inner walls of blood vessels. It regulates numerous essential cardiovascular functions including vasodilation (blood pressure), inhibition of platelet aggregation and adhesion to the vascular wall, which prevents atherosclerosis and unwanted blood clots. eNOS dysfunction and disruption of nitric oxide release within the blood vessel wall is associated with the genesis of many aspects of CVD and its alarming death toll. **Purpose:** The goal is to develop a model that incorporates eNOS into miniature lipid membranes that we call nanodiscs. The concept is based on nascent discoidal high-density lipoprotein (nHDL) particles wherein the scaffold proteins that wrap around lipid bilayers are derived from human apolipoprotein A-I (apo AI). Our aim is to study the activity of the enzyme heme domain in its native microenvironment provided by this unique system and to quantify the effect of lipid membranes in the functional regulation of eNOS. This will shed light on the role of eNOS in maintaining vascular tone as well as on how eNOS dysfunction is involved in the onset of CVD and its progress. **Methods:** Nanodisc and eNOSoxy/nanodisc complex formation were prepared by mixing phosphatidylcholine/detergent micelles and apo AI engineered protein with and without eNOSoxy in a defined molar ratio. The self-assembly was initiated by dialyzing overnight and the purification was achieved by applying to a Superdex 200 10/30 gel filtration column on ÄKTA FPLC system using Tris-buffer at 0.5 ml/min. eNOSoxy concentration in nanodisc was verified by the characteristic ferrous heme-CO adduct absorbing at 444 nm via an extinction coefficient of $\varepsilon 444 = 76\ mM^{-1}\ cm^{-1}$.The activity of eNOSoxy was determined using a Spectra max plus 384 plate reader based on the Griess reaction. Catalysis of NO production from N-hydroxyarginine (NOHA) and H_2O_2 by eNOSoxy and eNOSoxy-bound nanodisc were assayed in 96-well microplates. Samples were run in triplicate (50 µl final volumes) and the assay plate was read at 540 nm. **Results:** The specific activity of eNOSoxy/nanodisc or free eNOSoxy is calculated in nmol NO/min/nmol of enzyme in the reaction volume at 37°C. The activity was determined by quantifying NO in the form of nitrite through a standard calibration curve (R^2=0.9979). The detected specific activity of nanodisc-bound eNOSoxy (49.0 ± 1.3) nmol/min/nmol was > 50% lower in comparison to the free eNOSoxy enzyme (132.4 ± 2.4) nmol/min/nmol. **Conclusions**: The analyzed data showed a decrease in NO generation by the nanodisc-bound eNOSoxy in contrast to free eNOSoxy. This study suggests that the membrane lipids affect the catalytic properties of eNOS heme domain. Clinically, since NO bioavailability correlates with endothelial dysfunction and consequently CVD, membrane lipid abnormalities could have possible implications on eNOS functionality. Future studies will test eNOS with various lipids combinations embodied in human endothelial cells.

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Molecular characterization and genotyping of alpha and beta thalassemias among anemic patients

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Background:-

Thalassemia is a hereditary anemia resulting from defects in hemoglobin production. Thalassemia is among the most common genetic disorders worldwide; 4.83 percent of the world's population carry globin variants, including 1.67 percent of the population who are heterozygous for α-thalassemia and β -thalassemia. In addition, 1.92 percent carries sickle hemoglobin, 0.95 percent carry hemoglobin E, and 0.29 percent carry hemoglobin C. Thus, the worldwide birth rate of people who are homozygous or compound heterozygous for symptomatic globin disorders, including α-thalassemia and β -thalassemia, is not less than 2.4 per 1000 births, of which 1.96 have sickle cell disease and 0.44 have thalassemias . Beta thalassemias are very heterogeneous at the molecular level. In most cases, mutations are single nucleotide substitutions, deletions or insertions of single nucleotides or small oligonucleotides. This disease diversity and the consequent variable degree of globin chain imbalance are the main determinants for milder phenotypes.

Method:-

One hundred patients recruited from the hematology polyclinic of private hospitals in Jeddah; KSA. DNA was analyzed using validated multiplex polymerase chain reaction (PCR) amplification of 22 Beta-globin gene mutations and 21 α globin gene mutations using biotinylated primers followed by reverse hybridization on test strip derived from ViennaLab Diagnostics GmbH Vienna, Austria. A confirmatory genetic testing was done after preliminary screening test using validated Sebia Capillary's technique for hemoglobin fractionation. Serum ferritin was determined in patients who had low mean cell volume (MCV) and low mean cell hemoglobin (MCH) to exclude presence ferrokinetic abnormalities.

Results:-

This study showed that the overall prevalence of SCD with thalassemia in KSA was 44% and according to DNA analysis the prevalence of (HbS+α-thalassemia) was 44% with HbSS in 12% and HbS in 32 % of cases; (HbS/β0-thalassemia) was detected in 4 % of cases, SCD alone was in 4% of cases respectively; while thalassemias alone was in 44% of cases; Combined both α and β thalassemias in 8% while β thalassemias alone in 12% and α thalassemias alone in 32% of cases . This study confirms presence of different combination between sickle cell gene, β0 Thalassemia and α-Thalassemia genes which means presence of a genetic variability among saudi population. The most common mutation among Beta-globin gene was the (IVS II-1 A>T) type with frequency of (6%), IVS I-5 (G>C) with a frequency of (4%), and the most common mutation among the α globin gene was deletion Δ 3.7 (-3.7 kb), found in 32% of cases. The α-thalassemia was more frequent among HbSS patients (44%) than S/β0-thalassemia patients (4%).

Conclusion:-

This study was done on small scale which did not by any means give an accurate account of the frequencies of HbS gene, β–gene, and α-gene in population of KSA. For this reason other population based studies are needed in all regions of KSA to elucidate the prevalence of SCD, alpha and beta thalassemia to build a solid scientific data which help in creating a national registry of Hemoglobinopathies and thalassemia in KSA.

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Validation of fluorescence in situ hybridization assay for detection of the t(11;14) (CCND1/IGH) translocation in a clinical laboratory

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Background: The translocation t(11;14)(q13;q32) fuses the CCND1 and IGH genes, leading to cyclin D1 overexpression. This translocation is mainly found in mantle cell lymphoma, but also in B-prolymphocytic leukemia, plasma cell leukemia, chronic lymphocytic leukemia and multiple myeloma. Fluorescence in situ Hybridization (FISH) is a widely used tool to study hematologic diseases. FISH can be performed in dividing and nondiving cells. This is relevant to diseases with usually low mitotic

index and poor quality of metaphases, making it difficult to chromosomal analysis by conventional cytogenetic. The interphase FISH assay provides a reliable and routinely applicable tool for diagnosis of the t(11;14) translocation. Although the performance of most FISH probes has been evaluated by the manufacturer prior to marketing, they also must be validated prior to implementation of assay for clinical use. Clinical laboratories must independently adopt protocols in order to verify the performance of the assay. Objective: To validate FISH assay for detection of CCND1/IGH translocation following recommendations from the American College of Medical Genetics (ACMG). Methods: We used the CCND1/IGH Translocation, Dual fusion probe manufactured by Cytocell®. In the familiarization phase the analysts should become familiar with the probe labeling, testing probe strategy and result reporting. Metaphase cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its analytical sensitivity and specificity. To establish a reference range (normal cutoff) were estimate the false positive rate from 10 uncultured bone marrow samples and 10 uncultured blood samples that would be unlikely to harbor the CCND1/IGH translocation. Two analysts score 500 interphase cells (250 per analyst). All CCND1/IGH probe signal patterns were recorded. The cutoffs for each signal pattern were calculated using the beta inverse (BETAINV) function available in Microsoft Excel. Results: The CCND1/IGH translocation probe presents the CCND1 (11q13) gene probe labeled with red fluorophore, and the IGH (14q32) gene probe labeled with green fluorophore. A normal result of using this probe should show 2 green and 2 red signals (2G2R). Two fusion signals in addition to the one green and one red signals (2F1G1R) indicate the presence of the translocation. The probe demonstrated 100% specificity and analytical sensitivity. In the analysis of bone marrow and blood samples were respectively identified ten and six atypical signal patterns. We didn't observe change in the cutoffs with the increase in cell count. The cutoffs obtained with BETAINV function were validated for counting 200 cells. The signal patterns and its cutoffs for bone marrow samples are 1F1G1R (5,1%), 1F1G2R (2,34%), 1F1R (1,49%), 2G1R (3,76%), 1G2R (4,43%), 1G1R (2,34%), 2G3R (1,49%), 3G2R (2,34%), 4G3R (2,34%) and 4G4R (2,34%). The signal patterns and its cutoffs for blood samples are 1F1G1R (6,26%), 2G1R (3,1%), 1G2R (3,1%), 1G1R (2,34%), 3G2R (1,49%) and 2F (1,49%). The analyses by FISH were in agreement with the conventional cytogenetic. Conclusion: The FISH assay for detection of the CCND1/IGH translocation was approved for clinical use.

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Performance of the Alpha-1-Antitrypsin assay for use on the Binding Site Optilite® turbidimetric analyser

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Alpha-1-Antitrypsin (A1AT) is a serine protease inhibitor primarily acting on neutrophil elastase, protecting the lung from enzyme damage. Measurement of A1AT is of use in the diagnosis of several conditions including adult cirrhosis of the liver. Here we describe the performance of an A1AT assay for use on the Binding Site's Optilite analyser. Precision was determined with a CLSI protocol using 5 pooled sera samples with 2 runs of duplicate testing per day on 3 kit lots and 3 analysers for 21 days, results are shown in table 1. Linearity was verified by assaying a serially-diluted patient sample pool across greater than the width of the reportable measuring range and comparing expected versus observed results, the assay was linear over the range of 0.32-5.74 g/L; weighted linear regression gave y = 1.01x - 0.00 (r = 1.000) and analysis using StatisPro software in accordance with CLSI guidance confirmed linearity. This provides an assay measuring range of 0.35-5.00g/L using a 1/10 sample dilution. Interference was tested by spiking base pools at three levels (0.68, 0.89 and 1.42 g/L) with 200mg/L bilirubin, 5g/L hemoglobin, 500mg/dL intralipid and 1000mg/dL triglycerides and comparing with a negative control. Interference at all analyte concentrations of <3.5% was seen with bilirubin, hemoglobin and triglycerides, for intralipid the interference was <8.40%. Correlation to the Binding Site A1AT assay for the SPAPLUS® was performed using 124 samples; 102 from disease state patients and 22 from healthy blood donors (total range 0.40-4.73g/L). This demonstrated acceptable greement when analyzed by Passing-Bablok regression; y=0.98x + 0.01. We conclude that the A1AT assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.

Table 1: Precision of A1AT Optilite assay

Analyte Concentration	0.565g/L	0.675g/L	0.829g/L	1.139g/L	4.328g/L
Total precision (%CV) (Acceptance <10%)	4.7%	4.2%	4.5%	3.6%	4.0%
Within run precision (%CV) (Acceptance <5%)	1.4%	0.9%	1.4%	1.1%	1.3%
Between run precision (%CV) (Acceptance <8%)	2.6%	2.7%	2.4%	1.8%	1.9%
Between day precision (%CV) (Acceptance <8%)	3.7%	3.1%	3.5%	3.0%	3.2%

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Performance of a Haptoglobin assay for use on the Binding Site Optilite® turbidimetric analyser.

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Haptoglobin is an acid α2 acute-phase plasma glycoprotein and binds specifically to free plasma oxy-hemoglobin. The high molecular weight complex prevents filtering of hemoglobin by the kidneys. Low levels of Haptoglobin are associated with hemolytic anemias and liver disease. Here we describe the performance of a Haptoglobin assay for use on the Binding Site's Optilite analyzer. Precision was determined with a CLSI protocol using 8 pooled sera samples with 2 runs of duplicate testing per day on 3 kit lots and 3 analysers over 21 days, results are shown in table 1. Linearity was verified by assaying a serially-diluted patient sample pool across greater than the width of the reportable measuring range and comparing expected versus observed results, the assay was linear over the range of 0.139-4.472g/L; weighted linear regression gave y = 1.00x - 0.05 (r = 0.999). This provides an assay measuring range of 0.26-4.0g/L using a 1/10 sample dilution, with a sensitivity of 0.026g/L. The upper limit of the range is 8g/L at 1/20. Interference was tested by spiking base pools at five levels covering the range 0.1 - 5.00g/L with 200mg/L bilirubin, 2000mg/dL intralipid and 500mg/dL triglycerides and comparing with a negative control. Interference of <6.38% was seen at all analyte concentrations with bilirubin and triglycerides. Lipemia interference with intralipid was successfully detected by the blank absorbance flag utilized in this assay's parameters. Correlation to the Binding Site Haptoglobin assay for the SPAPLUS® was performed using 148 samples; 99 from disease state patients and 49 from healthy blood donors (total range 0.128g/L to 6.604g/L). This demonstrated acceptable agreement when analyzed by Passing-Bablok regression; y=0.99x + 0.01. We conclude that the Haptoglobin assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.

Table 1: Precision of the Haptoglobin Optilite assay

Analyte Concentration	0.109 g/L	0.262 g/L	0.388 g/L	1.024 g/L	1.447 g/L	2.448 g/L	3.246 g/L	5.520 g/L
Total precision (%CV) (Acceptance <15%)	5.8%	3.4%	9.9%	5.1%	4.0%	3.2%	3.1%	5.3%
Within run precision (%CV) (Acceptance <6%)	1.3%	0.8%	1.7%	1.1%	1.7%	1.1%	1.3%	1.7%
Between run precision (%CV) (Acceptance <6%)	2.8%	1.6%	4.7%	2.9%	1.9%	1.8%	1.6%	1.6%
Between day precision (%CV) (Acceptance <10%)	4.9%	2.9%	8.6%	4.0%	3.1%	2.4%	2.3%	4.8%

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Performance of serum IgA Kappa and IgA Lambda assays for use on the Binding Site Optilite® protein analyser

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Measurement of serum IgA Kappa (IgAκ) and IgA Lambda (IgAλ) has been shown to be of use in the detection and monitoring of monoclonal ammopathies. Elevated monoclonal protein production is indicative of an underlying abnormality such as MGUS, multiple myeloma & other disorders. International guidelines recommend SPE densitometry is performed to quantify monoclonal proteins, however monoclonal IgA can often be obscured by other proteins in the β region of a SPE gel. Turbidimetry

can be used in these instances to measure both IgA Kappa & IgA Lambda and give a more accurate representation of tumour production. Furthermore, calculation of the IgA Kappa/Lambda ratio & comparison with values found in normal subjects can give a more sensitive indication of clonality and will also compensate for any changes in plasma volume. Here we describe the performance characteristics of IgAκ and IgAλ assays (Hevylite®, The Binding Site) for use on the Binding Site's Optilite® analyser. The assays have measuring ranges of 0.18-11.20g/L for IgAκ (reference interval 0.480-2.82g/L) and 0.158-10.40g/L for IgAλ (reference interval 0.360-1.980g/L) at the standard 1/10 sample dilution, with sensitivities of 0.018g/L and 0.015g/L respectively. High samples are automatically remeasured at a dilution of 1/60 or 1/100, with upper measuring ranges of 1.80-12.00g/L (IgAκ) and 1.58-104.00g/L (IgAλ). Precision was assessed according to CLSI (EP05-A2), measuring samples at 5 concentrations, on 3 kit lots and 3 analysers over 21 days. Precision acceptance was <10% CV. Linearity was assessed by assaying a serially-diluted sample pool across the width of the measuring range and comparing expected versus observed results, with recovery required to be <10% at each level. Interference was tested by running the common interferents of triglyceride (10g/L), bilirubin (0.2g/L), haemoglobin (5.0g/L) and 17 potential drug interferents at 4 levels, acceptance being <10% difference to a negative control. Correlation to the Binding Site IgAκ and IgAλ assays for the Siemens BN™II was performed using 140 samples from normal subjects and patients with multiple myeloma as well as other monoclonal gammopathies (Range 0.043-57.46g/L κ, 0.038-20.793g/L λ). Acceptance was a Passing-Bablok regression slope of 0.9-1.1. Within-run CVs were 1.4% (0.31g/L), 2.8% (0.85g/L), 4.3% (1.63g/L), 2.0% (2.59g/L) and 1.4% (9.17g/L) for IgAK, 1.6% (0.28g/L), 3.3% (1.01g/L), 3.8% (1.86g/L), 1.8% (2.48g/L) and 1.8% (8.75g/L) IgAλ. Total precision CVs were 6.7% (0.29g/L), 3.8% (0.85g/L), 6.5% (1.65g/L), 5.1% (2.45g/L) and 4.9% (9.11g/L) for IgAK 6.8% (0.30g/L), 4.7% (0.93g/L), 9.3% (1.63g/L), 9.1% (2.32g/L) and 3.6% (8.58g/L) IgAλ. The assay was shown to be linear over the standard measuring range of the assays; y=1.00x+0.001 (R^2=0.999) IgAκ and y=1.02x+0.00 (R^2=1.000) IgAλ. No significant interference was observed at any level with the interferents studied. Correlation with the IgAκ and IgAλ BNII assays demonstrated good agreement when analysed by Passing-Bablok regression; y=1.09x+0.05 IgAκ and y=1.05x-0.02 IgAλ. We conclude that the IgAκ and IgAλ assays for the Optilite analyser provide a reliable, accurate and precise method for quantifying IgAκ and IgAλ in serum and the presence of an abnormal ratio may be useful in identifying patients with IgA myeloma.

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Evaluation of a latex-enhanced IgD assay for use on the Binding Site Optilite® turbidimetric analyser.

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Measurement of IgD is of use in both the monitoring of IgD myeloma and in the diagnosis of Hyperimmunoglobulinaemia D syndrome. Here we describe the evaluation of an IgD assay for use on the Binding Site's Optilite® analyzer. The assay time is 10 minutes and was read at end-point. The assay range is 13 - 210 mg/L using a 1/10 sample dilution, with a sensitivity of 13mg/L. The upper limit of the range is 16800 mg/L, utilizing auto-redilutions. Correlation to the Binding Site IgD assay for the SPAPLUS was performed using 93 samples including 43 from healthy donors (total range 12.355 - 14049.78mg/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; y=0.95x + 1.22. Precision was assessed with a protocol based on CLSI (EP05-A2) using 3 pooled sera samples with 2 runs of duplicate testing per day on 3 kit lots and 3 analysers over 21 days, results are shown in table 1. Linearity was assessed by assaying a serially-diluted patient sample pool across greater than the width of the reportable measuring range and comparing expected versus observed results, the assay was shown to be linear over the range of 12.594-244.847mg/L which exceeds the assay measuring range; weighted linear regression gave y = 1.00x - 0.30 (r = 1.000) and analysis using StatisPro software in accordance with CLSI guidance confirmed linearity . Interference was tested by spiking base pools at three levels (83.36, 133.38 and 165.46mg/L) with 200mg/L bilirubin, 5g/L hemoglobin, 2000mg/dL intralipid and 1000mg/dL triglycerides and comparing with a negative control. Interference at all analyte concentration of <1.5% was seen with haemoglobin and bilirubin, for intralipid and triglycerides the interference was less than <4%. We conclude that the IgD assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.

Table 1: Precision of IgD Optilite assay

Analyte concentration	23.27mg/L	110.20mg/L	165.27mg/L
Total precision (%CV)(Acceptance <10%)	4.0%	3.2%	3.2%
Within run precision (%CV)(Acceptance <5%)	2.7%	2.1%	2.0%
Between run precision(%CV) (Acceptance <8%)	2.9%	1.6%	1.6%
Between day precision(%CV) (Acceptance <8%)	0.0%	1.8%	1.9%

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Multivariable Graphic and Numerical Statistical Techniques for Comparing the Performance of Two Hematology Instruments. A Practical Example with Sysmex EX-5000®and Sysmex XT4000®.

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Background: Sysmex XE-5000® and Sysmex XT-4000® determine WBC, platelets, reticulocytes, and RBC counts, HGB, HCT, and the WBC differential for patient specimens in an 'open' and 'closed' mode. Consequently, for evaluating their performance two modes for each analyte have to be simultanously compared. We employed multivariable statistical models and their graphic representations to asses simultaneously the performance of the two instruments operating in the two modes. ***Methods***: The precision was evaluated by assaying quality control material (E-CHEK® (XE) and (XT) Lot # 2201, Sysmex) with five independent runs for each day for five consecutive days. Linearity was evaluated by assaying at least five levels of linearity material (Range Check XII® Lot # 2184, and Lot # 2192, Sysmex) with five independent runs in one day. The performance with patient specimens was assessed by assaying 52 patient specimens in parallel and within 30 minutes. The observations were transferred to Minitab® (Version16, Minitab Inc.) and analyzed with the general linear model (GLM) to compare multiple means, the polynomial regression model, their diagnostics and their graphical representations. *Results*: For the precision study the GLM showed that while for WBC, platelets, and HGB there were no statistically significant differences between instruments and modes (P>0.05) for RBC, HCT and reticulocytes showed statistically significant differences between instruments and/or modes (P<0.001). The parallel box plots by day, instrument, and mode clearly illustrated the differences between the means of instruments and modes. The polynomial regression analysis showed linear performance (pure error test, P>0.05) and statistically significant differences between instruments for RBC, HCT, and reticulocytes regression lines (P<0.001). Since the differences could have been significant for patient care, patient specimens were assayed in parallel with both instruments. The regression model corroborated the differences between instruments: RBC y=-0.2+1.1x; HCT y=0.2+1.1x; reticulocytes y=0.05+0.98x. The plots of the differences showed for XT-4000 a mean bias of 4% for RBC (minimum 0.7 maximum 7.8%), and of 5.5% for HCT (minimum 1.4 maximum 8.3%) and no clinically significant bias for reticulocytes (mean 0.01, minimum -0.5, maximum 0.4). Since the XE-5000 was considered our reference instrument the manufacturer representative was consulted to recalibrate the XT-4000. After recalibration, comparison with patient specimens showed no clinically significant differences for XT-4000; RBC: mean bias 1.5% (minimun 0%, maximum 3%), HCT mean bias -0.8% (minimum -2.5%, maximun 1.5%). *Conclusion*: These results clearly showed that the graphical and numerical multivariable statistical analysis techniques could immediately demonstrate differences between instruments performance. Further multivariate and univariate analysis on patient specimen determined that the differences were clinically significant. The secondary instrument was recalibrated, and the differences were clinically acceptable. Finally, the availability of statistical software, such as Minitab®, allowed the laboratorian to perform numerical and graphical data analysis simultaneosly on several instruments and parameters.

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Myeloperoxidase enzyme deficiency detected by peroxidase cytochemistry method available in Hematology system

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Background: The myeloperoxidase (MPO) is an enzyme found in white blood cells (neutrophils, monocytes and eosinophils). This enzyme is involved in the killing of several micro-organisms and foreign cells, including bacteria, fungi, viruses, red cells, and malignant and nonmalignant nucleated cells. Despite the primary role of the oxygen-dependent MPO system in the destruction of certain phagocytosed microbes, there are subjects with total or partial MPO deficiency. Infectious diseases, especially

with species of Candida, have been observed predominantly in MPO-deficient patients who also have diabetes mellitus, but the frequency of such cases is very low, less than 5% of reported MPO-deficient subjects. This study investigates the incidence of the myeloperoxidase deficiency (MPO-def) in patients of LANAC Laboratory.

Methods: Approximately 40.000 blood counts were performed from October to December, 2014, by LANAC Laboratory using the equipment ADVIA® Hematology (Siemens Healthcare Diagnostics). 22 samples were identified with MPO-def through the flags and charts released by the equipment.

Results: The report of these 22 samples contained the following observation: "Sample result suggestive of Myeloperoxidase Deficiency" because they presented the MPO Def flag, MPXI and Perox cytochemistry characteristic, revealing weak staining of the cells by hydrogen peroxide method "cytochemistry of peroxidase" available in electronic analyzers ADVIA Hematology line. In this method, leukocytes are identified based on the size and intensity of the peroxidase reaction. As neutrophils, eosinophils and monocytes are stained revealing the existing concentration of peroxidase. Other white blood cell does not contain peroxidase and thus do not stain. The cytology of available Perox therefore has a characteristic saturation area associated with the flag MPO Def, allows the analyst to suggest MPO deficiency on the report. These 22 samples represent 0.05% of this lab routine during the study period.This profile had prevalence in pregnant patients, children and patients who were making use of some anti-inflammatory.

Conclusion:

Through the peroxidase cytochemistry Methodology available in ADVIA Hematology line, it was possible to suggest in report the deficiency of the enzyme myeloperoxidase for these 22 patients. This observation certainly contributes to the clinical conduct, making this methodology an excellent alternative for clinical laboratory routine.

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Evaluation of an IgG4 assay with increased measuring range for use on the Binding Site SPAPLUS® automated analyser.

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Measurement of IgG4 is useful in the detection and monitoring of primary immunodeficiency (PID) disorders, but is increasingly used to detect disorders associated with hyper-elevation of serum IgG4, notably autoimmune pancreatitis (AIP). Here we describe the evaluation of an improved IgG4 assay for use on the Binding Site SPAPLUS platform, incorporating an increased measuring range and prozone protection to facilitate measurement of AIP samples. The assay measuring range is 0.030 - 2.6g/L using a 1/20 sample dilution, with sensitivity at 1/1 of 0.003g/L and an upper limit of 13g/L using automatic sample redilution (1/100). Prozone protection is programmed to monitor reaction kinetics and identify samples with a fast, early response indicative of antigen excess. Such samples are indicated with a 'P' flag and are automatically rediluted if preset criteria are exceeded. Precision was assessed according to CLSI (EP05-A2) using 8 samples (0.02 - 4g/L), on three kit lots and three analysers over 21 days. Interference testing was carried out at five analyte concentrations with Intralipid (1%), triglyceride (1%), bilirubin (200mg/L), haemoglobin (5g/L) and a panel of 17 commonly prescribed drugs. Linearity was tested across a range exceeding the reportable range using a series of dilutions of elevated and depleted pools. Prozone functionality was challenged on three kit lots by running 60 samples with an antigen concentration up to an equivalent of 45g/L. Correlation to the original SPAPLUS IgG4 kit (measuring range 0.030 - 0.850g/L) was carried out using 229 serum samples, incorporating 72 disease state samples, of which 20 were IgG4 deficient and 34 were above the normal range, including AIP positive samples up to a level of 8.5g/L. The precision testing returned within run CV's of 0.9% (0.024g/L), 1% (0.030g/L), 4.0% (0.056g/L), 1.7% (0.298g/L), 1.2% (0.756g/L), 1.2% (0.998g/L), 1.8% (1.889g/L) and 2.2% (4.216g/L). Total precision CV's were 7.2% (0.024g/L), 6.7% (0.030g/L), 10.1% (0.056g/L), 5.7% (0.298g/L), 5.2% (0.756g/L), 6.3% (0.998g/L), 5.8% (1.889g/L) and 6.6% (4.216g/L). No significant interference (<10%) was observed with any of the chemical or biological interferents tested. Linearity was demonstrated across the range 0.024 - 2.7g/L with a correlation y=1.0092x+0.0511. Prozone protection was demonstrated to a minimum of 45g/L with three kit lots, on two occasions each on separate analysers. All elevated samples from 2.6 - 45g/L were correctly reported as >2.6g/L or 'P' flagged and auto-rediluted at 1/100. Analysis of reaction kinetics confirmed there was no undetected antigen excess. Correlation to the original SPAPLUS IgG4 kit using Passing-Bablok regression returned an agreement of y=0.99x+0.00 on a sample range from 0.016g/L to 8.5g/L. We conclude that the extended range IgG4 assay for the Binding Site SPAPLUS shows good performance and agreement with existing assays and allows accurate measurement of elevated IgG4 conditions without the possibility of false low results through prozone.

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Performance of a low level albumin assay for use on the Binding Site Optilite® turbidimetric analyser.

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Serum is the predominant source for albumin present in the cerebrospinal fluid (CSF), as regulated by the permeability of the blood-CSF barrier and CSF flow rate. An increase in CSF protein levels can be indicative of barrier dysfunction and/or local synthesis of immunoglobulin within the central nervous system. Early detection and treatment of nephropathy is important in preventing renal failure in insulin-dependent diabetics. Elevated urinary albumin concentration is a good indicator of glomerular damage in such patients. Increased albumin excretion is also a marker of future cardiovascular problems in non insulin-dependent diabetes mellitus and also occurs in other chronic conditions such as hypertension, malignancy and chronic obstructive airways disease. Here we describe the performance of a low level albumin assay for measurement of CSF and urine samples on the Binding Site's Optilite analyser. Precision was verified using a protocol based on CLSI (EP05-A2) testing samples spiked with purified albumin to give CSF levels of 145.49, 281.51, 439.90, 593.11 and 975.24mg/L and urine levels of 22.98, 39.04, 153.40, 275.05, 1490.18mg/L, with 2 runs of duplicate testing per day on 3 kit lots and 3 analysers for 21 days. The acceptance criteria for total precision was a CV of <10%. For both CSF and urine samples at all levels the total precision gave CV's of ≤8.2%. Linearity was verified by assaying a serially-diluted CSF or urine sample across greater than the width of the reportable measuring range and comparing expected versus observed results, the assay was linear for CSF over the range of 9.23-373.11mg/L. For urine, the assay was linear over the range of 8.09-397.77mg/L. This provides an assay measuring range of 11-332mg/L at neat, with an upper limit of 16600mg/L utilizing auto-dilutions. CSF interference was tested by spiking CSF pools at two levels (159.52 & 371.26mg/L) with 200mg/L bilirubin and 5000mg/L haemoglobin. Urine interference was tested by spiking urine pools at two levels (29.33mg/L and 495.80mg/L) with 200mg/L bilirubin, 200mg/L ascorbic acid, 1000mg/L total protein and 250mg/L haemoglobin. All results were compared against a negative control. Interference at all analyte concentrations in CSF was <8% and in urine was <11%. Correlation to the Siemens BNII albumin CSF assay was performed using 124 samples (total range 30.3-1340mg/L). This demonstrated acceptable agreement when analyzed by Passing-Bablok regression; y=1.01x + 10.63. Correlation to the Siemens BNII albumin urine assay was performed using 162 samples (total range <2.12-7350mg/L). This demonstrated acceptable agreement when analyzed by Passing-Bablok regression; y=1.06x - 0.37. We conclude that the low level albumin assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.

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The frequence of factor V de Leiden and prothrombin gene mutation (PTM) in results from a large Brazilian laboratory database.

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Background: The factor V Leiden (FVL) is the most common inherited risk factor that predisposes venous thromboembolism (VTE) in Caucasian individuals. The prevalence of the mutation (G1691A) varies between different ethnic groups and geographically. The FVL is very rare in African blacks, Asians, and native populations of America and Oceania. On the other hand, the highest prevalence is found in European with allele frequency ranging from 1.4% to 7%. The presence of FVL may be associated with VTE and possibly obstetric complications. Sometimes it is assumed that the identification of a positive result will always be useful, but if positive, can lead to anxiety by the diagnosis of a genetic disease. Although thrombophilia testing can identify positive cases, the evidence that an individual has the diagnosis rarely influences the clinical management.

Objectives: To evaluate the incidence of FVL and prothrombin gene mutation (PTM) in heterozygous and homozygous states, and the association of the two mutations in 3065 samples evaluated in a Brazilian laboratory.

Methods: We analyzed 3065 samples in 28 days in 2014; 1973 with the search for FVL mutation and 1092 samples for the PTM. The prevalence of the presence of mutations in heterozygous and homozygous states, and its association in the same individual was evaluated.

Results: The prevalence of the FVL mutation, searched in 1973 samples was 5.06% (n = 100) in heterozygous and 0.05% (n = 1) in homozygous state. The mutation of the prothrombin gene (1092 samples) was heterozygous in 4.39% (n = 48), homozygous 0.18% (n = 2), and the double heterozygous (FVL and PTM) was found in the same individual in 0.097% of samples (n = 3 in 3065 samples).

Conclusion: Heterozygous FVL mutation is associated with 4 to 5 times increased risk of VTE and prothrombin gene mutation 2 to 3 times. The presence of mutations in homozygous and double heterozygous for FVL and PTM indicate a much higher risk of thromboembolic events, and the change in medical management in these individuals with a previous thromboembolic event is necessary, requiring anticoagulation indefinitely and need for antithrombotic prophylaxis during pregnancy and postpartum. The prevalence of the FVL mutation in the Caucasian population is 5%, similar to that found in our sample. In a previous study in the Brazilian population, the incidence of FVL and PTM in heterozygous states was observed in 1.2% and 0.5%, in 400 blood donors. Since the Brazilian population is mixed, we can conclude that the tested individuals at our study should correspond to a population with a higher incidence of VTE or obstetric complications.

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Development of A Novel Device to Assess Hemostatic Function from Whole Blood

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Background: Previous studies have shown the benefit of rapid information to guide treatment of excessive bleeding in the clinic, including targeted transfusion and better outcomes. However, no point of care (POC), comprehensive, whole blood assay exists. Current tools provide limited information slowly and often require skilled technicians. HemoSonics LLC is developing the QuantraDx™ point-of-care (POC) system, which provides time critical, quantitative information on hemostatic subsystems in whole blood.

Aims: To demonstrate the ability of the QuantraDx to provide rapid and comprehensive results on the treatable causes of bleeding at the POC.

Methods: The QuantraDx applies ultrasound technology to estimate the shear modulus (stiffness) of whole blood during the process of coagulation. The device incorporates a multi-well cartridge with embedded reagents. The clotting time and stiffness values measured in each well are used to quantify the treatable components of hemostasis. QuantraDx reproducibility was assessed with kaolin and thromboplastin activation with a novel blood-derived control sample (n = 21 for kaolin, n = 28 for thromboplastin) and whole blood (n = 11 for kaolin, n = 12 for thromboplastin) from healthy volunteers. Additional whole blood samples (n = 5) were tested with varying amounts of abciximab (0, 24, 32, 64ug/ml), a potent platelet inhibitor, to challenge the device with lower stiffness clots and assess accuracy at low dynamic range threshold.

Results: Control tests show average clotting times of 3 to 5 min with kaolin or thromboplastin with 3.5 and 9.4% CVs respectively. Average clot stiffness was 690 Pa for kaolin (8.1% CV) and 781 Pa for thromboplastin (10% CV). When testing with whole blood, intra-cartridge average clotting time for kaolin activation was 3 minutes (2% CV), while average clot stiffness with thromboplastin was 2944 Pa (4.75% CV). Increasing the amount of abciximab in the sample progressively reduced clot stiffness from ~2900Pa to ~300Pa (4.5% CV), equivalent to a 90% reduction in clot stiffness.

Conclusions: The QuantraDx provides quantitative measures of clot time, overall clot stiffness and platelet and fibrinogen contribution to clot formation in 15min with low variability. A multi site clinical study is planned to further evaluate clinical performance.

A-195

Heparin Interference in Anti Xa Assays for Rivaroxaban and Apixaban

K. Finnegan[1], G. A. Cutsforth[2]. *[1]Stony Brook University, Stony Brook, NY, [2]Diagnostica Stago Inc, Vernon Hills, NJ*

The purpose of this study was to evaluate the degree of interference from therapeutic and supra-therapeutic levels of unfractionated and low molecular weight heparins (UFH, LMWH) on rivaroxaban and apixaban assays. The package insert for the rivaroxaban and apixaban calibrators states "other anticoagulants (vitamin K antagonist, UFH, LMWH, fondaparinux, direct thrombin inhibitors...)" can interfere with the assay. Rivaroxaban and Apixaban are target specific oral anticoagulants recently approved by the FDA for use in specific indications. Rivaroxaban (Xarelto®) is approved for stroke prevention in atrial fibrillation patients, prevention and treatment of venous thromboembolism (VTE) and for VTE prevention after total hip and knee replacement. Apixaban (Eliquis®) is approved for stroke prevention in atrial fibrillation patients. The anti-Xa activities of rivaroxaban and apixaban are higher than the activities of UFH and LMWH because they are specific for Factor Xa. Therefore, measuring the new anti-Xa anticoagulants with UFH or LMWH calibrators and controls is not possible. This protocol is designed to measure the anti-Xa activity of therapeutic UFH and LMWH levels in the assays calibrated for rivaroxaban and apixaban. A total of 88 samples were analyzed. Samples were collected from the clinical laboratory identified by prolonged aPTT and samples previously run for anti-Xa assay. Samples with anti-Xa levels above the therapeutic range were purchased: 20 samples from 1.5-2.0, 10 samples from 2.0-2.5, and 10 samples from 2.5-3.0 IU/ml . Each sample was analyzed by aPTT and anti-Xa. Three calibration curves were used in the anti-Xa assay: a hybrid curve for UFH and LMWH, rivaroxaban and apixaban. Assays were performed in singlicate and the values were compared by linear regression. Linear regression analysis demonstrated that there was no correlation between the APTT and the anti-Xa results using either of the three calibration curves. When the heparin samples were assayed using either the rivaroxaban or apixaban calibration curves, heparin anti-Xa values ≤ 0.5 IU/ml produced values ≤ 25 ng/ml, the lower limit of quantification for the assay. Heparin samples ≥0.5 IU/L/ml demonstrated a linear relationship to both rivaroxaban and apixaban values with a correlation coefficient of .956 and 0.959 respectively. The conclusion of the study is that the overlap of UFH and LMWH therapy and the use of rivaroxaban or apixaban will result in anti Xa results that are due to combined anti Xa activity of the anticoagulants.

A-196

DxH PROService Solutions: A Case Study - Proactive Monitoring of System Data

J. Colom, C. Ramirez, J. Lu, J. Castillo, S. Guntupalli, P. ONeil. *Beckman Coulter, Miami, FL*

Background:

The UniCel DxH Series of Coulter Cellular Analysis Systems provides CBC, WBC differential, NRBC and reticulocyte analysis. Turnaround time is critical and physicians require prompt and accurate results. An inoperable system directly impacts the laboratory's turnaround time and it's essential to provide a solution that efficiently analyzes, in near real time, large amounts of system data for root cause analysis.

PRO*Service* is a remote management and diagnostics system that facilitates the continuous transfer and analysis of system performance data from Beckman Coulter instruments through Beckman Coulter's PRO*Service* servers. The PRO*Service* framework is designed for real time monitoring of instrument system functions and includes large-scale, multi-dimensional data analysis over time. Incoming data is channeled through PRO*Service* applications where the output is used by Beckman Coulter Technical Service and Product Development engineers to proactively diagnose and resolve system issues.

Methods:

Instrument subsystem performance data from over 1800 DxH 800 instruments is collected, collated and analyzed through PRO*Service* applications utilizing a Hadoop server to manage, standardize, analyze and graphically present large scale instrument data analysis. The analysis identifies the primary drivers and related parameters by region over time.

Results: A data set of ≈120 million data records was analyzed. Figure 1, identifies graphically, the three key subsystem parameters that correlate to the increased calls. The data details, by parameter (Y axis), over time (X axis) and by system (red require maintenance; blue are properly maintained) specific system information for effective problem identification and resolution. The data also documents the corrective measures implemented were effective.

Conclusion: The PRO*Service* Solution provides real time system monitoring on the DxH series including expanded data analysis and identification capabilities facilitating large-scale, complex investigations. Several programs working in conjunction enable efficient, large scale data analysis and management for the proactive identification of factors contributing to system down time.

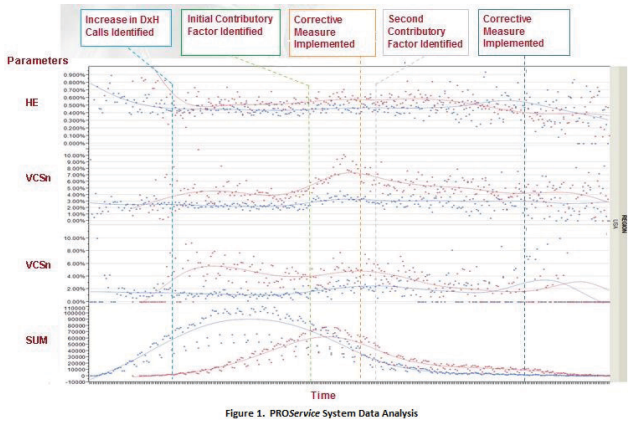


Figure 1. PROService System Data Analysis

A-197

Reducing Non-conforming Laboratory Samples through Educational Activities: A Quality Initiative

H. K. Al Ghaithi. *Sultan Qaboos University Hospital, Muscat, Oman*

Background: Patient safety and well-being mandates that medical laboratories maintain accuracy and reduce errors in all phases of specimen analysis. However, despite all efforts, the most testing errors occur in the pre-analytical stage, with specimen identification and labelling being the biggest culprits. Incorrect identification of a blood sample can result in incorrect treatment, wrong blood transfusion and may even lead to the patient's death.

Aim: The objective of this study was to determine the incidence of non-conforming blood samples in the general haematology laboratory, at the Sultan Qaboos University Hospital from 2007 to 2014 and by taking appropriate corrective action, evaluate the efficiency of the preventive measures that were implemented.

Method: Data of the specimen records for the year 2007 to 2014 were collected and analyzed. Non conforming samples were defined using the following categories: clotted, haemolyzed, wrong anticoagulant, quantity not sufficient, wrong or no label and no sample received. Based on the available records for the year 2007, corrective actions were initiated in the year 2008, including several educational training activities like proper labeling, collection and handling of blood samples.

Results: A total of 63,800 laboratory specimens were received in 2007 with 184 (0.29%) non conforming specimen. After starting the corrective measures in 2008, we found that the total specimen received with the percentage of non conforming samples were respectively 81,024 (0.29%), 93,763 (0.25%), 102,400 (0.21%), 114,588 (0.23%), 116,820 (0.11%), 122,316 (0.08%) and 131,064 (0.07%) for the years 2008, 2009, 2010, 2011, 2012, 2013 and 2014.

Figure 1: Percentage of Non-Conforming samples in General Haematology Laboratory from 2007 to 2014

Figure2: Total number of incorrectly labelled samples in General Haematology Laboratory from 2007 to 2014

Summary/Conclusion: Despite the increase in the specimen numbers through the years up to 2014 by 48.6%, the study showed a clear reduction in the non conforming specimen from 2007 to 2014 by 0.22%, indicating a 75.9% improvement. Specimen identification errors showed a reduction of 0.056% with 96.55% improvement. The study demonstrates the value of the educational activities taken by the haematology laboratory staff and emphasizes the need for providing attention to specific categories of the non conforming specimen samples to get better results.

A-198

A Validation Study for the Quantitative Measurement of Prothrombin Time/International Normalized Ratio (PT/INR) test on the Xprecia Stride™ Coagulation Analyzer* for Warfarin Monitoring.

C. Lessard, I. Akunovich. *Siemens Healthcare Diagnostics, Norwood, MA*

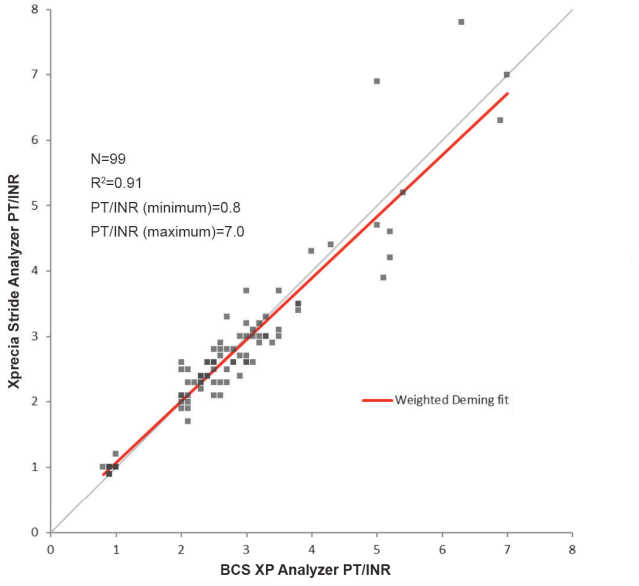
Background: The Xprecia Stride™ Coagulation Analyzer from Siemens Healthcare Diagnostics (SHD) is a novel, handheld POC device that generates rapid PT/INR results from fingerstick samples for oral anticoagulant therapy monitoring (OAT). This external validation study, conducted under the International Conference on Harmonization/Good Clinical Practice (ICH/GCP) guidelines, assessed the clinical substantial equivalence of the Xprecia Stride analyzer PT/INR test against an established laboratory hemostasis method (BCS® XP System).

Methods: Test methods were based on Clinical Laboratory Standards Institute (CLSI) guidelines. Evaluation of Precision Performance of Quantitative Measurement Methods (EP05-A2), Measurement Procedure Comparison and Bias Estimation Using Patient Samples (EP09-A3) and Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory (EP28-A3c).

Results: Weighted Deming regression analysis yielded a slope of 0.95 and an intercept of 0.12, with R^2=0.91 across the range of 0.8 to 7.0 INR. Repeatability using whole blood demonstrated %CVs were ≤5.9 across the reportable range. LQC at two levels demonstrated repeatability precision % CVs that were ≤3.6 and within laboratory %CVs that were ≤7.0. The Expected Range for the PT/INR on the Xprecia Stride analyzer was 0.9 to 1.1 for subjects not on OAT.

Conclusion: The Xprecia stride analyzer PT/INR test results were substantially equivalent to the BCS XP system.

*Not available for sale in the U.S. Product availability varies by country



A-199

Application of Procalcitonin and neutrophil VCS parameters in the diagnosis of bacterial infection in non-small cell lung cancer postoperative patients

L. Liu[1], N. Cong[2], D. Li[3], X. Song[4]. *[1]Clinical Laboratory, Shandong Cancer Hospital, Ji'nan, China, [2]Department of Surgical Oncology (Interventional Therapy), Shandong Cancer Hospital, Ji'nan, China, [3]Clinical Laboratory, Shandong Cancer Hospital, ji'nan, China, [4]Clinical laboratory, Shandong Cancer Hospital, Ji'nan, China*

Background:

We explored the predictive value of procalcitonin and VCS parameters of neutrophil in infected non-small cell lung cancer patients after operation.

Method:

To analysis treated 37 infected NSCLC patients after thoracotomy operation in Department of thoracic surgery from 2014 June to October, collected venous blood samples 1 - 3 days before operation and first days after surgery , analyzed blood cells by Beckman Coulter LH750, and collected neutrophil VCS parameters, including the mean neutrophil volume (MNV), neutrophil volume distribution width (MNV-SD), mean neutrophil conductivity (MNC), neutrophil conductivity distribution width (MNC-SD), mean neutrophil scatter(MNS) and neutrophil scatter distribution width(MNS-SD) ; and detected serum procalcitonin (PCT) concentration. Meanwhile obtained the area under the ROC curve, to evaluate the ability of each marker in the diagnosis of bacterial infection.

Results:

To compare the indicators of WBC ,neutrophil VCS parameters and serum PCT before and after the operation, the differences were statistically significant (P<0.05). Area under the ROC of MNV, MNV-SD and serum PCT was respectively 0.95,0.946, 0.892, this suggest that they have a good diagnosis effect. The positive rates were compared between Serum PCT, MNV and MNV-SD in diagnosing infection, there were not significant differences (P>0.05).

Conclusion :

The serum PCT, neutrophil MNV and MNV-SD were significant in the early diagnosis of bacterial infection in the thoracotomy NSCLC patients.

A-200

Variant hemoglobin prevalence in Brazil and its regions: An update from a large laboratory database.

A. C. Rocha, M. D. C. Freire. *DASA, Cascavel, Brasil, Cascavel, Brazil*

Background:

Hemoglobinopathies are caused by genetic structural abnormalities of the chain of hemoglobin, affecting its functionality, that occurs when there are changes in genes encoding trainers for the formation of hemoglobin variants. The structural abnormalities of hemoglobin are among the most common genetic diseases in human populations. Brazilian population, because of miscegenation, show great diversity of gene translocations in the formation of hemoglobin, which can complicate the clinical and laboratory diagnosis of these diseases. This study aims to assess the prevalence and diversity of hemoglobinopathies in Brazilian population, and its distribution among country regions.

Methods:

We actively searched LIS database, from January to December 2014, of a national central laboratory in Brazil, that receive samples for all country regions. Hemoglobinopathy diagnose was determined by hemoglobin High-Performance Liquid Chromatography (HPLC) results, obtained by Variant II Beta Thalassemia Short Program by Bio-Rad.

Results:

From 148,317 individual hemoglobin HPLC results, 21,451 showed hemoglobinopathies, and were classified by hemoglobin found and country region.

We observed that 6.86% were suggested positive for sickle cell trace(AS) , 2.70% were suggested positive for beta thalassemia screening and 2.13% were suggested positive for C Hemoglobine trace. The incidence of hemoglobinopathies in females was higher, 13,271 (61,87%), when compared to males, 8,180 (38,13%). Northeast region showed 8,528 (5.75%) OF hemoglobin variant results, 6,178 (4.17%) of positive results were found in Southeast patients, 2,866 (1.93%) in the Central West patients, 2,753 (1.86%) in South and 1,126 (0.76%) in North of Brazil.

Conclusion:

Thus these results show the great variability of hemoglobin variants presented in Brazil, thus aiming to provide an update on regional and national hemoglobinopathy epidemiology. The higher prevalence in the Southeast and South of Brazil can be explained by the demographic formation of the area, those regions have the largest population concentration in the country, built by the great mass immigration of Africans, Italians and Oriental peoples in the process of colonization of Brazil. The Northeast region of the country has the largest prevalence of variations of S hemoglobin in the study, even as the Southeast, which can be explained because of the ethnicity of the population, mostly black, which is more propitious for developing the S hemoglobin. In turn, the North region concentrates the lower portion of the findings, which may be due to the low population of the region.

A-201

Evaluation of Serum IgM Hevylite® assays; IgM Kappa and IgM Lambda for use on the Binding Site Optilite® analyser

J. R. Kerr, S. K. Ahaliwal, S. Kausar, F. Murphy, S. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Elevated monoclonal protein production is indicative of an underlying abnormality such as MGUS, multiple myeloma & other disorders. SPE densitometry is recommended to quantify monoclonal proteins however, monoclonal IgM can be obscured by other proteins on SPE gels. Measurement of IgM Kappa (IgMκ) and IgM Lambda (IgMλ) may give a more accurate representation of tumor production. Moreover, calculation of the IgMκ/IgMλ ratio and comparison with values found in normal subjects can give a more sensitive indication of clonality. Here we describe the performance characteristics of the IgM Kappa and IgM Lambda Hevylite assays for use on the Binding Site's Optilite analyser. Precision was determined following CLSI (EP05-A2) using 8 pooled sera samples in the range 0.10-3.94g/L for IgMκ and 0.06-3.90g/L for IgMλ with two duplicate runs a day on 3 kit lots and 3 analysers over 21 days. Acceptance criteria were <10% CV for total precision and <5% CV for within run precision. Total precision was ≤6.1% CV for IgMκ and ≤5.0% CV for IgMλ at all levels tested. Within run precision was ≤1.7% CV for IgMκ and ≤1.5% CV for IgMλ at all levels tested. Linearity was verified by assaying a serially-diluted patient sample pool across the width of the measuring range and comparing expected versus observed results. Acceptance was defined as recovery within 10% at each level. The assay was shown to be linear using StatisPro software in accordance with CLSI guidance over the range 0.14-5.80g/L for IgMκ and 0.16-4.98g/L for IgMλ. This validated measuring ranges of 0.2-5.0g/L for IgMκ and 0.18-4.50g/L for IgMλ at 1/10 with sensitivity of 0.020g/L and 0.018g/L and upper limits of 150.0g/L and 135.0g/L respectively, utilizing auto-redilutions. Interference testing was carried out using 200mg/L bilirubin, 5g/L haemoglobin, 1500mg/dL intralipid, 1000mg/dL triglycerides and 15 other common drug and metabolite interferents spiked into serum pools at 5 levels covering ranges of 0.10-2.48g/L for IgMκ and 0.06-1.47g/L for IgMλ with comparison to negative controls. Acceptance was <10% difference to the negative control. Interference was found to be acceptable for all interferents at all levels on both assays. Correlations to the Binding Site IgMκ and IgMλ assays for the SPAPLUS were performed using 263 samples on IgMκ (range 0.02-51.75g/L) and 249 samples on IgMλ (range 0.03-33.03g/L. Acceptable agreement was seen when analyzed by Passing-Bablok regression; kappa gave a slope of y=0.93x + 0.01 and lambda gave y=1.01x - 0.00. We conclude that the Optilite IgM Hevylite assays provide a reliable, accurate & precise method for quantifying IgM Kappa & IgM Lambda and show good agreement with existing turbidimetric assays.

A-202

Prevalence, clinical impact of NPM1 and FLT3-ITD mutations in acute myeloid leukemia

H. Gou, J. Zhou, X. Hu, M. Shang, J. Zhang, Z. Zhao, X. Song, Y. Zhou, X. Lu, B. Ying. *West China Hospital, Sichuan University, Chengdu, China*

Background: Internal tandem duplications (ITDs) of the FLT3 gene mutations and the nucleophosmin (NPM1) gene mutations, most of which are frequently found in acute myeloid leukemia (AML), associate with clinical outcomes. This study aims to analyze the prevalence and the clinical impact of FLT3-ITD and NPM1 mutations.

Methods: We retrospectively analyzed 313 patients with AML, in which there are 255 cases with newly diagnosed de novo AML, in West China Hospital, Sichuan University, who receive with standard chemotherapy induction therapy consisting of cytarabine and daunorubicin except the patients with FAB M3.

Results: FLT3-ITD mutations were found in 9.9% and NPM1 mutations in 18.1% of the patients, and there were 12 cases with the FLT3-ITD mutations and NPM1 mutations. FLT3-ITD aberrations were more found in patients with FAB M1, and fewer were found in patients with FAB M5 and M6, and NPM1 aberrations were more found in patients with FAB M1 and M5, and fewer were found in patients with FAB M3 and M6. The presence of FLT3-ITD was associated with high white blood cell count (p<0.01), high bone marrow blast count (p=0.02), high Peripheral blood blast count (p<0.01) at diagnosis. Deleting the patients with FAB M3, patients with FLT3-ITD had a worse influence on DFS (Disease-free survival) (p<0.01). The cases expressed NPM1 had a better influence on the CR (Complete remission) rate (p=0.01) when give the first standard chemotherapy induction therapy. But FLT3-ITD and NPM1 had no influence in OS (overall survival). NPM1 +/FLT3-ITD- in AML associated with high CR(p<0.02).

Conclusions: Patients with FLT3-ITD seem to have a poor prognosis, and mutations in NPM1 have a good influence on prognosis. This may lead to a refined disease classification and help to provide more pathway-targeted therapies.

A-203

Complex Biological Profile of Hematological Markers and Indices Across Pediatric, Adult, and Geriatric Age: Establishment of Robust Reference Intervals based on the Canadian Health Measures Survey

J. E. Raizman[1], Y. Chen[1], V. Higgins[1], M. Nieuwesteeg[1], M. Abdelhaleem[1], S. L. Wong[2], D. Blais[2], K. Adeli[1]. *[1]CALIPER Program, Divisions of Clinical Biochemistry and Hematopathology, Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada, [2]Health Analysis Division, Statistics Canada, Ottawa, ON, Canada*

Background: The Canadian Health Measures Survey (CHMS) is a project designed by Statistics Canada with the objective to collect comprehensive nationwide health information and blood specimens from children and adults in the household population. The Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) has collaborated with Statistics Canada to develop a comprehensive database of reference intervals for haematological biomarkers of disease across healthy pediatric, adult, and geriatric age groups. This study aimed to address the challenges of obtaining fresh whole blood specimens for analysis of hematological marker reference intervals and provides a robust dataset based on a very large cohort of children and adults.

Methods: Between 2007-2011, the CHMS collected health data from approximately 12000 respondents aged 3 to 79 and obtained fresh whole blood specimens. Sixteen hematology markers (including calculated parameters) were measured using the Beckman Coulter DxH300C or HmX analyzers on fresh whole blood. After exclusion criteria were applied and outliers were removed, statistically relevant age and gender partitions were determined and reference intervals, including 90% confidence intervals, were calculated using CSLI C28-A3 guidelines.

Results: Concentrations of hematology markers showed dynamic changes from childhood into adulthood, as well as between genders, necessitating distinct partitions throughout life. Most age partitions were found during childhood, reflecting the hematological changes that occur during growth and development. Hemoglobin, red blood cell (RBC) count, hematocrit, and indices (MCV, MCH, and MCHC) increased with age, but as expected females had lower hemoglobin and hematocrit starting at puberty. Platelet count increased with age and required multiple gender partitions during adolescence and adulthood. White blood cell (WBC) count and fibrinogen also declined with age, requiring distinct age and gender partitions. Eosinophil and basophil levels were low, as these markers were not detected in many of the participants. Upper limits, however, were highest in early life.

Conclusion: The robust dataset generated in this study has allowed observation of dynamic biological profiles of several hematology markers and resulted in the establishment of comprehensive age- and sex-specific reference intervals that may contribute to accurate monitoring of pediatric, adult, and geriatric patients. The rich dataset collected also allows for data mining using a systems biology approach, and therefore, has the potential to uncover relationships between hematology biomarker values, clinical outcomes, and risk factors for many disease states.

A-204

Patient posture during phlebotomy influence routine coagulation testing and jeopardize patient safety

G. Lima-Oliveira[1], G. C. Guidi[1], G. L. Salvagno[1], E. Danese[1], E. J. Favaloro[2], G. Lippi[3]. *[1]University of Verona, Verona, Italy, [2]Westmead Hospital, Westmead, Australia, [3]University of Parma, Parma, Italy*

Background: Routine hemostasis testing is an essential part of the clinical decision making process in patients with both hemorrhagic and thrombotic disorders. Several lines of evidence now attest that the quality of hemostasis testing may be dramatically impaired by a number of preanalytical variables, mostly related to collection, transportation, and patient preparation. Nevertheless, the patient posture during phlebotomy is rarely regarded as a potential source of laboratory error. This study was aimed to verify if routine coagulation tests are influenced by patient position during blood collection by venipuncture.

Methods: Three citrate blood tubes were collected by venipuncture from all 19 volunteers without tourniquet application. The first tube was drawn after the volunteers remained in supine position for 25 min, the second after 20 min resting in a comfortable sitting position, and the third after 20 min stationary standing in upright position. The citrated samples were separated at 1500xg for 15 min, at 20°C, then assayed for prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen (Fib) using ACL TOP 700 (Instrumentation Laboratory). Differences on routine coagulation tests due to patient posture during blood collection by venipuncture were reported as bias (Figure 1). The significance of differences was assessed with Mann-Whitney test. Statistical significance was set at $P < 0.05$.

Results:

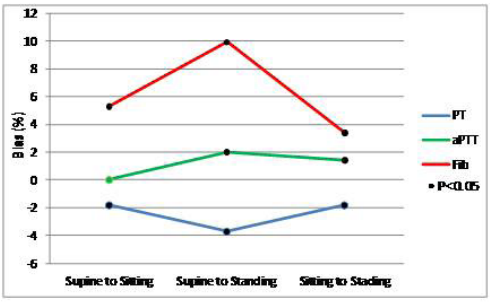


Figure 1. Differences on routine coagulation tests due to patient posture .

Conclusion: Patient posture may have a significant impact on results of routine hemostasis testing, especially if patient position is changed from supine to standing, thus is necessary to standardize the patient position for blood collection by venipuncture. A minimum period between 15 to 20 min of resting in reference position should be observed before collecting diagnostic blood samples. From a organizational perspective, this resting period could seriously impact on the workflow at blood collection sites - but this can be obviated by scheduling blood collections, thus starting an appropriate personalization of laboratory analysis.

Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Immunology

A-205

Application of Assay Appropriate Statistical Analysis Dramatically Improves Turn Around Time

L. J. Ouverson, M. R. Snyder. *Mayo Clinic, Rochester, MN*

Background: Antibody responses to *Streptococcus pneumoniae* are often measured as an assessment of response to vaccination, particularly for patients with suspected immunodeficiency. The concentrations of antibodies are a function of each patient's immunological response to the vaccine; results between patients and between serotypes for the same patient can vary widely. To account for this, our method includes a series of dilutions for each patient, with the final result based on the weighted average of the dilutions. Unfortunately, this is a time-consuming process, taking up to 3 hours for a 54 patient load. The purpose of this study was to find an alternate method for calculation of the weighted average that would allow for improvement in turn-around-time, while maintaining analytical accuracy. Methodology: *Streptococcus pneumoniae* antibodies are measured by dispensing 5 serial dilutions of each patient sample into a 96-well vacuum filter plate. A mixture of Streptococcal polysaccharide-conjugated microspheres (representing each of the 23 polysaccharides in the Pneumovax-23 vaccine) is added to each patient dilution. Following incubation and washing, a fluorescein-conjugated anti-hIgG is added to each dilution. After a second incubation and wash, the mean fluorescence intensity (MFI) for each bead is measured on a Luminex platform. Calculation of patient results is performed using the statistical analysis program StatLia (Brendan Scientific). The MFI for each bead is compared to the MFI of the standards via a 5PL weighted regression, which yields a concentration for each of the 5 dilutions. The final result for each serotype, taking into account the results from each dilution, is determined through a series of weighting, curve fitting, and parallelism calculations. An alternate to StatLia was identified at http://www.itl.nist.gov/div898/handbook; this approach relates the weight as a function of the variability of the response at each standard (W=1/StdDev) A weighting function is obtained that can be related to the MFI at each of the standards. These are plotted Weight(Y) vs MFI(X) and fitted with a power equation (y=cx^b) to obtain a function that will calculate the appropriate weight to assign each of the 5 results. *Streptococcus pneumoniae* antibody results from patients (n=1122) were calculated using both methods and the results for each serotype were compared by linear regression. Results: Linear regression analysis comparing results calculated by StatLia and the new weighting algorithm showed slopes ranging from 0.836 to 1.060 for the 23 serotypes, with correlation coefficients ranging from 0.865 to 0.998. Overall qualitative concordance ranged from 96.8 to 99.2%. The amount of time required to calculate a 54 patient load dropped from 3 hours with Statlia to 2.8 minutes for the new weighting algorithm (N=4).The average turnaround time has improved from 4.8 days to 3.4 days for a representative 25 loads timed before and after the conversion. Conclusion: By researching alternate method of statistical analysis our laboratory was able to improve the turnaround time by more than 1 day while maintaining high quality results. Laboratories should look at their processes and evaluate them on a regular basis to determine if efficiencies can be gained.

A-206

Analytical Validation of an ELISA for the Quantitation of Calprotecin as a Marker of Intestinal Inflammation.

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Background: Fecal calprotectin has emerged as a promising marker for organic diseases of the bowel, including inflammatory bowel disease (IBD). This protein is produced by neutrophils, which are recruited to the intestine during a localized immune response. Upon activation, calprotectin is released, eventually entering the intestinal lumen. The amount of calprotectin present in feces is proportional to the amount of intestinal inflammation. The purpose of our study was to validate an ELISA for the quantification of calprotectin in feces for evaluation of suspected IBD. **Methods:** Fecal samples from patients (n=38, 18-70 years) with suspected or confirmed IBD and healthy donors (n=20, 27-82 years) were collected. Calprotectin was extracted by diluting between 80-120 µg of feces 1:50 with extraction buffer. The mixture was then homogenized and centrifuged. Finally, the supernatant was poured off, aliquoted, and frozen at -20°C until testing. Calprotectin was quantitated using the QUANTA Lite® Calprotectin ELISA (INOVA Diagnostics, Inc.). All experiments were performed manually; end-point colorimetric determinations were read on the Synergy H1 Hybrid plate reader (Bio-Tek). Data analysis was performed using Gen 5 software (Bio-Tek). The packet inserts were followed with no deviations. For validation, intra- and inter-assay precision was assessed by testing 20 replicates of 10 unique extracts (five for intra-assay and five for inter assay) with calprotectin concentrations across the analytical measuring range (15.6 – 500 µg/g). A method comparison was assessed qualitatively and quantitatively by testing 29 extracts on both the QUANTA Lite® Calprotectin ELISA and the PhiCal™ ELISA kit (Eurospital SpA). The reference range (<120 µg/g) was assessed using extracts from healthy donors (n=20). Linearity was assessed by diluting 4 extracts with high calprotectin concentrations (259.5 to 1,453.0 µg/g) until extinction. The limit of blank (LOB) was determined by testing 20 replicates of the assay diluent and using the following equation: Mean of the blank + 1.645 * SD of the blank. The limit of detection (LOD) was determined by testing 20 replicates of one extract with a low calprotectin concentration and using the following equation: LOB + 1.645 * SD of low extract. The limit of quantitation (LOQ) was determined by converting the LOD to µg/g. **Results:** Intra- and inter-assay precision coefficient of variation (CVs) were <9.4% and <10.5%, respectively, for all extracts. The method comparison showed 93.1% qualitative concordance between the two ELISA kits. Quantitative comparison assessed by linear regression showed a slope of 1.21 and R^2 of 0.987. 100% of the results from the normal donors were less than the positive cutoff, confirming the manufacturer's reference range. Linearity of the assay ranged between 81.8%-104.0% at concentrations from 1,453 µg/g-2.0 µg/g. The LOB was 0.130 optical density (O.D.). The LOD was 0.162 O.D. Conversion of the LOD to µg/g led to an LOQ of 9.8 µg/g. **Conclusion:** The QUANTA Lite Calprotectin ELISA kit is both a robust and reliable test for the quantitation of calprotectin in feces. The test may be useful prior to more invasive procedures (i.e. colonoscopy, endoscopy) used to evaluate patients suspected of IBD.

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Quantitation of Eculizumab in serum by microLC-ESI-Q-TOF MS

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Background: Eculizumab (Soliris, Alexion Pharmaceuticals) is a recombinant humanized therapeutic monoclonal immunoglobulin (mAb) IgG2/4 kappa targeting factor C5 in the terminal pathway of the complement cascade. It is approved for treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. As this is a high cost therapy that is at times life-long, effective therapeutic drug monitoring is good practice for monitoring treatment efficacy.

Objective: The goal of this study was to establish that the intact light chain portion of the mAb could be used to quantify eculizumab in patient serum using microLC-ESI-Q-TOF mass spectrometry.

Methods: Stock eculizumab was obtained from the manufacturer at 9.8mg/mL. Dilutions were made in pooled serum to produce standards and controls. Pooled serum spikes at 10 concentrations between 25 and 1000mcg/mL were evaluated over multiple runs to establish LOD, LOQ, and analytical measuring range. Pooled serum spikes at 3 concentrations where used to simulate quality control and give initial data for intra- and inter-assay precision. A subset of neuromyelitis optica patients receiving eculizumab previously quantitated by ELISA was obtained (N=8). Serum aliquots frozen at -20°C were thawed and 20mcL were treated with 200mcL Melon Gel (Thermo Fischer) to purify immunoglobulins. A volume of 20mcL of supernatant, 10mcL 200mM DTT, and 20mcL 50mM ammonium bicarbonate were placed into a 96-well plate and incubated for 15 minutes; 55°C. A volume of 5mcL reduced sample was injected on an Eksigent microLC system and separated by reverse phase chromatography. Mass spectra were collected on an ABSciex Triple TOF 5600 mass spectrometer. Analyst TFv1.6 was used for instrument control and data review.

Results: The average molecular mass of the light chain amino acid sequence, as provided by Alexion, was calculated to be 23,134.75Da. The +11 charge state of eculizumab, verified from spikes in pooled serum, was used to generate extracted ion chromatograms, instead of the average molecular mass of the deconvoluted mass spectrum. The peak observed from the extracted ion chromatogram was summed, and the area under the +11 charge state peak was manually integrated.

Imprecision was evaluated, using the coefficient of variation, for both intra- (N=12 replicates) and inter-assay (N=8 runs) at 75mcg/mL (4%; 6%), 150mcg/mL (7%; 8%), and 300mcg/mL (13%; 12%). A linear response was observed from 75mcg/mL to 400mcg/mL; R2=0.9957. LOD was defined as 50mcg/mL and LOQ as 75mcg/mL. Interference was evaluated by spiking eculizumab along with three other mAbs into serum. All were chromatographically separated and identified by their unique

+11 charge state mass. Accuracy was determined by comparison to ELISA in patient samples; y=1.0972x-33.857; R2=0.8855. Recovery studies also verified the accuracy of the assay; 104%.

Conclusions: We have demonstrated that microLC-ESI-Q-TOF MS can quantify eculizumab using the response for the +11 charge state of the intact light chain. While more clinical studies of patients taking eculizumab are warranted to better define therapeutic thresholds, this analytical approach has the potential to be quickly adaptable to other drugs in this class and to significantly improve patient care.

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CEREBROSPINAL FLUID FREE KAPPA CHAINS AS A POSSIBLE BIOMARKER TO ASSAY RISK CONVERSION TO MULTIPLE SCLEROSIS

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Background:

Multiple Sclerosis (MS) is the most common demyelinating disease of the central nervous system. In most patients, MS initiates with a clinically isolated syndrome (CIS). A certain number of CIS develop the disease over time, while another group of patients never convert to MS. Therefore, the search for novel biomarkers who predict conversion from CIS to MS is of major importance for early treatment in MS. Aim: Assay the predictive value of free kappa chains (FKC) for the conversion of CIS patients to MS, and compare with other parameters: oligoclonal IgG bands (OCGB), IgG index and magnetic resonance imaging (MRI) criteria used in clinical practice.

Methods:

FKC levels were analyzed in cerebrospinal fluid (CSF) from 176 patients: 41 with normal pressure hydrocephalus (NPH), 77 with CIS, and 29 with relapsing-remitting MS. FKC levels were quantified by nephelometry using the Freelite® Human Kappa Free kit (Binding Site Ltd) on the Siemens® BN II analyzer. SPSSv20 was used for statistical analysis.

Results:

Using a cut-off value of 0.38mg/L determined from the mean CSF-FKC levels of the NPH patients plus 2SD, 45 CIS patients were found above this threshold and 32 below. CIS patients above 0.38 mg/L were found to be at greater risk of conversion to MS than patients with FKC values below this limit ($p<0.0001$). This predictive value of the FKC is further strengthened by the fact that both CIS groups show statistical significant differences when comparing Barkhof-Tintore criteria, MRI and OCGB criteria. Furthermore, high FLC levels are also strong predictors of positive OCGB and IgG index. Further results for both groups are presented in table 1.

Conclusion:

In our cohort, high CSF-FKC values above 0.38 mg/L increased the risk of conversion to MS. These results suggest CSF-FKC as a possible biomarker to predict conversion to MS.

Table 1: clinical and laboratory data of CIS patients

	Group 1: patients with CSF-FKC > 0.38 mg/L	Group 2: patients with CSF-FKC < 0.38 mg/L	p value
Patients (n)	45	32	
Gender (females %)	37 (82.2)	23 (71.9)	
Age [mean (CI95%)]	34.6 (31.8-37.3)	36.2 (31.9-40.6)	
Oligoclonal IgG bands in CSF (+/-)	45/0	3/29	<0.0001
IgG index (> 0.5/< 0.5)	45/0	17/15	<0.0001
≥ 3 Barkhof-Tintore criteria (%)	29 (44)	7 (10.6)	0.0040
Fulfilled MRI and OCGB criteria (yes/no)	29/14	3/20	<0.0001
Conversion to MS (%)	51.5	4.6	<0.0001

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CXCL10 Gene Promoter Polymorphism -1447A>G is Associated with Susceptibility to Malaria in Ghanaian Children

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BACKGROUND

Plasmodium falciparum malaria kills nearly a million people annually. Over 90% of these deaths occur in children under five years of age in sub-Saharan Africa. In Ghana, malaria accounts for about 60% of all outpatient visits in public health facilities, with 40% of the affected being children under age 5 years. The disease accounts for 13.2% of all mortalities in Ghana and ranks fifth as the commonest cause of death in children under 5 years of age. The risk factors for severity of malaria pathogenesis and the wide variation in clinical manifestations of malaria are poorly understood. The influence of host genetics on susceptibility to P. falciparum malaria has been extensively studied over the past twenty years.

RATIONALE:

We have recently shown that interferon gamma inducible chemokine, CXCL10, is a predictor of both human and experimental cerebral malaria severity. Additionally, we have reported that the -1447A>G polymorphisms in the CXCL10 gene promoter is associated with increased serum CXCL10 production, which is predictive of severity of malaria in Indian malaria patients. In the present study, we hypothesized that the -1447A>G polymorphism is present in Ghanaians and that it correlates with susceptibility to malaria. We expect that there might be some differences between the Ghanaian cohorts analyzed and that of the Indian cohort previously reported.

METHODS:

We assessed basic demographics that may impact our assessments including age, gender, hemoglobin levels, sickle cell status and cxcl10 polymorphism. We determined whether polymorphisms in the CXCL10 gene are associated with the clinical status of malaria patients. We assessed a single nucleotide polymorphism in the CXCL10 promoter (-1447A>G [rs4508917]) among 311 malaria and 62 non malaria cases using PCR-restriction fragment length polymorphism assay. Adjusted Odds Ratio (AOR) was used to find out if there was any association between CXC10 promoter polymorphism -1447 A>G and malaria.

RESULTS

There was no significant difference with regards to hemoglobin level between malaria patients (12.6g/dL) and non-malaria patients (12.2g/dL), $p=0.0484$. The -1447A>G genotype of the CXCL10 gene was significantly associated with susceptibility to malaria (adjusted odds ratio =8.5, 95% CI=3.6-20.4, $p<0.0001$). These results suggest that the -1447A>G polymorphism in CXCL10 gene promoter is associated with susceptibility to malaria in Ghanaian malaria children. These results further confirm what was found in the Indian cohort study that CXCL10 gene promoter -1447 A>G polymorphism was a marker for susceptibility to malaria.

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Epitopes of human and microbial transglutaminase are similarly recognized by celiac disease sera

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Introduction: The use of microbial transglutaminase (mTg) in the food industry is expanding alongside its ingestion in Western diet. Being a member of the human endogenous tTg, the mTg shares multiple functional similarities. However, immunogenic comparison of the two enzymes in celiac disease (CD) is lacking.

Materials and methods: Complexing mTg and gliadin results in mTg neo-epitope (mTg neo). These complexes were purified by asymmetric field flow field fractionation and confirmed by multi angle light scattering and SDS-PAGE. Sera of 81 CD patients (mean age 30± 17) and 81 healthy controls (mean age 29± 21) were analysed using the following ELISAs: AESKULISA® tTg New generation (tTg-neo-epitopes) IgA and IgG, AESKULISA® Gliadin IgA and IgG, AESKULISA® DGP IgA and IgG and AESKULISA®s against mTg and mTg neo-epitopes (Research use only (RUO) Kits as IgA and IgG).

Results: Purified mTg-neo IgG and IgA (AUC=0.92, 0.93, respectively) showed an increased immunoreactivity compared to single mTg and gliadin (p<0.001) but similar immunoreactivity to the tTg-neo IgG and IgA ELISA (AUC=0.94, 0.95, respectively). Using a competition ELISA, the mTg- and tTg-neo-epitopes have identical outcomes in CD sera both showing a decrease in optical density of 55±6%, (p<0.0002). Sera with high antibody titre [U/ml] against the tTg neo-epitope show also high antibody activities of the mTg neo-epitope and vice versa indicating the presence of similar epitopes within the Tg-gliadin complexes.

Conclusions: mTg and tTg display a comparable immunopotent epitope. mTg neo-epitope IgA and IgG antibodies are immunogenic in CD. If substantiated it will impact the food industry additive policy and regulation

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Anti-neo-epitope tTG complexed to gliadin are more reliable then tTG for celiac disease diagnosis

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Introduction: The guidelines of ESPGHAN for the diagnosis of pediatric celiac disease (PCD) rely on anti-human tissue transglutaminase (tTg) as the prime and unique antibody for screening PCD population. None of the CD-associated antibodies has challenged tTg premiership. tTg complexed to gliadin presents neo-epitopes and antibodies against the complex are called tTg neo-epitope (tTg-neo). Reliability of anti-tTg and tTg-neo antibodies in diagnosis of PCD was compared.

Material and methods; 95 pediatric CD patients (mean 8.3y), 99 normal children (NC) (8.5y) and 79 normal adults (NA) (28y) were tested using the following ELISAs detecting IgA, IgG or both IgA and IgG: AESKULISA® tTg (tTg; RUO) and AESKULISA® tTg New Generation (Neo-epitope tTg complexed to gliadin). The results were compared to the degree of intestinal injury, using revised Marsh criteria.

Results: A significantly higher OD activity was detected for tTg-neo IgA, IgG and IgA+ IgG than for tTg (p<0.0001, p<0.0001, p<0.001, respectively). tTg-neo IgA, IgG correlated better with intestinal damage than tTg (r2=0.968, 0.989 compared to 0.909, 0.488 (p<0.001), respectively). Antibody Sensitivity, Specificity, Positive predicted value, Negative predicted value and AUC for tTG-neo IgA+G were: 95.8, 99, 98.9, 96.1, 0.984, and for tTG IgA+G: 83.2, 100, 100, 86.1, 0.961, and the Significance between the two antibodies performances: p<0.0001 0.0001 0.0001 0.0001 0.0001, respectively. Coclusions: The tTg-neo IgA, IgG and IgA+IgG isotypes exhibited a higher OD activity and better reflected intestinal damage in PCD, compared to tTg isotypes. The tTg-neo combined IgA+IgG ELISA kit had higher sensitivity and a comparable specificity for the diagnosis of childhood CD. tTg neo should be included in the ESPGHAN diagnostic flow chart.

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The incidence/prevalence of autoimmune diseases is increasing worldwide

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Introduction: Epidemiological data provide strong evidence of a steady rise in autoimmune disease (AD) throughout westernized societies over the last three decades. Multiple publications exist, describing past or actual incidences/prevalence of individual ADs, however, long term studies on selected populations are scarce.

Aims: to calculate the % increases per year of AD frequencies worldwide and analyze the differential increases of AD per country and disease, and identify geoepidemiological trends.

Methods: A systematic review was performed to identify incidence and prevalence of ADs. Studies from the last 30 years were identified using Medline, Google, and Cochrane Library databases. Only long-term regional or national long-term follow-ups are reported

Results: The means ± s.d. of the increased %/year incidence and prevalence of ADs worldwide were 6.1±4.9 and 11.2±12.8, respectively. Allocating these annual % increases to neurological, endocrinological, rheumatic and gastrointestinal ADs revealed the following trends: 9.65, 6.2, 4.0, and 3.7%, respectively. In all of these, differences between old vs new frequencies were highly significant (p< 0.0001). The diseases showed high to low increased %/year frequencies: myasthenia gravis, celiac disease, SLE, IDDM and IBD, with 17.4, 12.2, 3.7, 2.6 and 2.4%, respectively. Geoepidemiologically, the following countries had high to low %/year ADs frequency increases: Japan, Italy, Brazil, Denmark, Norway, Sweden, UK, Israel, USA, Canada, Finland and Serbia with 23, 20, 12.4, 10.6, 10.3, 9.8, 9, 5.6, 5, 3.3, 3.1 and 0.8%, respectively.

Conclusions: Despite multiple reports on ADs frequencies, long-term longitudinal follow-ups are scarce. Reviewing available literature, it can be deduced that incidences and prevalences of ADs have increased significantly over the last 30 years. Neurological (MS), endocrinological (IDDM) and gastrointestinal (CD, IBD) ADs and Japan, Italy, Brazil and Denmark increased most. These observations point to a stronger influence of environmental factors as opposed to genetic factors on AD development.

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Association of gene polymorphisms in E26 transformation specific sequence 1 with rheumatoid arthritis in Chinese Han population

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Background: Rheumatoid arthritis (RA) is a complicated autoimmune disease which can lead to incapacitation and decreased life expectancy compared with the normal population. E26 transformation specific sequence 1 (ETS-1) belongs to the ETS family of transcription factors that regulate the expression of various immune-related genes. We aimed to identify whether polymorphisms in ETS-1 play a role in RA by describing in Chinese Han population.

Methods: 68 RA patients and 136 healthy controls were included for this analysis. Clinical information was gathered and disease activity was determined according to the disease activity score for 28 painful/swollen joints (DAS28). Four single nucleotide polymorphisms (SNPs) in ETS-1 were genotyped by high resolution melting (HRM) curve method.

Results: Rs73013527 TT genotype was less common in RA group (8.1% versus 18.9%, P<0.001 in a dominant model). Furthermore, we found that T allele of rs73013527 was overrepresented in RA group as well (25.7% versus 42.6%, P<0.001). Besides, an association was found between rs73013527 TT genotype and DAS28 (P=0.001). No statistically significant difference was observed in the distribution of other three SNPs (rs10893872, rs4937333 and rs11221332) alleles or genotypes between case and control groups were found in this study (all P>0.05).

Conclusions: This study suggests that polymorphisms in the ETS-1 region may be associated with the RA susceptibility. The patients with rs73013527 TT genotype may be not prone to RA. However, larger studies, most likely through multicenter collaboration will be needed to fully validate the significance of these findings.

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Determination of Deamidated Gliadin Peptide Antibodies in Dried Blood Spots

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Background: With increasing prevalence and heightened awareness of celiac disease, consumers and professionals are seeking a convenient, precise, and accurate method for celiac serologic testing. The differential diagnosis for celiac disease includes the analysis of blood for the detection and quantitation of antibodies to deamidated gliadin peptide (DGP). Our objective was to develop and validate DGP antibody testing in dried blood spot (DBS) samples. Single-use, self-retracting bloodletting devices enable the self-collection of capillary blood from lay users. Highly-standardized filter paper collection and transport media are increasingly used for clinical laboratory testing. The employment of sensitive chemiluminescent immunoassay testing facilitates expansion of DBS offerings in clinical laboratories for the reliable analysis of specific proteins, including DGP immunoglobulin A (DGP-IgA) and DGP immunoglobulin G (DGP-IgG).

Methods: Capillary blood samples are collected using SurgiLance™ sterile lancets, spotted onto PerkinElmer® 226 Spot Saver Cards, and permitted to dry. Cards are desiccant packaged and shipped via standard postage to the laboratory. From a 50µL blood drop, four 3-millimeter spots are punched using a calibrated hole punch and extracted into a buffered solution, equivalent to the established on-board sample dilution of the analyzer. Extracts are analyzed on an INOVA Bio-Flash® chemiluminescent analyzer in microsampling mode, bypassing further on-board dilution. Results are read from 5-point DBS calibration curves, derived from high-titer standards of DGP-IgA and DGP-IgG obtained from INOVA. Analytical precision, linearity, recovery, accuracy, and stability of DGP-IgA and DGP-IgG were assessed.

Results: For DGP-IgA, intra-assay and inter-assay precision coefficients of variation (CV) (n=20) at 289.0 concentration units (CU) were 1.4% and 3.2%, and at 34.8 CU were 3.0% and 6.1%, respectively. For DGP-IgG, the intra-assay and inter-assay CV (n=20) at 596.0 CU were 0.9% and 3.8%, and at 11.6 CU were 2.0% and 8.2%, respectively. DGP-IgA was confirmed linear between 5.2 - 1144 CU, with recovery of expected units ranging from 96.4% - 100.8% (n=6). DGP-IgG was confirmed linear between 2.8 - 1646 CU, with recovery ranging from 95.1% - 101.9% (n=6). Known celiac-positive donors provided samples to permit sample matrix comparison. Least-squares regression analysis comparing DGP-IgA values in serum to DBS (n=44, range 5.2 - 762.0 CU) yielded a correlation coefficient of 0.969, y = 1.13x + 7.77; standard error of estimate ($S_{y/x}$) = 44.9. Least-squares regression analysis comparing DGP-IgG values in serum to DBS (n=44, range 3.0 - 769.0 CU) yielded a correlation coefficient of 0.978, y = 1.05x - 4.21; $S_{y/x}$ = 33.0. Both DGP-IgA and DGP-IgG demonstrated 30-day stability with collection cards stored desiccated in sealed Ziploc™ bags at 25ºC or lower.

Conclusion: The analytical method developed and validated for DBS DGP-IgA and DGP-IgG testing on the INOVA Bio-Flash provides a precise and accurate means of determining serologic status for celiac disease. The collection system for this method has proven to be well-accepted by lay users, with 99% of submitted samples of adequate quantity and quality. The transportation system provides extended stability to preserve sample integrity, enabling shipping from remote locations to a central laboratory for analysis.

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Can Serum Light Chain Tests Replace Diagnostic Tests for Urine Free Light Chains? Positive Urine Free light Chains but Negative Serum Free Light Chain levels in two cases

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Background: Serum testing including SPE, IFE and free light chains [FLCs] are considered the important tests in diagnosis and follow-up of plasma cell dyscrasias. Among these serum FLCs provide more sensitive alternative to 24 hour urine testing in the diagnosis and monitoring of light chain MM. In contrast urine K/L ratios are not as dependable and UPE, UIFE and Urine free light chains analysis are increasingly considered as wasteful and therefore expendable. In our laboratory we perform the full gamut of serum and urine testing on myeloma patients for diagnosis and follow-up

Methods: We report two cases, both had initial diagnosis of monoclonal gammopathy of uncertain significance [MGUS] or light chain disease and were monitored with serum and urine free light chains as well as with SPE and IFE. Free light chains were [measured by immunoassay on Beckman Image platform]. In both cases serum light chains and ratio were normal and the urine free light chain assays were diagnostic emphasizing the importance of urine testing in these cases.

Results: The first case is of a 60 year old gentleman, who presents with a kappa light chain disease. He had initially presented in 2010 with an MGUS (IGMK) with normal albumin, calcium, CBC. He now has a normal SPEP and normal serum FLC [K 12.1 and L 11.9mg/L; K/L ratio 1.02]. Urine IFE shows an atypical restricted band with urine FK 63 mg/L and FL 1.6 mg/L and K/L ratio of 40. The patient had a complex cystic kidney disease with unremarkable serum albumin, creatinine, calcium, CBC, eGFR.

The second case is a 58 year old gentleman with a diagnosis of Multiple myeloma [IgG Lambda]being treated with chemo and radiation. He has anemia but normal chemistries. An atypical restriction is seen on serum SPE and IFE showed IgG Lambda and an additional band in Kappa but the serum FLC are within normal limits [S-FK <2.9 and S-FL 6.6 mg/L; K/L ratio <0.44]. The urine free light chains are abnormal (U FK 61.8 and UFL 3.2 mg/L and a high K/L ratio of 19.31.

Conclusion: In both cases serum free light chains and ratios were normal and the urine free light chain assays were diagnostic emphasizing the importance of urine testing in these cases. Why these patients repeatedly fail to show serum free light chains is not clear and requires further investigation.

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Alternatives to Oligoclonal Banding Electrophoresis in CSF: Method Comparison with Quantitative Free Light Chains and Accurate Molecular Mass Measurement of Immunoglobulins

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Background: Isoelectric focusing coupled with IgG specific immunoblotting (IgG-IEF) is routinely used to identify immunoglobulins specific to the CNS compartment as part of the diagnostic criteria for multiple sclerosis (MS); i.e. oligoclonal banding (OCB). However, it is a labor-intensive technique with subjective interpretation of IgG bands from paired cerebrospinal fluid (CSF) and serum. Measurement of the concentration of free light chains (FLC) in CSF by nephelometry has been reported as an alternative measurement to support the diagnosis of MS. In addition, microLC-ESI-Q-TOF mass spectrometry can be used to identify both monoclonal and polyclonal immunoglobulins using accurate molecular mass. We compared the diagnostic performance of the IgG-IEF reference method with FLC by nephelometry and microLC-ESI-Q-TOF mass spectrometry to identify immunoglobulins in CSF.

Methods: Forty-four residual paired CSF/serum samples previously analyzed as positive OCB (OCB^+, N= 25) and negative (OCB^{neg}, N=19) by IgG-IEF (Helena SPIFE 3000) were used for this study. FLC kappa and lambda were measured by nephelometry (The Binding Site) in serum and CSF. Serum immunoglobulins were purified using Melon Gel (Thermo Fischer). Samples were reduced with dithiothreitol then analyzed by microLC-ESI-Q-TOF MS on an AB SCIEX Triple TOF 5600 mass spectrometer. Clones unique to CSF and serum were identified using accurate molecular mass (monoclonal immunoglobulin Rapid Accurate Mass Measurement (miRAMM)). Readers were blinded to OCB results.

Results: The mean±SD number of IgG bands observed by IEF was 9.2±3.6 for OCB^+ samples, whereas in the OCB^{neg} cohort it was 0.2±0.4. Concentrations of kappa and lambda were ~12-fold and ~6-fold higher in OCB^+, respectively (p<0.0001). Receiver Operating Characteristic (ROC) curve analysis showed an AUC of 0.976 for kappa FLC concentration in CSF, and a cut-off ≥0.0623 mg/dL provides a sensitivity of 100% with specificity of 83% in comparison to IEF. Analysis of the sum of FLC in CSF provided similar results (AUC 0.970) when a cut-off ≥0.1200 mg/dL is applied. Concentrations of FLC in serum did not correlate with OCB results (p>0.05). MicroLC-ESI-Q-TOF oligoclonal profiles were in 100% agreement with IEF. In the OCB^{neg} cohort, CSF did not contain any light chain clones in 16 samples (84%). 3 samples had clones whose accurate mass (*m/z* ratio) matched in both serum and CSF and therefore were interpreted as negatives. For OCB^+, paired CSF/serum analysis showed that 4 samples had unique clones in CSF, none detected in serum. 21 CSF samples had clones in both serum and CSF, however in CSF there were additional unique clones whose accurate masses were not identified in serum, and reported as positives.

Conclusion: FLC measurement in CSF by nephelometry shows excellent correlation with IEF with the benefit of potentially eliminating the need of a paired serum for interpretation. MicroLC-ESI-Q-TOF had equivalent performance to IEF to measure immunoglobulins light chains in CSF, with the advantage of being automated and allowing for unambiguous identification of the accurate mass of the clones produced intrathecally.

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Cryoprecipitate as a Quality Control Material for Cryoglobulin Analysis

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Background: Cryoglobulins are serum immunoglobulins that precipitate at a temperature less than 37ºC. The presence of cryoglobulins in vivo are associated with serum hyperviscosity and a variety of clinical symptoms including, but not limited to, purpura, arthralgias, myalgias, weakness, Raynaud's phenomenon, visual disturbances, headache, vertigo, loss of consciousness and stroke. These clinical symptoms are not specific to the presence of cryoglobulinemia; thus accurate detection of cryoglobulinemia is important. Cryoglobulin analysis involves incubation of separate aliquots of patient serum at 4ºC and 37ºC for up to 72 hours. Cryoglobulins, if present, will precipitate at 4ºC while the aliquot at 37ºC should remain clear. A challenge of cryoglobulin analysis is the lack of suitable commercially available quality control materials. In our search for a material that would reliably precipitate at 4ºC but remain clear at 37ºC we investigated whether expired or returned cryoprecipitate from the hospital blood bank would serve as a suitable positive QC

material. Per protocol, cryoprecipitate returned to the blood bank is discarded as it expires 6 hours after thawing. **Objective:** The objective of our study was to determine if expired or returned cryoprecipitate from the blood bank could serve as a positive control material for cryoglobulinemia analysis.

Methods: Returned cryoprecipitate was quarantined and stored at 4°C until retrieved by chemistry laboratory staff. Thawed cryoprecipitate bags were pooled and frozen as 2.5 mL aliquots at 20°C until use. Validation of the cryoprecipitate for use as control material consisted of incubating separate thawed aliquots of cryoprecipitate on 5 non-consecutive days in Wintrobe tubes at 4°C and 37°C for 72 hours. At the end of the 72 hour incubation period the Wintrobe tube held at 4°C was centrifuged in a refrigerated (4°C) centrifuge at 590 RCF for 30 minutes after which cryocrit was assessed visually.

Results: Analysis of the 5 cryoprecipitate aliquots in Wintrobe tubes after 72 hours of 4°C incubation consistently demonstrated a white flocculent material visually consistent with the presentation of a cryoglobulin. The white flocculent material re-dissolved when warmed to 37°C and separate aliquots of cryoprecipitate incubated at 37°C remained clear; indicating that the flocculation observed at 4°C was a satisfactory surrogate for the cryoglobulin precipitation phenomenon. Centrifugation of the flocculent material revealed that four of five aliquots had a reproducible cryocrit of 10%. The fifth aliquot had a cryocrit of 6%. Investigation of the lower cryocrit value in this one aliquot revealed that the fifth aliquot was thawed in a 37°C water bath as opposed to being thawed at room temperature. This difference in procedure revealed that the thawing process of the cryoprecipitate can contribute to the variability in the QC cryocrit value.

Conclusion: Cryoprecipitate unused for patient care can be repurposed as an assay control for the detection of cryoglobulinemia. Addition of a material that reliably precipitates at 4°C and re-dissolves at 37°C replicates the analytical procedures needed for assessment of cryoglobulinemia and ensures appropriate sample handling of the cryoglobulin tubes during the extended 72 hour incubation and analysis time.

A-218

Development and Validation of an Innovative Allergen-specific IgE Assay on the HOB Lumiray®1260 Automated Chemiluminescence Immunoassay Analyzer

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Background:

Besides the skin tests, in vitro determination of allergen-specific IgE (sIgE) represents an important approach to the diagnosis and treatment of allergy. Recently, the innovative HOB Allergy testing, coupling with the fully automated, random-access Lumiray®1260 chemiluminescence immunoassay system has been launched. The HOB Allergy testing is based on the nanoparticle magnetic properties and chemiluminescence technology by using the indirect method. The magnetic streptavidin coated nanoparticles enable the separation and washing procedures; the bounded sIgE reacts with selected biotin-labeled allergen and is detected by the anti human IgE conjugated alkaline phosphatase triggers the chemiluminescence substrate for quantitative determination of IgE antibodies in serum.

Methods:

In this study, the HOB allergy assay was evaluated and compared to the ImmunoCAP (Phadia AB, Sweden) according to the CLSI guidelines. The analytical performance of ten allergens was compared. It covered five inhalant allergens including D1 (*Dermatophagoides pteronyssinus*), D2 (*Dermatophagoides farina*), E1 (cat epithelium), E5 (dog dander) and M6 (*Alternaria alternate*) and five food allergens including F3 (codfish), F4 (wheat), F13 (peanut), F23 (crab) and F24 (shrimp).

Results:

The HOB Allergy sIgE assay performed excellent linearity in the assay ranging from 0.1- 100 kU/L and high precision (the within-run CV% were <5.1%, the between-run CV% were <7.4%, and the total precision CV% were <9.8%). The limit of detection was < 0.01 kU/L. Bilirubin (up to 50 mg/dL), hemoglobin (up to 400 mg/dL), and lipid (up to 2000 mg/dL) did not affect the IgE quantitative detection in serum. It showed no cross-reactivity with other human classes of immunoglobulins (IgG, IgD, IgA and IgM) at physiological concentrations. Total of 1547 clinical samples were compared between HOB Allergy assay and Phadia ImmunoCAP at the cut-off of 0.35 kU/L for the ten allergens, the sensitivity concordance were ranged from 76.2% (F3) to 97.3% (E1), the specificity concordance were ranged from 92.0% (D1) to 100% (F4), the total agreement were ranged from 87.4% (D1) to 96.3% (F13).

Conclusion:

The HOB Allergy system is an innovative allergen-specific IgE assay performing a linear reaction, an extended working range, good precision and reproducibility on Lumiray® 1260. Excellent agreements are observed between HOB Allergy and ImmunoCAP for the detection of specific IgE. The HOB Allergy system with automated, random access and multiprofiling capabilities provides the fast and accurate detection of sIgE that leads to greatly improve the diagnosis and treatment of allergy patients.

A-219

SLE-KeyTM Rule-Out Serologic Test for Excluding the Diagnosis of SLE

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Background:Systemic Lupus Erythematosus is associated with a large spectrum of autoantibodies, but currently there is no reliable serologic diagnosis. Each autoantibody individually fails to discriminate with sufficient specificity and/or sensitivity SLE patients from healthy controls or from subjects afflicted with other autoimmune diseases. Currently a diagnosis of SLE is based on multiple criteria and can take years of monitoring.

Methods: We developed the previously described iCHIP™1 as an effective SLE rule-out diagnostic test by profiling with an antigen microarray multiple, distinct autoantibody reactivities in the sera of SLE patients compared to healthy controls followed by informatics analysis to rule out a diagnosis of SLE. An initial set of 200 antigens associated with SLE was selected from the literature bolstered with sets of proprietary markers developed by ImmunArray. In addition, we analyzed a subset of ANA(+) samples from the Healthy Control pool. We collected serum samples from 250 SLE patients from four independent sources [Albert Einstein College of Medicine, Medical University of South Carolina, Johns Hopkins University and Emory University] and compared them with age race and gender matched sera of 250 healthy control samples independently sourced. We tested these samples using the ImmunArray SLE-Key™ - a proprietary microarray that displays multiple antigens representing a range of SLE-associated biochemical pathways. The SLE-KeyTM chip is printed using a SciFLEXARRAYER SX (Scienion AG, Berlin Germany. After processing, chips were scanned on a G2565 Series C Microarray Scanner (Agilent Technologies, Santa Clara, CA). Multiple classification algorithms were used to develop a set of classifiers. Training and verification was performed on a subset of 200 SLE patients and 200 healthy controls using 4 independent classification methods (Support Vector Machine (SVM), Logistic Regression (LR), Quadratic Discriminant Analysis (QDA), Linear Discriminant Analysis (LDA)). Validation was performed on an additional set of 50 SLE patients and 50 healthy control samples. For the ANA(+) subset of Healthy Control samples, 26 tested positive (>1:40 dilution; ANA IFA Screen, Test Code 249, Quest Diagnostics, Madison, NJ) out of 136 (19.1%) samples with sufficient residual volume for testing. These positive samples were also analyzed on the SLE-Key TM chip.

Results: The SLE-Key TM classifier successfully differentiated SLE patients from healthy subjects with a sensitivity of greater than 90% and specificity of greater than 70%. For the ANA(+) healthy control samples, the SLE-KeyTM classified 50-80% of these samples as healthy control depending on classification method chosen.

Conclusion: The SLE-Key TM multiplex test can be used to assist physicians in ruling out serologically a diagnosis of SLE with a sensitivity of >90%. The SLE-Key TM may also show utility in evaluating ANA(+) otherwise healthy patients after further confirmatory studies are performed. Work comparing the testing performance of the SLE-Key TM in direct comparison to standard serologic testing in SLE patients is ongoing.

1 Fattal, I, et al; Immunology 2010, 130, 337-343

A-220

Comparison of NK Vue, quantitative IFNγ ELISA, with flowcytometric NK activity test

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Backgrounds: Natural killer (NK) cells play a key role in innate immune responses. NK cell activity is generally measured by flowcytometric CD107a degranulation assay or ^{51}Cr release assay. Conventional NK activity measurement methods require complex experimental setups, such as peripheral blood mononuclear cells (PBMC) purification, NK cells stimulation with K562 cells or cytokines, and professional

interpretation of flowcytometric analysis or handling with radioactive materials. In this study, we evaluated analytical performances of NK Vue (ATgen, Sungnam, Korea), which detect NK cell activity by measuring the secretion of IFNγ from *ex vivo* stimulated NK cells in whole blood using ELISA.

Methods: NK Vue assay was done using the supernatant from whole blood following incubation with PROMOCA (ATgen), a bioengineered recombinant cytokine that activates NK cells, for 24 hours in a 37℃ CO_2 chamber. Precision and linearity studies of NK Vue were conducted according to Clinical and Laboratory Standards Institute guidelines. For the comparison of NK Vue with flowcytometry, 83 participants were enrolled with informed consent to study. For flowcytometry, PBMC separated from the matched heparinized blood were incubated with K562 cell line for 6 hours and stained with CD45-FITC, CD3-ECD, CD16/CD56-PC7, CD107a-PC5, and cytoplasmic IFNγ-PE. CD107a expression and intracytoplasmic IFNγ level in $CD56^{+}CD16^{+}$ NK cells as parameters of NK cell activity were determined by flowcytometry.

Results: Within laboratory precision of NK Vue for low, middle and high level was 7.5%, 9.1% and 6.3% CV, respectively. The measurement range of NK Vue from 35.5 pg/mL to 884.6 pg/mL showed a clinically relevant linearity ($R^2 = 0.9987$). Secretory IFNγ measured by NK Vue showed good correlation with CD107a expression determined by flowcytometry (Pearson correlation coefficient 0.413, $P<0.001$), especially in a group of IFNγ ≥100 pg/mL (Pearson correlation coefficient 0.759, $P<0.001$). However, secretory IFNγ by NK Vue did not correlated with cytoplasmic IFNγ by flowcytometry.

Conclusions: The precision of NK Vue was less than 10%, and linearity confirmed over a range of 35.5 pg/mL to 884.6 pg/mL. The significant correlation of secreted IFNγ by NK Vue with CD107a expression by flowcytometry was noted. The findings in this study suggest that NK Vue assay, less laborious and more compactible than traditional NK cell activity measurement, could be a feasible method to estimate and monitor the NK cell function in clinical setting.

A-221

Profiling Sialylation Status during Monocytes Differentiation into Macrophages

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Background: Sialic acids (SAs), a family of 9-carbon containing acidic monosaccharides, often terminate the glycan structures of cell surfaces and are involved in many biological functions including early fetal development, cellular recognition and adhesion, and its utilization by microbes. While it is clear that cell surface SAs are highly involved in the immune system, the sialylation status related to individual immune cells and their activation state and functions are still unknown. In this study, we combined a newly developed LC-MS/MS method, along with flow cytometry and confocal microscopy to profile the changing pattern of SAs during monocytes differentiation and polarization. This is the first report of applying LC-MS/MS to the determination of SAs in monocytes and macrophages. This work will lead to a better understanding of the physiological and pathological roles of SAs in the immune system.

Objective: To study the level and pattern change of SAs during monocytes differentiation and polarization with LC-MS/MS, flow cytometry and confocal microscopy.

Methods: THP-1 monocytes were used as a model immune cell. The differentiation of monocytes to macrophages was accomplished by PMA. The further polarization to classically activated M1 macrophages and alternatively activated M2 macrophages was achieved by addition of LPS and IFN-γ, IL4 and IL13, respectively. A newly developed LC-MS/MS method was employed to quantify free SA in the culture medium and cellular SA in the cell lysate. Flow cytometry was optimized to quantify α-2,3 and α-2,6 linked SAs on the cell surfaces with MAA and SNA lectin labeling, respectively. Confocal microscopy was performed to visualize SAs on the cell surfaces and inside the cells. Sialidase activity was measured to confirm the change in SA amounts in the culture medium.

Results: After PMA treatment, free SA in the culture medium increased from 4.18±0.01 ng/ml to 11.57±0.78 ng/mL, α-2,3 SAs on the cell surface decreased 35%, and α-2,6 SAs decreased 25%. These results were confirmed by sialidase activity assay, which showed the activity of major sialidase (Neu1) increased by more than a factor of 2. Cellular SAs increased from 718±6 ng/mL to (1.59±0.5)×103 ng/mL. This change was verified by confocal microscopy, which showed the increase of both α-2,3 and α-2,6 SAs inside the cells. Moreover, after M1 and M2 polarization, cellular SA decreased 26% in M1 macrophages and increased 13% in M2 macrophages compared with only PMA treatment.

Conclusions: When THP-1 monocytes differentiate into macrophages, both the level and pattern of sialylation changes. The increase of free SA in the medium and decrease of α-2,3 and α-2,6 SAs on the cell surface indicate the elevation of the sialidase activity. The increase of cellular SAs is confirmed by both LC-MS/MS and confocal microscopy. However, the SA change in M1 and M2 polarization can only be determined by LC-MS/MS owing to the technique's sensitivity and accuracy. Overall, this study provides for the first time a global investigation of the cellular sialylation status of monocytes and differentiated and polarized macrophages. It has potential significance in understanding the pathology and diagnosis of disorders involving monocytes and macrophages.

A-222

Circulating BCMA Binding to Its Ligand BAFF Prevents Normal Antibody Production in Multiple Myeloma Patients

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Background:

A hallmark of multiple myeloma (MM) is the low levels of uninvolved immunoglobulin (Ig) levels. B-cell maturation antigen (BCMA) is a receptor expressed in mature non-malignant and malignant B lymphocytes, including plasma cells. We previously demonstrated that BCMA is present in the serum of MM patients (pts) and that its levels predict overall survival (Sanchez et al. Br J Haematol 2012). We hypothesized that circulating BCMA binds to its ligands, preventing normal plasma cell development and antibody production in MM patients.

Methods:

BCMA-Fc and control Ig were obtained (R&D Systems). Human BCMA and mouse BAFF, and mouse plasma IgA, IgA and IgM levels were measured with ELISA (R&D Systems & Bethyl Laboratories). rhBCMA-mBAFF complexes were determined using an ELISA. Plates were pre-coated with a monoclonal mouse antiBAFF capture Ab. Plasma samples were incubated and an antihuman-BCMA detection Ab was added. Human serum IgA and IgG levels were determined in MM patients using nephelometry (Immage 800, Beckman Coulter). Hevylite® Assays (Binding Site) were used to quantify the levels of heavy-light chain isoform pairs.

Results:

To determine the effects of human BCMA on plasma Ig levels in immune competent mice, rhBCMA-Fc or control Ig-Fc (100 µg) was injected into C57 Bl or Balb/c mice. rhBCMA-Fc resulted in significant decreases in IgA, IgG and IgM levels. Decreases in IgA levels were observed when compared to baseline levels on days 4 and 6 (P = 0.0031 and P = 0.0064, respectively), and the controls (P = 0.0087 and P = 0.0221). Mouse IgG levels also showed a reduction compared to baseline (P = 0.0023), the Ig-Fc (P = 0.0014) and control (P = 0.0129) groups. IgM levels showed similar decreases when compared to the untreated (P = 0.0001) and IgFc (P = 0.0088) groups. We determined if rhBCMA-mBAFF complexes formed *in vivo*. Complexes were detected by ELISA at high levels in plasma from mice dosed with BCMAFc, whereas none were found in samples in control IgFc or untreated mice. Next, we determined the relationship of serum BCMA levels to uninvolved Ig levels in MM pts. For pts with IgA (n = 134) or IgG (n = 313) MM, higher BCMA levels (≥ 100 ng/ml) correlated with below normal levels of uninvolved IgG in IgA MM and uninvolved IgA in IgG MM, whereas lower BCMA levels (< 100 ng/ml) correlated with normal uninvolved levels (P < 0.0001). Using the Hevylite Assay, similar results were observed BCMA levels compared to uninvolved IgG isoforms in both pts with involved IgG lambda (n = 62, P = 0.0006) and IgG kappa (n = 117, P < 0.0001) MM.

Conclusion:

We demonstrate the formation of circulating BCMA-BAFF complexes in MM, and administration of recombinant BCMA to normal mice results in marked reductions in their antibody levels. We also show that BCMA levels inversely correlate with uninvolved Ig levels in MM pts. Thus, the lack of normal antibody production in MM pts results in part from circulating BCMA binding its ligands, preventing production of normal antibody-producing cells.

A-223

Comparison between singlex ELISAs and a microfluidics-based multiplex cytokine assay

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Recently, technologies have enabled multiplexing of cytokine immunoassays with substantially less sample volume requirement than the conventional 96-well plate-based single measurand ELISA (singlex ELISA). The study objective was to compare the analytical performance of CyPlex, a microfluidics-based multiplex immunoassay, to singlex ELISAs on measurement of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-α (TNF-α). 68 serum samples were assayed by singlex ELISAs (R&D systems, Minneapolis, MN) and by multiplexed assay (CyPlex by CyVek, Wallingford, CT). Manufacturer-provided calibrators (ELISA) or lot-specific pre-calibration (CyPlex) was used. IL-1β and IL-6 assay antibodies in both methods were from the same supplier. Singlex ELISAs required 600 µL of serum (100 µL each for IL-1β and IL-6, 200 µL each for IL-10 and TNF-α), whereas Cyplex required 25 µL for four cytokines total. The singlex ELISA accommodated 80 samples per plate, excluding blanks, calibrators and quality controls (QCs), and each plate took 4-6 hours to run; Cyplex allowed for 16 samples per cartridge, and each cartridge took 1.5 hours to run (80 samples for 7.5 hours). Table 1 listed assay characteristics including coefficient of variation (CV) and analytical measurement range (AMR). The limited AMR is more of a concern for cytokines with low serum concentrations (IL-1β and IL-10). Assays using the same antibody supply in the two methods showed good linear regression: $R^2 = 0.98$ for IL-1β, 0.98 for IL-6. Assays with different antibody sources showed poor correlation: $R^2 = 0.02$ for IL-10, 0.37 for TNF-α. We concluded that the microfluidics-based CyPlex multiplexed immunoassay substantially conserved sample volume than traditional singlex ELISAs for measurement of IL-1β, IL-6, IL-10, and TNF-α (25 µL versus 600 µL). When the same antibodies were used, the two methods showed comparable performance on precisions and reportable ranges, and good correlation between each other.

Table 1. Assay characteristics of the singlex ELISA and Cyplex.

	Inter-assay CV (number of QCs)		AMR (pg/mL)		Number of samples outside AMR (%)	
Cytokine	ELISA	Cyplex	ELISA	Cyplex	ELISA	Cyplex
IL-1β	4.1 - 8.4% * (20)	8.0% (6)	0.125-8	0.21-2000	28/43 (65%)	45/66 (68%)
IL-6	9% (30)	6.2% (6)	0.156-10	0.52-2000	8/65 (12%)	2/66 (3%)
IL-10	14.5% (24)	9.1% (5)	0.195-50	0.21-2000	32/64 (50%)	0/66 (0%)
TNF-α	6.6% (23)	6.2% (6)	0.50-32	1.31-5000	9/43 (21%)	3/66 (5%)

* The inter-assay precision of the IL-1β singlex ELISA was provided by the manufacturer and was not verified by our lab due to limited reagents.

A-224

Analytical Evaluation of The Helena SPIFE 3000 Electrophoresis/ Immunofixation System

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Background: Protein Gel electrophoresis (PEP) and immunofixation (IFE) are commonly used in clinical laboratories for the diagnosis and management of patients with monoclonal gammopathies. There are two semi-automated agarose gel electrophoresis systems for PEP/IFE in routine clinical use_the Sebia Hydrasys (Norcross, GA) and Helena SPIFE (Beaumont, TX). The Helena SPIFE 3000 system has the ability to run more PEP (60 vs 30) and IFE samples (15 vs 9) than the Sebia Hydrasys system. Therefore, SPIFE 3000 may potentially allow us to handle the rapidly increasing PEP/IFE test volumes in our institution with an acceptable turn-around-time for our clinicians. The aim of this study was to assess analytical performance of the SPIFE 3000 and compare its performance with the Hydrasys system.

Methods: The evaluation included precision studies, linearity analysis, protein fraction and immunofixation comparison studies. One normal and one abnormal serum sample with hypergammaglobulinemia were used to assess precision for each protein fraction (albumin, alpha-1, alpha-2, beta-1, beta-2, gamma). One serum sample with paraprotein was serially diluted (1:2; 1:4; 1:8; 1:16) and analysed in triplicates to evaluate the linearity for paraprotein quantification in gamma region. 36 serum and 35 urine samples were used for the immunofixation comparison study. The quantification of protein fractions and paraproteins obtained with both Hydrasys and SPIFE 3000 were performed by experienced laboratory technologists.

Result: The total precision of SPIFE 3000 on all protein fractions was between 2.0% and 5.6% CV; the linearity was excellent up to an paraprotein value of 5.1 g/dL ($r^2 = 0.997$). The mean bias of SPIFE 3000 versus Hydrasys was -0.650 g/dL for albumin, 0.095 g/dL for alpha-1, 0.069 g/dL for alpha-2, 0.344 g/dL for beta, and 0.143 g/dL for gamma fraction, respectively. Immunofixation comparison showed discrepant results in 17% of all samples tested (n=71). SPIFE 3000 accurately characterized all 18 IgG, 6 IgA, and 1 IgM monoclonal bands in serum and 5 IgG, 1 IgA, and 9 kappa monoclonal bands in urine, respectively. However, missing monoclonal bands were seen in: 1/10 kappa, 6/18 lambda in serum; and 2/8 lambdas in urine using SPIFE 3000. Missing bands were also observed with Sebia system: 1/1 IgM, 1/10 kappa, and 1/18 lambda in serum; 3/5 IgG, 1/8 kappa and 1/7 lambda in urine.

Conclusions: For routine protein electrophoresis, the results of our evaluation showed that Helena SPIFE 3000 is a suitable alternative method. Differences in protein fractions were found between two systems; therefore reference intervals should be established for each system. For immunofixation, our study showed that SPIFE 3000 is prone to miss some monoclonal free lambdas. All missing lambda bands observed in this study were coupled with another monoclonal band (i.e. monoclonal IgG lambda, IgG kappa, free lambda) in the serum sample. This could be due to the specificity of the antisera against lambda used in SPIFE 3000 reagent, however, a specific antisera reagent against free light chains (kappa and lambda) provided by Helena did not resolve this problem for the samples tested. Further study is ongoing to understand and resolve this issue.

A-225

Diagnostic Utility of Biomarkers of Systemic Mastocytosis

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Background: Systemic mastocytosis (SM) is a myeloproliferative neoplasm resulting in the accumulation of clonally derived mast cells. When the mast cells are triggered, the released cellular contents cause a variety of symptoms including, flushing, tachycardia, gastrointestinal distress, or loss of consciousness. Current markers of SM include the mast cell secretory molecules tryptase, N-methylhistamine (NMH), 2,3-dinor-11-beta prostaglandin F2α, (2,3-BPG) and leukotriene E4 (LTE4). Patients often present after taking over-the-counter anti-histamines and cyclooxygenase inhibitors. These medications may influence biomarker concentrations.

Objective: To assess the diagnostic utility of all SM biomarkers and assess the influence of medication on analyte concentrations.

Methods: This study was approved by the Institutional Review Board. Urine samples from 169 patients, with a clinically ordered NMH and tryptase were collected. NMH, 2,3-BPG and LTE4 were measured in urine by LC-MS/MS (AB Sciex API 5000). Concentrations were normalized to creatinine (enzymatic method, Roche). SM diagnoses were confirmed by an allergist according to blinded chart review. Clinical sensitivity and specificity for SM diagnosis was determined using cutoffs previously established (tryptase >20ng/mL, LTE4 >104 pg/mg creatinine, NMH >200 ng/mg creatinine, and 2,3-BPG >1382 pg/mg creatinine).

Results: All analytes were significantly higher in patients with SM (Table 1). Tryptase and NMH were not significantly altered by medication status. LTE4 was significantly different in patients taking aspirin while 2,3-BPG was significantly different in patients taking Benadryl. The diagnostic sensitivity (92%) and specificity (60%) of the three urinary markers together was not significantly improved by addition of tryptase (92% sens; 61% spec).

Conclusion: Urinary markers individually or in combination aid in the detection of SM. However, adding serum tryptase to all three urinary markers does not increase performance. Medications could alter the concentrations of urinary markers; however, the effect on diagnostic performance for SM remains to be elucidated.

Table 1: Sensitivity and Specificity for Systemic Mastocytosis Detection Markers

	Systemic Mastocytosis			Aspirin		Benadryl	
	Yes (n=26)	No (n=143)		No (n=124)	Yes (n=45)	No (n=87)	Yes (n=82)
	Mean		Sensitivity,% Specificity, %	Mean		Mean	
Tryptase, ng/mL	55	7	65 96	13	20	14	15
p-value		**<0.0001**			0.102		0.582
NMH, ng/mg cr.	840	54	77 92	193	287	231	203
p-value		**<0.0001**			0.080		0.686
2,3-BPG, pg/mg cr.	1517	860	46 84	955	977	852	1076
p-value		**<0.0001**			0.433		**0.024**
LTE4, pg/mg cr.	203	109	42 81	103	179	132	114
p-value		**0.016**			**0.017**		0.713

A-226

Study of bronchoalveolar lavage flow cytometry analysis in the diagnosis of patients with diffuse interstitial lung diseases

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Background:

Diffuse interstitial lung diseases are a heterogeneous group of respiratory diseases with difficult diagnosis. The study of bronchoalveolar lavage (BAL) by flow cytometry may define typical patterns in different diseases, providing some help in the differential diagnosis. The aim of this study has been to retrospectively analyze the clinical usefulness of flow cytometry analysis of the cellular and lymphocyte subpopulations

Methods:

We have study 104 patients retrospectively. Subjects were diagnosed of interstitial lung disease during 2.5 years. BAL cellular analysis by flow cytometry, histology and CT scan were analysed in different disease subgroup. Percentage of T cells, B cells, NK cells, CD4, CD8 and CD4/CD8 ratio were analysed by flow cytometry as CD3+, CD19+, CD4+, CD8+, CD3+CD4−CD8−, and CD3-CD16+56+.

Results:

The low incidence of diffuse interstitial lung disease in our area was confirmed (1/10000 per year). Idiopathic pulmonary fibrosis (30%) and sarcoidosis (16%) were the most frequent diagnosis. Lymphocytosis and the CD4:CD8 ratio was the most useful parameter. Thus, this ratio was high in sarcoidosis, and it was inverted in extrinsic allergic alveolitis. It was also low in the bronchiolitis obliterans organizing pneumonia. Neutrophils were the predominant population in idiopathic pulmonary fibrosis

Conclusion:

The study of BAL is helpful to discriminate between interstitial lung diseases

A-227

Application of Bioenergetic health index (BHI) in Healthy individuals and Chronic Kidney Disease

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Background: Mitochondria play a critical role in maintaining cellular homeostasis by regulating the bioenergetic, signaling and cell death mechanisms. Mitochondrial dysfunction in chronic metabolic and inflammatory diseases such as diabetes, kidney disease, obesity, alcoholic liver disease and cancer indicates the significance of mitochondria in maintaining normal physiology. Interestingly, these diseases exhibit extensive diversity in terms of who is susceptible and the rate of disease progression. Genetic, epigenetic and environmental factors are critical regulators of mitochondrial function. A potential relationship between mitochondrial bioenergetic function and disease severity could be exploited as a personalized predictive biomarker. At present no clinical test capable of determining the overall mitochondrial function of individuals exists. We have addressed this problem by measuring the various mitochondrial functional parameters and using these data to calculate a single mitochondrial bioenergetic health index or BHI. Although BHI incorporates interdependent but distinct parameters of mitochondrial function, the impact of physiological variables such as age, gender, ethnicity, physical activity and BMI on BHI are also not known. It is hypothesized that distinct correlations exist between the individual bionergetic parameters of BHI in healthy and diseases states.

Methods: In this study, the biological variability of BHI of human leukocytes and platelets isolated from healthy subjects and the correlation between individual bioenergetic parameters in the context of physiological variables and chronic kidney disease are demonstrated. The bioenergetic (mitochondrial respiratory) parameters of isolated leukocytes and platelets were determined using the extracellular flux analyzer (Seahorse Biosciences) and the BHI was calculated using the formula BHI = (Reserve Capacity x ATP-Linked Respiration)/(Proton Leak x Non-Mitochondrial Respiration). Multivariate statistical analysis was used to test the correlation between individual parameters. The linear correlation was determined using Pearson correlation coefficient and the significance levels were calculated.

Results: We demonstrate that BHI can stratify individuals based on their mitochondrial function (bioenergetic health). In chronic pathological conditions such as chronic kidney disease a significant decline in monocyte BHI (6.14±1.3) is observed compared to the age-matched healthy subjects (13.08±1.13). Multivariate analysis of the bioenergetic parameters in healthy subjects show a very high correlation between basal mitochondrial respiration and ATP-linked respiration (R^2=0.92, p=<0.0001). Basal respiration is also correlated well with maximal (R^2=0.428, p<0.0001) and with non-mitochondrial respiration (R^2=0.437, p<0.0002). Although, the relationship between basal respiration and ATP-linked respiration remained unaffected, correlation between other bioenergetic parameters is breaking down in chronic kidney disease.

Conclusion: These novel findings suggest that BHI and the individual bioenergetic parameters in peripheral blood leukocytes can be used to determine the bioenergetic health of individual subjects. It is also suggested that BHI may be used to determine the disease progression in chronic kidney disease and possibly other pathological conditions. The distinct relationships between mitochondrial bioenergetic parameters suggest their potential utility in gaining insights into the mechanism of diseases with bioenergetic dysfunction. Taken together, BHI is a novel parameter that shows promise for being used as a clinical tool in developing personalized management/ therapeutic strategies in patients.

A-228

Spontaneous in vitro conversion of various phenotype forms of Factor B

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Objective: Several phenotype variants of factor B (FB) can be identified in human serum. We have previously shown that there are differences in concentration and activity of various phenotypes FB. It was also observed that during serum storage a slow conversion rate between FB phenotypes is accelerated at 37 °C. The purpose of this work is to determine the difference of conversion rate between different FB phenotype variants. **Methodology**: 107 serum samples of healthy individuals were investigated. Electrophoresis with immunofixation was used for phenotyping. Activity of FB was determinated using the kinetic, nephelometric test with FB deficient serum. RID and electro immunoassay were used for determination of FB concentration. C3 component conversion was investigated by cross- electrophoresis. **Results**: In analyzed samples 60SS, 43FS, 3 FF and one F1S phenotype was found. Three serum samples of each phenotype were stored at 37°C. After 24h, 3 and 7 days in each sample FB concentration and activity and complement alternative pathway activity were determinated. During conversion it was noticed that complement activity was lost on the 7th days, while FB activity remained unchanged. Using cross-electrophoresis we found that on 7th days, C3 component was completely converted leading to the loss of complement hemolytic activity. **Conclusion**: Phenotype FF had the fastest conversion rate, FS phenotype slower and the slowest conversion rate had SS phenotype. The differences were most obvious on the third day of conversion. Various FB phenotypes had no influence on C3 component conversion.

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Association between functional polymorphisms in the Toll-like receptor 4 (TLR4) gene and HD severity

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Background:

Autoimmune thyroid diseases (AITDs), including Hashimoto's disease (HD) and Graves' disease (GD), are organ-specific autoimmune diseases. Disease severity can vary among patients, and it is very difficult to predict. The toll-like receptor (TLR) is an essential regulator of the innate immune system, and it functions to recognize pathogen-associated molecular patterns. TLR4 reacts with Lipopolysaccharide (LPS) as exogenous ligand and heat shock proteins and fibrinogen as endogenous ligands, and promotes cytokine production.TLR4 expression is associated with the several autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus. However, an association between TLR4 polymorphisms and the pathogenesis of AITD has not been reported. To clarify the association between TLR4 polymorphisms and AITD prognosis, we genotyped TLR4 polymorphisms.

Methods:

Among 151 HD patients who were positive for the anti-thyroid microsomal antibody (McAb) or anti-thyroglobulin antibody (TgAb), 59 developed hypothyroidism before the age of 50 and were treated with thyroxine (severe HD), 47 HD patients over the age of 50 were left untreated and demonstrated euthyroid (mild HD), and 45 HD patients could not be categorized to severe or mild HD at the time of analysis were examined. In addition, among 159 GD patients who were positive for anti-thyrotrophin receptor antibody (TRAb) at diagnosis, 68 were euthyroid, had been treated with methimazole for at least five years and were still positive for TRAb (intractable GD),42 GD patients maintained a euthyroid state and were negative for TRAb for more than two years after discontinuing the anti-thyroid drug therapy (GD in remission), and 49 could not be categorized to intractable GD or GD in remission at the time of analysis were examined. We also examined 94 healthy volunteers (control subjects) who were euthyroid and negative for thyroid autoantibodies. Written informed consent was obtained from all of the patients and control subjects. The study protocol was approved by the Ethics Committee of Osaka University. Initially, we performed direct sequencing to genotype 20 SNPs (rs144028493, rs11536888, rs1057312, rs189683599, rs1064290, rs1057313, rs1057314, rs55910231, rs56332471, rs41426344, rs1064292, rs1057316, rs55861596, rs113017335, rs7869402, rs1057317, rs41296047, rs11536889, rs60972665) from randomly selected AITD patients. Because the minor allele frequencies were >5% only for the rs41426344 and rs11536889 polymorphisms, we genotyped these polymorphisms in all AITD patients using the PCR-RFLP method.

Results:

1.In the rs41426344 polymorphism, the C allele was more frequent in the AITD and HD (5.6% and 6.4%, respectively) compared to control subjects (2.2%) (p=0.0459 and 0.0311, respectively).

2.In the rs41426344 polymorphism, the GC + CC genotypes and C allele were more frequent in the mild HD (26.7% and 14.4%, respectively) compared to the severe HD (6.8% and 3.4%, respectively) (p=0.0051 and 0.0037, respectively) and control subjects (4.4% and 2.2%, respectively) (p=0.0003 and 0.0002, respectively).

3.The distribution of the rs11536889 polymorphism was similar among all of the groups examined.

4.No association was observed between these polymorphisms and TRAb, McAb and TgAb levels or goiter size.

Conclusion:

The GC + CC genotypes of the TLR4 rs41426344 polymorphism protect against thyroid destruction and are less prone to hypothyroidism.

A-230

Anti-PLA2R antibodies in Chinese patients with membranous nephropathy

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Objective. The prevalence of autoantibodies against phospholipase A2 receptor (PLA_2R) among Chinese patients with primary membranous nephropathy (PMN) is still not known. This study used two standardized methods to evaluate anti-PLA_2R antibody in serum to determine whether immunological reactivity reflected by antibody titer correlates with kidney function parameters.

Methods. Overall, 82 subjects with biopsy-proven primary membranous nephropathy (PMN) , 22 cases with secondary membranous nephropathy (SMN), 40 non-MN patients with established glomerulonephritis, 20 healthy volunteers were recruited from the Division of Nephrology, Nanfang Hospital, China. Anti-PLA_2R antibody in the serum of each patient was evaluated by both recombinant cell-based indirect immunofluorescence assay (RC-IFA) and enzyme linked immunosorbent assay (ELISA). Kidney function was assessed by proteinuria for 24 hours, serum albumin, blood urea nitrogen (BUN), serum creatine, serum cystatin C. We assessed the correlation between anti-PLA_2R antibody levels and clinical parameter in the PMN patients.

Results. Fifty-three patients with PMN (64.6%) were positive for anti-PLA_2R antibody. The level of antibody determined by RC-IFA ranged from 1:10 to 1:1000 and 0 to 1423 RU/ml by ELISA. The two anti-PLA_2R test systems correlated very well with each other and reached an agreement of 95.7% for PMN patients. The level of antibody detected by ELISA in patients with PMN also significantly correlated with proteinuria and nephritic-range proteinuria (> 3.5g/day).

Conclusions. Anti-PLA_2R antibody is sensitive and extremely specific for diagnosis of Chinese patients with primary membranous nephropathy. Concentration of autoantibody against PLA_2R is an ideal marker for monitoring the activity of immunological disease.

Keywords: phospholipase A2 receptor, membranous nephropathy

A-231

The industrial food additive microbial transglutaminase is immunogenic in children with celiac disease

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Introduction: Microbial transglutaminase (mTg) is capable of cross-linking numerous molecules. It is a family member of human tissue transglutaminase (tTg), involved in Celiac disease (CD). Despite declarations of mTg safety, direct evidence for immunogenicity of the enzyme is lacking. Aims: To explore the immunogenicity of mTG compare to tTG in CD. Materials and methods: The serological activity of mTg, tTg, gliadin complexed mTg (mTg neo-epitope) and gliadin complexed tTg (tTg neo-epitope) were studied in: 95 pediatric celiac patients (CD), 99 normal children (NC) and 79 normal adults (NA). Sera were tested by ELISAs, detecting IgA, IgG or both IgA and IgG: AESKULISA® tTg (tTg), AESKULISA® tTg New Generation (tTg neo-epitope (tTg-neo)), microbial transglutaminase (mTg) and mTg neo-epitope (mTg-neo). Marsh criteria was used for the degree of intestinal injury. Results: Comparing pediatric CD patients with the 2 normal groups: mTg-neo IgA, IgG and IgA+IgG antibody activities exceed the comparable mTg ones ($p<0.0001$). All mTg-neo and tTg-neo levels were higher ($p<0.001$). tTg IgA and IgG+IgA were higher than mTg IgA and IgA+IgG (p tTg-neo IgA. Taken together, mTg-neo IgG and tTg-neo IgG correlated best with intestinal pathology ($r2=0.989$, $r2=0.989$, $p<0.0001$, $p<0.0001$, respectively). Conclusions: mTg is immunogenic in children with CD and by complexing to gliadin its immunogenicity is enhanced. Anti-neo-epitope mTg antibodies correlate with intestinal damage to the same degree as anti-tTg. Further studies are needed to explore the pathogenic potential of anti-mTg antibodies in CD.

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Comparison of the reliability of celiac disease serology to reflect intestinal damage

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Introduction: In view of the increasing importance of the serological biomarkers for the screening and diagnosis of celiac disease, their differential performance, and the lack of head to head comparison, the reliability of those isolated or combined antibodies to reflect the intestinal damage in children with CD was evaluated.

Material and methods: 95 pediatric CD patients (mean age 8.3), 45 nonspecific abdominal pain children (AP) (mean age 7.3), 99 normal children (NC) (mean age 8.5) and 79 normal adults (NA) (mean age 28) were tested by the following ELISAs, detecting IgA, IgG or both, IgA and IgG: AESKULISA® Gliadin (AGA),

AESKULISA® tTg (tTG; RUO), AESKULISA® DGP (DGP) and AESKULISA® tTg New Generation (Neo-epitope tTg complexed to gliadin=tTg-neo). The results were compared to the degree of intestinal injury, using revised Marsh criteria. Scatter diagrams and regression analysis comparing the 12 antibodies' optical density (OD) activities to the degree of the intestinal damage were correlated.

Results: Most of the assays were able to differentiate patients with low and high degree of intestinal damage. Comparing the different correlations between CD associated IgA and IgG antibodies' isotypes, the tTg neo IgA (r2=0.968, p<0.0025) and tTg-neo/DGP IgGs (r2=0.989, p<0.0001/ r20.985, p<0.0001, respectively) stood out, significantly, as the best indicators of the intestinal damage in CD.

The highest OD values (medium 2.94±1.2, p<0.0001) were achieved by using the tTg-neo IgA ELISA in patients with Marsh 3c.

Conclusions: It is suggested that tTg-neo IgA/IgG antibodies should be preferably used to reflect intestinal damage during screening, diagnosing and monitoring compliance in childhood CD.

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Technical performance evaluation of the Elecsys® Periostin immunoassay as a companion diagnostic for the investigational asthma drug lebrikizumab

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Background: Asthma is a chronic respiratory disorder with complex clinical and biological heterogeneity and differential response to treatment. The Type 2 cytokine interleukin-13 (IL-13) plays a central role in asthma pathophysiology in a subset of patients and is being investigated as a potential therapeutic target. Periostin is a systemic biomarker of Type 2-driven asthma that can act as a surrogate biomarker for IL-13 activity in the lung and is predictive of benefit from the investigational anti-IL-13 drug lebrikizumab (Corren *et al*. NEJM 2011). The Roche Elecsys® Periostin immunoassay is being developed as a companion diagnostic for lebrikizumab in asthma. A clinical trial version of the assay was used in the Phase IIb lebrikizumab trials LUTE and VERSE to stratify patients by baseline serum periostin levels based on a cut-off of 50 ng/mL established in the previous Phase II study MILLY (Corren *et al*. NEJM 2011). Given this defined cut-off and the narrow, normal distribution of serum periostin values, strict adherence to quality control (QC) procedures and a high level of precision, accuracy, and reproducibility is required for the assay. The periostin assay has previously demonstrated robust precision during routine testing (Sherman *et al*. AACC 2014). Here, we further evaluate the technical performance of the periostin clinical trial assay and describe its QC performance over an extended period of time.

Methods: The technical performance of the periostin clinical trial assay (limits of blank [LoB], detection [LoD] and quantitation [LoQ], linearity, interferences, sample stability, and lot-to-lot comparability) was evaluated according to CLSI guidelines. The assay was further used for clinical sample testing at three external testing laboratories on four different **cobas e** 601 analyzers, using two different reagent lots, between July 2013 and November 2014. Laboratory-specific QC target values and ranges were determined based on 20 QC runs performed over 10 days. QC rules were defined for three levels of human serum samples used as QC material, and applied during clinical sample testing.

Results: Based on CLSI EP-17 procedures, using two **cobas e** 601 analyzers, the specified LoB, LoD, and LoQ was confirmed, at 2 ng/mL, 4 ng/mL, and 10 ng/mL (at 30% total allowable error), respectively. Linearity was confirmed between 4 and 160 ng/mL (CLSI guideline EP-6). No significant differences in the recovered periostin concentration were observed in the presence of a broad range of potentially interfering substances and drugs (including asthma medications), and samples were stable across a range of storage conditions and durations. The assay demonstrated good lot-to-lot comparability. The variability of valid QC measurements in the external laboratories ranged from 1.7% to 2.5% CV, and the mean relative deviation of QC levels from the global target values (bias) was between -3.2% and 2.5%.

Conclusion: The clinical trial version of the Elecsys® Periostin immunoassay demonstrated robust technical and QC performance. These results support the suitability of the assay for patient stratification in lebrikizumab clinical studies. Disclaimer: This product is not cleared or approved for use in the USA.

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Adding an additional biomarker IL-18 does not improve the QuantiFERON TB test

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Background: The QuantiFERON-TB Gold In-Tube is a widely used test for mycobacterium tuberculosis (TB) exposure which measures interferon-ɤ (IFN-γ) production in peripheral blood following *in vitro* incubation with TB antigens. Although this test is widely used as a screening test, false-positive and indeterminate results are common which can lead to unnecessary follow-up testing. Interleukin 18 (IL-18), also known as IFN-γ inducing factor, is known to be a primary regulator of IFN-γ production in inflammatory reaction. The aim of this study is to evaluate if QuantiFERON test interpretation could be improved by simultaneous measurement of IL-18 and IFN-γ. **Method:** Residual materials from patient samples submitted for the QuantiFERON testing were collected. Each patient sample consisted of three conditions; a negative (Nil) control tube (containing saline), a mitogen control tube (containing PHA) and a TB antigen tube (containing a cocktail of ESAT-6, CFP-10, and TB7.7). The QuantiFERON test was performed and interpreted according to the manufacturer's instructions (QIAGEN Inc., Valencia, CA). Samples were classified into three groups according to the QuantiFERON results: TB positive (n=30), negative (n=32) and indeterminate (n=30). IL-18 concentration in each tube was determined using an electrochemiluminescence assay (Meso Scale Discovery. Rockville, MD). **Results:** A strong IL-18 release was detected in the mitogen control tube for all samples with an average of 810.8±641.4 pg/mL (Mitogen-Nil). IL-18 levels in the TB antigen tubes of all groups were significantly higher than that in the Nil control tubes (p<0.01). However, no differences in IL-18 levels were seen between different patient groups. The TB antigen induced IL-18 release (TB antigen-Nil) in the TB positive, negative and indeterminate group was 182.1±383.5, 168.7±211.8 and 214.6±413.2 pg/mL respectively. The TB antigen induced IL-18 release in the TB infected patients was similar to that in the non-infected subjects (p>0.05). Additionally, no correlation was found between the TB antigen-stimulated IFN-γ and IL-18 release in the TB-positive patients. To normalize possible impact from variation of cell number and immune response, we further calculated the ratio of TB antigen-induced to mitogen-induced IL-18 release. This ratio remained similar between the TB positive and negative patients. ROC analysis showed that the AUC was 0.48 (TB antigen induced IL18 release) and 0.49 (Ratio: TB antigen/Mitogen induced IL18 release) in differentiation of the TB positive and TB negative patients. **Conclusion:** No significant increase of IL18 secretion was elicited by TB antigen in TB infected patients. Quantitation of IL18 in addition to IFN-γ is not useful in improving QuantiFERON test interpretation.

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Prevalence and distribution of IgG, IgA and IgM Gammopathies in samples obtained from a large reference Laboratory in Brazil.

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Background: Monoclonal gammopathies (MG) are a group of disorders characterized by monoclonal proliferation in plasma cells resulting in the production of monoclonal immunoglobulin or immunoglobulin fragment (M protein), which can be detected in serum in the form of a band or monoclonal component (M component). Immunoglobulins or antibodies typically consist of two heavy polypeptide chains and two light polypeptide chains. There are five types of heavy chains named IgG, IgA, IgM, IgD, and IgE; and two light chain different types, called kappa and lambda. Thus, MG can be classified by the type of light and heavy chains produced. This identification is possible due to specific techniques that are able to define the type of abnormal proteins, identifying the heavy and light chains involved, such as immunofixation, which combines two other techniques: immunoprecipitation and electrophoresis. Confirmation of the presence of monoclonal protein is essential for differentiation of monoclonal gammopathies and polyclonal gammopathies. The first is a neoplastic or potentially neoplastic proliferation, and the last, results from infective or inflammatory processes.

Objective: We aimed to access the prevalence and distribution of IgG, IgA and IgM gammopathy in a large reference Laboratory in Brazil.

Methods: We identified from May 2013 to June 2014, 1548 serum immunofixation assay results from a large brazilian reference laboratory database. The types of chains were detected with 9IF immunofixation methodology, in conjunction with the semi-automatic Hydrasys system, from Sebia® manufacturer.

Results: Monoclonal gammopathy was detected in 452 samples in a total of 1548 samples analyzed, the prevalence was 29.20% in the present study, the highest prevalence of monoclonal component were observed in males, aged from 61 to 70 years old (40.34%). In gammopathy distribution, the most prevalent isotype found was IgG kappa (43.14%), followed by IgG lambda (26.33%), IgA kappa (14.60%), IgA lambda (12.17%), IgM, kappa (2.88%) and the less common IgM lambda (0.88%).

Conclusion: Among patients referred by physicians to our clinical laboratory with a prescription of serum-immunofixation, gammopathy was found in 29.20% of this population. IgG class is the most prevalent, followed by IgA and IgM and this results are being consistent whit the literature. The analysis option used for the diagnosis of MG is a technique which has high sensitivity and have wide application in the identification of M protein, normally present in small amounts, which are difficult to detect by other methods. A correct identification of monoclonal component class is important because each type is associated with little differences in disease patterns, being essential for guidance and monitoring of therapeutic or treatment efficacy.

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A rare case of paraneoplastic limbic encephalitis associated with squamous cell lung carcinoma

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Background: Limbic encephalitis (LE) is a paraneoplastic syndrome that is often associated with small cell lung cancer (SCLC), breast cancer, testicular tumors and thymoma. The common clinical manifestations of LE are subacute onset, cognitive dysfunction, seizures and psychiatric symptoms. The main intracellular antigens related to limbic encephalitis are Hu, Ma2, and less frequently CV2/CRMP5 and amphiphysin.

Methods:A 79 years old man with behavioral disorders, seizures and fever was admitted to the Emergency Service of our Hospital. The fever was confirmed (37.2 ºC), CT scan showed residual ischemic brain injury. The diagnosis was subacute encephalitis. However, the patient's condition deteriorated with aggressiveness, agitation and three episodes of oculocephalic version. The patient was admitted in the Service of Neurology to study the origin (infectious, autoimmune or neoplastic) of the episodes of subacute encephalitis.

Results:

1.Biochemistry study

LDH (696 U/L) and PCR (18.4 mg/dL) altered without significant alterations of the others parameters of the biochemistry. Normal thyroid function.

2.Hematology study

CBC without significant alterations.

3.Tumor markers

NSE (Neuron-Specific Enolase)=27.3 ng/mL.

AFP, CEA, CA-125, CA-15.3, CA-19.9, B-HCG and PSA in normal ranges.

4.Microbiological analysis (Study of meningitis and viral encephalitis)

PCR negative for Herpes simplex, Varicella-zoster, Toscana virus, Enterovirus.

5.Autoimmune study

Antinuclear antibodies (ANA), DNA and ENAs screening: negatives

Onconeuronal antibodies: Positive anti-amphiphysin antibodies 1/100 and confirmed by immunoblot assay. The presence of this antibody is associated to paraneoplastic LE. The presence of this antibody allow us to diagnose the patient's symptoms as LE.

6.Chest X-rays

Normal, without presence infiltrates or masses.

7.Thoraco abdominal CT SCAN

Condensation at left lung and presence of adenopathies in mediastinum and abdomen suggesting lung carcinoma.

8.Biopsy sample by bronchoscopy

A biopsy sample of bronchial fragments of the mucosa showed the presence of tumoral cells CK5/6+, P63+, TTF1- and CK7- associated to scamous cell lung carcinoma.

9.Diagnosis of the patient

With previous findings, the diagnosis of the patient was paraneoplasic limbic encephalitis with anti-amphiphysin antibodies positives associated to scamous cell lung carcinoma.

Conclusion: The presence of anti-amphiphysin antibodies allows us to diagnose a scamous cell lung carcinoma. The paraneoplastic antibodies appear to be a useful tool for diagnosing a neurological disorder as paraneoplastic and for indicating the probable type of underlying tumor.

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The Grace Bio-Labs ArrayCAM Multiplex Protein Microarray System surpasses the diagnostic throughput and accuracy shortfalls of ELISA and Multiplex Bead Assays

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Background: Simultaneous multiplexed profiling of protein markers will become standard method for physicians to more accurately diagnosis disorders and/or monitor therapeutic responses, yielding improved survivability or manageability of disease. Autoimmune connective tissue diseases are an example, where profiling multiple auto-antibodies to dsDNA and numerous extractable nuclear antigens (ENA's) defines the presence of specific disease subset(s). Here ELISA is customarily the standard screening method, but lacks the through-put economy and diagnostic power of multiplex applications. This has driven market appraisal of Luminex, providing through-put economy and multiplexing diagnostic advantages, but exhibiting deficiencies in accuracy and consistency.

Grace Bio-Labs has developed the ArrayCAM Microarray System for multiplexed protein profiling consisting of complimentary optimized components, providing the solution to these predicate device shortfalls. Here, we illustrate efficacy of the ArrayCAM Microarray System for high through-put, sensitive and specific multiplexed detection of protein biomarkers, providing next-generation diagnostics.

Method: The multiplex Grace BioLabs Microarray Assay System components begin with antigens arrayed onto ONCYTE high binding porous nitrocellulose slides producing multiple co-localized assay reaction sites within each well. Sample analyte detection is achieved using single wavelength excitation quantum nanocrystals, providing extremely high signal emission in discrete wavelength bands. This enables multi-color multiplexing readable with an economical single laser image acquisition instrument. This data capture and analysis is achieved using the ArrayCAM imager and automated software, which rapidly acquires and analyzes data, then provides objective test reports, thereby eliminating the subjective influence of technical operators. In this illustration we simultaneously measure presence of serum autoantibodies to SM, SMRNP, SSA60 kDa, SSA52 kDa, SSB, SCL-70, JO1, RIBO-P and dsDNA (semi-quantitatively) using multiplex reverse capture immunoassay methods. Test wells also contain human-IgG calibrators, anti-human IgG controls, interference controls and alignment fiducials. To demonstrate performance, 64 human ANA disease positive samples were sourced from commercial vendors characterized for anti-dsDNA and anti-ENA autoantibodies via ELISA (Inova, ImmunoConcepts). Samples were then screened with the ArrayCAM Microarray System and data expressed as semi-quantitative binary auto-antibody positivity established by known positive/negative cut-offs determined from predicate device sample characterizations. Anti-dsDNA measurements were reported semi-quantitatively using regression of sample signal against Hu-IgG intra-well curve calibrators at known concentrations.

Results: The microarray performed to clinical standards with observed specificity ranging through 90.6% to 100% (mean, 94.9%) and sensitivity ranging through 66.7% to 100% (mean, 93.3%). Sample measurements for anti-dsDNA exhibit semi-quantitative measurements correlating to those produced by ELISA. Also, the ability of the microarray to integrate unique controls and multiple antigen epitopes illustrates potential false negative and positive measurements by ELISA.

Conclusions: The Grace Bio-Labs ArrayCAM Protein Microarray System provides the economical and diagnostic advantages of multiplexing not possible with single-plex ELISA along with superior sensitivity and specificity than Luminex by virtue of unique assay controls. The shortfalls of these two predicate systems are overcome while delivering clinical performance specifications with familiar, easy-to-use methods, requiring no significant technical training.

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Evaluation of Anti-HTLV (I/II) CMIA testing in a comparative study with results of HTLV blot assay: Anti-HTLV (I/II) CMIA gray zone needed?

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Background: Human T-lymphotropic virus (HTLV)-1 and HTLV-2 were the first oncogenic human retroviruses identified in the early 1980's. HTLV-1/2 are retroviruses linked etiologically to various human diseases, and both of them can be transmitted by vertical route, sexual intercourse, blood transfusion and intravenous drug use. This infection has a worldwide distribution, with an estimate of up to 15-20 million people affected.

Diagnosis and screaning is important in protecting the safety of blood products and in the disease diagnostic, but better specificity and sensitivity assays are still necessary. This study aims to suggest the creation of a gray-zone to the CMIA HTLV based in a comparison of a gold standard HTLV Blot test.

Methods: Using the database of a clinical laboratory, we assessed 1103 patients results of HTLV during the year of 2014 from Brazil. The tests performed were HTLV Blot (MPD HTLV Blot 2.4) and CMIA (Architect ® rHTLV-I/II, Abbott), following strictly manufacturers' instructions and evaluated with internal control. For CMIA testing, firstly results were interpreted according to package insert (considering cut-off at 1.0 S/CO), and secondly, gray-zone intervals were created and evaluated to determinate inconclusive results.

Results: From 1103 samples tested by HTLV Blot, 799 showed negative, 243 positive and 55 were inconclusive. When samples were tested by CMIA we obtained 757 negative and 346 positive results.

Afterwards CMIA results were reclassified allocating the results in seven different groups as shown on the table 1.

		HTLV Blot		
	Results (S/CO)	**POS**	**INC**	**NEG**
rHTLV I/II CMIA	**0,0 - 1,0**	**0**	**16**	**741**
	1,0 - 5,0	**0**	**13**	**56**
	5,0 - 10,0	**0**	**10**	**0**
	10,0 - 20,0	**0**	**3**	**2**
	20,0 - 50,0	**6**	**11**	**0**
	50,0 - 100,0	**80**	**2**	**0**
	> 100,0	**163**	**0**	**0**

Table - Classification groups

Conclusion: CMIA and HTLV blot results showed that we had a significant number of discrepant results. We think that the reclassification is necessary, thus we suggested a new distribution for the CMIA results creating a gray zone of 1,0 - 20,0 S/CO for allocating inconclusive results. This new gray zone makes possible a reliable and assertive diagnostic, reducing substantially the possibility of false negative result.

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Reduction of Immunoglobulin Carry-over on an Automated Tecan Pipetting Platform

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Background: Measurement of IgG responses to the *Streptococcus pneumoniae* vaccine are often assessed for patients with suspected immunodeficiency. The degree of response to a vaccination can vary significantly. Because patients with very high concentrations of antibody may be analyzed together with patients having undetectable responses, carry-over on automated pipetting platforms is a concern. When carry-over is identified, the affected patient sample must be repeated, adding cost and time to the testing process. The purpose of this study was to identify a procedure that would eliminate carry-over in a 23-plex *Streptococcus pneumoniae* antibody assay. Methodology: *Streptococcus pneumoniae* antibodies are measured by dispensing 5 serial dilutions of each patient sample into a 96-well vacuum filter plate via a Tecan Freedom Evo. A mixture of Streptococcal polysaccharide-conjugated microspheres (representing each of the 23 polysaccharides in the Pneumovax-23 vaccine) is added to each patient dilution. Following incubation and washing, a fluorescein-conjugated anti-hIgG is added to each dilution. After a second incubation and wash, the mean fluorescence intensity (MFI) for each bead is measured on a Luminex platform. Calculation of patient results is performed using the statistical analysis program StatLia (Brendan Scientific). A recent publication demonstrated that 0.17M NaOCl washes could eliminate carry-over of IgG molecules on the Tecan Freedom Evo platform (JALA 10.2010,p379-389). This modification was applied to our assay, with a 0.17M NaOCl wash of the probes between samples and immediately prior to the water rinse step. To assess the impact of carry-over, aliquots of relatively low and high concentrations were placed strategically throughout a run to induce carry-over. Observed carry-over, carry-over limit, and carry-over ratio were calculated for each serotype. Observed carry-over was calculated as the mean differences of the low concentrations followed by high samples and the low concentrations followed by low samples. The carry-over limit equals 3 times the standard deviation of the mean difference of the low concentration when followed by a low sample. The carry-over ratio equals the observed carry-over/carry-over limit. A method comparison was conducted by assessing the *Streptococcus pneumoniae* antibody concentrations in patient samples (n=54) performed with and without the 0.17M NaOCl wash. The results for the 23 serotypes were compared by linear regression. Patient sample repeat rates were calculated pre and post NaOCl wash (n=2441). Results: The average carry-over ratio for all 23 serotypes before the addition of the NaOCl wash was 3.5. After the addition of NaOCl, the ratio was reduced to 0.2, indicating that the majority of the carry-over had been eliminated. . Linear regression analysis showed slopes ranging from 0.857 to 1.067 for the 23 serotypes and correlation coefficients ranging from 0.952 to 0.998, demonstrating that the NaOCl wash did not affect the analytical performance of the assay. The elimination of carry-over has reduced our repeat rate from 8.3% to 6.0% which equates to about 600 fewer repeats per year. Conclusion: Carry-over can be a source of laboratory errors and identifying it is sometimes difficult. Implementation of a NaOCl wash may be effective in mitigating carry-over of immunoglobulins on automated pipetting platforms.

A-240

Evaluation of the Analytical Performance of Multiple Optilite Systems under routine conditions

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Background: **T**he Binding Site Optilite® system is a fully integrated immunoturbidimetric analyser that has been specifically optimised to undertake specialised protein assays. The intention of this study was to evaluate the performance characteristics of the analyser under simulated routine conditions using multiple analysers, assays and operators over a prolonged period of time. **Methods:** Four Optilite analysers were used by a total of 4 operators with 12 analytes - IgG, IgG1, IgG2, IgG3, IgG4, IgA, IgM, C3c, C4 Beta-2-Microglobulin and the Freelite® Kappa and Lambda. The testing occurred over a 2 week period utilising a workload derived from a high volume laboratory (280 - 350 tests/day, 80-110 samples/day). All systems and reagents were final and operators were trained according to the manufacturer's final procedures. Testing was carried out using the Quality control materials provided for the precision testing and patient samples for the comparability testing. Samples were a mixture of both native and contrived materials in order to span the measuring ranges and challenge dilution capabilities of the system. **Results:** For each analyte, precision was calculated over the 10 day period and the composite standard deviation and CV derived across all 4 systems. Similarly for each analyte, patient samples were correlated using Passing-Bablok regression - all system combinations were examined. Agreement was estimated using Bland-Altman. **Conclusion:** All assays on all the Optilite Systems demonstrated excellent precision. Similarly correlation between systems was very good and showed homogeneity of performance. Under these demanding conditions, the Optilite systems proved to be robust and reliable

Analyte	QC material results			Sample results	
	QC	Mean	CV (%)	Sample range	Range of r*
Freelite Kappa	High	26.418 mg/L	4.0	0.991 – 1189mg/L	0.767 – 0.995
	Low	14.924 mg/L	5.1		
Freelite Lambda	High	61.934 mg/L	7.0	1.37 – 14.248mg/L	0.761 – 0.983
	Low	29.132 mg/L	5.4		
IgG1	High	7122.160 g/L	4.3	1628 – 5392.6g/L	0.925 – 0.965
	Low	2938.048 g/L	6.9		
IgG2	High	4169.356 mg/L	3.2	1093.39 – 4480.43mg/L	0.983 – 0.997
	Low	2394.085 mg/L	3.9		
IgG3	Elevated	1642.858 mg/L	5.1	225.97 – 1145.28mg/L	0.926 – 0.978
	High	701.955 mg/L	5.5		
	Low	350.000 mg/L	6.7		
IgG4	Elevated	1868.307 mg/L	3.5	92.71 – 2527.53mg/L	0.999 – 1.000
	High	433.890 mg/L	3.3		
	Low	237.559 mg/L	3.2		

**Range of r is calculated from 6 combinations of platform comparisons by Pearson Product-moment correlation coefficient.*

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Total Vs Antigen Specific Pneumococcal Antibody Response: A Comparison of Two Different Assay Types

M. M. Reynolds[1], D. L. Murray[1], M. A. V. Willrich[1], A. Parker[2], B. K. DuChateau[2], M. R. Snyder[1]. *[1]Mayo Clinic, Rochester, MN, [2]The Binding Site, Birmingham, United Kingdom*

Background: The absence or modulation of antibody response to Pneumococcal pneumoniae (PCP) antigens may be observed in patients with an immunodeficiency. Different types of assays have been developed to evaluate Pneumococcal antibody responses. Commercial assays generally measure total PCP antibody titer, whereas some laboratory developed tests (LDT) measure antibody responses to specific PCP serotypes.

Objective: To correlate results of a commercially available assay that renders a total PCP antibody result to an LDT assay that differentiates individual antibody responses to specific Pneumococcal antigens.

Methods: 151 serum specimens were interrogated using the Anti-PCP IgG EIA (The Binding Site, UK) and results were compared with historic data obtained using a LuminexTM-based LDT (Mayo Clinic) which quantifies individual responses to 23 common Pneumococcal antigens.

Results: There was moderate agreement between the summated serotype results of the LDT (Median 108.4, min 4.5 - 1764.7 max, average 232.7, µg/ml) and the total anti-PCP EIA (Median 50.7, min 5.8 - max 1055.0, average 146.8, µg/ml); (n=151 Pearson's Correlation 0.61, linear regression R2 0.37).

In a second analysis, Mayo-derived, sero-specific cut-offs were applied to each of the 23 individual Pneumococcal antibody results and the percentage of Pneumococcal antibodies rendering a positive result was determined for each of the 151 serum specimens. When these interpretations were applied to the LDT results and compared to the total anti-PCP EIA results, Pearson's correlation showed a relationship of 0.58.

In an additional comparison, Mayo-derived, sero-specific cut-offs were applied to the results of the LDT assay. Specimens rendering positive results in ≥50% or <50% of the Pneumococcal antigens tested were considered PCP responders and PCP non-responders respectively. For the total anti-PCP EIA results, specimens rendering a result ≥100 µg/ml or <100 µg /ml were considered PCP responders and PCP non-responders respectively. The 100 µg/ml cut-off was empirically determined and not optimized in context of clinical outcome. When these definitions were applied to the 151 specimens interrogated, 39 and 73 specimens rendered results consistent with the responder or non-responder definitions on both assays respectively. Collectively these data demonstrate a concordance of 74.2% among the LDT assay and the total anti-PCP EIA. Thirty-nine specimens demonstrated discordant results with 22 and 17 specimens rendering (non-responder total anti-PCP EIA/ responder LDT assay) and (responder total anti-PCP EIA/non-responder LDT assay) respectively.

Conclusion: The analytical correlation of results obtained using the Anti-PCP IgG EIA (The Binding Site, UK), which renders a total Pneumococcal antibody result, and a Luminex-based LDT (Mayo Clinic), which differentiates individual antibody responses to specific Pneumococcal antigens, display varying agreement, depending upon the interpretation criteria applied to the results. The establishment of clinically optimized cut-offs for the total anti-PCP EIA may improve the concordance of the assays.

Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Mass Spectrometry Applications

A-242

Development and Validation of a Liquid Chromatography Tandem Mass Spectrometry Bioanalytical Method for 21-Deoxycortisol, 11-Deoxycortisol and Corticosterone in Serum or Plasma

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Background:

Congenital Adrenal Hyperplasia (CAH) is most often caused by mutation of the 21-hydroxylase gene (CYP21), which results in underproduction of cortisol with overproduction of precursor steroids and their metabolites by the adrenal glands. Similarly, the second most common cause of CAH is a defect in 11-Hydroxylase (CYP11B1), for which the screening tests are 11-Deoxycortisol or 11-Deoxycorticosterone. Historically the most common biomarker used for detecting CAH in pediatric patients is 17-Hydroxyprogesterone (17OHP). In recent years the use of Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) has afforded the clinical laboratory the opportunity to simultaneously quantify multiple additional biomarkers such as Androstenedione, 11-Deoxycortisol, Cortisol, DHEA and Testosterone to improve the specificity and sensitivity of clinical diagnosis. Another less commonly used biomarker for 21-Hydroxylase deficiency is 21-Deoxycortisol, which increases from very low levels in normal patients to high levels in affected patients as 17OHP rises to very high levels. In this study we modified our existing multiplex assay for 11-Deoxycortisol and Corticosterone to include 21-Deoxycortisol, thus screening for three important markers.

Method:

An analytical method was developed using a TX-4 HPLC system (Thermo-Fisher) with Agilent® 1100 pumps (Agilent Technologies, Inc.) and a Sciex® API5000 (Danaher) triple quadrupole mass spectrometer. Independent calibration curves were prepared for each analyte in depleted serum (Golden West Biologicals). Sample preparation consisted of isotope dilution using a cocktail of three heavy isotope internal standards followed by Liquid-Liquid Extraction. A Fluophase® RP (Thermo-Fisher) analytical column (2.1x50mm, 5 um) was used with a water/methanol solvent gradient to achieve chromatographic separation of all isobars in under 6 minutes. Positive mode atmospheric pressure chemical ionization (APCI) was used for detection in Multiple Reaction Monitoring (MRM) mode.

Validation Data:

Analytical sensitivity was 10 ng/dL for each analyte and the analytical measurement range was up to 2,000 or 5000 ng/dL (up to 20,000 ng/dL with dilution). Inter-assay precision ranged from 2.4-9.8% for 11-Deoxycortisol, 2.4-11% for Corticosterone and 6.1-7.0% for 21-Deoxycortisol. Accuracy ranged from 92.6-97.8% for 11-Deoxycortisol, 96.5-98.5% for Corticosterone and 91.6-102.7% for 21-Deoxycortisol. Reference intervals for both adults and pediatric patients were developed for 11-Deoxycortisol, Corticosterone and 21-Deoxycortisol. Reference intervals were also developed for 11-Deoxycortisol to support Metyrapone stimulation testing.

Clinical Significance:

The use of 21-deoxycortisol may be beneficial in reducing the rate of false positives in CAH diagnosis when used in concert with other steroid hormones, including 17-Hydroxyprogesterone and Cortisol.

A-243

Identifying Three Salvia Peptides as Biomarkers for Severe Early Childhood Caries Early Diagnosis by MALDI-TOF MS

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Background:

Pediatric dental caries is one of the most common chronic diseases among children, which may result in functional, esthetic, and psychological disturbances. However, there is no molecular detection and diagnostic tests available for severe early childhood caries (s-ECC) with high sensitivity and specificity that can be used as routine clinical screening tools. The aim of this study was to apply magnetic bead fractionation coupled with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) to evaluate the profiles of salivary peptides in children with s-ECC before and after treatment for 1 and 4 weeks, to screen and identify which salivary peptides are associated with caries.

Methods:

Children recruited for this study were selected from the following conditions: 1) age between 36 and 60 months, who had been diagnosed with s-ECC; 2) no influenza or upper respiratory tract infection, who had not received antibiotic therapy within 1 month or fluoride prophylaxis within 1 year; 3) no history of congenital and genetic problems; and 4) able to expectorate and comply with treatment. Totally, 16 children who fulfilled these criteria were included in the study. Whole saliva samples were collected at 08:30 AM, after the breakfast and rinse the mouth. All saliva samples were fractionated using weak cation exchange magnetic bead (SPE-C100; Bioyong Tech, Beijing, China) according to the manufacturer's instructions. The resultant supernatant was transferred into a fresh tube, and the peptides were analyzed directly on a ClinTOF Mass Spectrometer(Bioyong Tech, Beijing, China). A three-peptide mixture (MW of 1532.8582 Da, 2464.1989 Da, and 5729.6087 Da, Cat Nos. P2613, A8346, and I6279, Sigma-Alderich) was used as internal standard. The salivary peptide profiles were analyzed by three groups: s-ECC, s-ECC treated for 1 week, and s-ECC treated for 4 weeks. A diagnostic model for s-ECC was established using the K nearest-neighbor (KNN) method. The detected peptides were identified by linear ion trap-Orbitrap-Mass Spectrometry (LTQ-Orbitrap-MS).

Results:

An average of 81 peptide mass peaks were detected when the three groups were compared. The peak intensities differed significantly for seven peptides (1370.4, 1723.7, 1851.4, 1886.5, 2331, 2995.7, and 3310.0 Da). Four of the seven mass peaks (1723.7, 1851.4, 2331.0, and 2995.7 Da) were used to establish a diagnostic model by the KNN method. Validation of the diagnostic model was carried out in six s-ECC patients before and after treatment for 1 and 4 weeks. Five of the six s-ECC patients (sensitivity 83.3%) were diagnosed as s-ECC, and five of those treated for 1 and 4 weeks (specificity 83.3%) were correctly grouped by this diagnostic model. Moreover, using LTQ-Orbitrap-MS detection, three of the four peptides used in the KNN method(1723.7, 1851.4, and 2331.0 Da) were successfully identified as Histatin-1 precursors, Myoglobin, and Prolactin-inducible protein.

Conclusion:

The three peptides identified here (1723.7, 1851.4, and 2331.0 Da) may be novel biomarkers of s-ECC. In future, the salivary peptide profile may allow identification of children at high risk of developing caries, and ensure the establishment of specific preventive and treatment strategies for such cases.

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MALDI-TOF MS analysis of HbA1c in Diabetes Mellitus

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Background: Optimal diagnosis and monitoring of diabetes mellitus relies on measurements of HbA1c, the relative amount of glucose attached to the N-terminal valine of the beta-subunit of hemoglobin. Here we report a novel approach to determine HbA1c using MALDI-TOF mass spectrometry. MALDI-TOF analyses of diluted, whole blood samples determine the ratio of hemoglobin subunit(s) with CVs of ≤ 3%.

Methods: Whole blood samples at a final dilution of 1:2000 in 5 mg/mL sinapinic acid were analyzed on a SimulTOF 200 (SimulTOF Systems, Sudbury, MA) from m/z 5000-20,000 in positive, linear mode. Each spectrum was the average of 20,000 laser-shots evenly distributed across the surface of the sample spot. Spectra were calibrated using the M1+ and M2+ ions of hemoglobin alpha- and beta-subunits. HbA1c was calculated from glycated alpha- or beta-subunit (M+162) using the following formula: [(M+162)/ (M + (M+162))]. Results obtained by MALDI-TOF assay are compared with those obtained in a clinical laboratory used clinically accepted HPLC methodology.

Results: Analytical standards and clinical patient samples for quantitation of total glycation on the beta-subunit of hemoglobin exhibited linearity (y = 0.79x + 1.4; R2 = 0.99) from 1.36% to 17.94% with CVs <1.66% (Figure 1).

Conclusion: While MALDI-TOF MS detects all glycation of the alpha and beta-subunit of hemoglobin, we have established the direct relationship between the MALDI-TOF method for the determination of the glycated beta chain and the HPLC method for determination of HbA1c. Additionally, the strong correlation between glycation of the alpha- and beta-subunits allows their ratio to be used as a quality control check for the determination of the glycated beta chain.

A-245

Simultaneous Sensitive Quantitation of Testosterone and Estradiol in Serum by LC-MS/MS Without Derivatization

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Background: Very sensitive measurements of serum estrogens and testosterone are important in adult and pediatric endocrinology and oncology. Sensitive measurements of estradiol (E_2) are needed for determination of menopausal status, estrogen deficiency, estrogen measurements in men, during antiestrogen treatment and in the diagnosis of other sex-hormone-related disorders. E_2 levels in postmenopausal women, men and children are typically <20 pg/mL. Ultrasensitive testosterone (Te) measurements are needed for adult women, whose values are routinely <50 ng/dL, in children, and men undergoing antiandrogen therapy whose values are usually less than 10 ng/dL. Many automated immunoassays lack the required sensitivity and specificity for the measurement of these very low concentrations of E_2 and Te. Our objective was to develop a very sensitive LC-MS/MS assay for both Te and E_2 in serum in a single analysis without the need for chemical derivatization and extended extraction protocols.

Methods: Serum samples (500 microL) were prepared by the addition of deuterated internal standards of both compounds followed by a liquid-liquid extraction using hexane:ethyl acetate (9:1, v/v). The supernatant was evaporated at 35°C under a stream of nitrogen and reconstituted in methanol:water (1:1, v/v). 80 microL was injected into the LC-MS/MS system. Chromatographic separation was performed on a Thermo Scientific TLX-2 HPLC system interfaced to an ABSciex 6500 mass spectrometer operated in both positive and negative ion ESI mode. Chromatographic separation was achieved using an Accucore C18 (50 X 3 mm i.d.) analytical column. Mobile phase comprised of A: 0.02 mM ammonium fluoride in water and B: acetonitrile. The HPLC gradient elution was 15-50% of mobile phase B over 2 minutes which was then held for 1.5 minutes. MRM transitions were as follows: Te 289.2>97.1 and 289.2>109.1 m/z; E_2 271.0>145.1 and 271.0>143.1 m/z. Calibrators and controls were purchased from Chromsystems using their multilevel calibrators and tri-level controls.

Results: The LOQs of Te and E_2 were 1 ng/dL and 5 pg/mL, respectively. The analytical measurement range (AMR) for Te was 1-1,170 ng/dL and 5-600 pg/mL for E_2. The calibration curves were linear over the AMR with correlation coefficients $R^2 \geq 0.998$. Assay accuracy was determined both by comparison with a LC-MS/MS method performed at a national laboratory and through recovery studies. Comparison with samples analysed by LC-MS/MS at the reference laboratory showed the following: Te had a regression slope of 1.01, R^2=0.996; E_2 had a regression slope of 0.92, R^2=0.996. Te recoveries at three concentrations spanning the AMR were between 101.4 and 105.4% and E_2 recoveries were between 100.3 and 105.2%. Within-day (N=10) and between-day (N=20) CVs at concentrations spanning the AMR were less than 5% for both analytes. A solution containing 13 naturally-occurring steroids with the potential for isobaric interferences or with similar retention times were analyzed and showed no interferences.

Conclusion: We have developed an accurate and highly sensitive assay to simultaneously measure Te and E_2 levels in serum by LC-MS/MS without the need for chemical derivatization. Unlike many immunoassays this method is free of cross reactivity from structurally similar analogs.

A-246

Tandem mass spectrometry-based molecular networking to detect drugs of abuse and analogues

J. Y. Yang[1], D. A. Herold[2], R. L. Fitzgerald[1]. *[1]UCSD, La Jolla, CA, [2]UCSD/VASD, La Jolla, CA*

Background: There is a need to quickly identify newer generations of illicit drugs such as "bath salt" analogs since routinely used multiple reaction monitoring (MRM) LC-MS/MS techniques are by definition targeted approaches. The development of MRM based methods requires a primary standard, which does not exist for most designer drugs when they first appear. In order to shorten the duration between the appearance of and the ability to identify a new designer drug analog, we propose the application of a novel approach that combines LC-MS/MS with molecular networking. Molecular networking exploits the premise that molecules with similar structures fragment similarly and computationally connects related molecules based on pair-wise comparisons of MS/MS spectra. The primary goal of this study is to evaluate the ability of LC-MS/MS and molecular networking to identify structurally related drugs from mixtures.

Methods: MS/MS reference spectra were generated on an AB SCIEX QTRAP 6500 by direct infusion of eleven standards purchased from Cerilliant diluted in methanol to 10 ng/mL. Data were converted to mzXML universal format using MSConvert from ProteoWizard, and MS/MS spectra were compared and analyzed using computational algorithms according to gnps.ucsd.edu. The computational algorithms simplify the data by generating consensus spectra from identical MS/MS spectra with a cosine similarity score of 0.97 or higher, where a score of 1 indicates identity. Then the consensus spectra, which are merged scans from multiple files, were compared pairwise and cosine similarity scores were assigned per pair. The results from molecular networking (via gnps.ucsd.edu) were imported into Cytoscape and displayed as a network of nodes and edges. The nodes labeled with precursor mass represent MS/MS consensus spectra, and the edges indicate cosine similarity score above 0.7, with the thicker lines reflecting higher scores. The mass difference between nodes or precursor masses may be used to predict the differences in functional groups.

Results: In the network, the nodes that represented consensus spectra for individual standards corresponded to methylone (m/z 208.1), ethylone (m/z 222.13), 4-methylephedrine (m/z 180.14), N-ethylcathinone (m/z 178.13), 3,4-DMMC (m/z 192.15), and R- and S-cathinone (m/z 149.19). Mass spectrometry detects m/z and not stereochemistry; it was surprising that R- and S-cathinone were represented by independent nodes. R- and S-methcathinone shared two nodes. There were five nodes representing methedrone (m/z 193.24), two nodes for diethylproprion (m/z 205.26), and the node for mephedrone (m/z 178.12) was combined with spectra from N-ethylcathinone.

The spectral patterns for methylone, ethylone, 4-methylephedrine, and N-ethylcathinone all show a strong initial loss of 18. Methylone and ethylone both have subsequent losses of 30 then 28, where as N-ethylcathinone has a subsequent loss of 28.

Conclusion: A molecular networking mass spectrometry based approach may be used to identify structural analogs of chemicals. Future directions will involve the analysis of compounds with both similar and diverse structures as compared with those used to develop the molecular network to determine if this approach will be useful for classifying new chemical compounds.

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Screen and Identification of Four Biomarkers for Discriminating Non-small Cell Lung Carcinomas and Pulmonary Nodules by MALDI-TOF MS

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Background:

Non-small cell lung carcinomas (NSCLC) is one of the most common Lung cancer. Early detection of NSCLC renders an opportunity to dramatically reduce the disease mortality. In our study, serum peptides were analyzed for the early detection or monitoring of NSCLC comparing with pulmonary nodules, so as to provide alternative detective options rather than the computed tomographic scanning.

Methods:

Serum samples from 15 patients with solitary lung nodules and 12 patients diagnosed with NSCLC were colleted in this study. All blood samples were obtained prior to

their treatment or surgery. Serum samples were fractionated using weak cation exchange magnetic bead (SPE-C100; Bioyong Tech, Beijing, China) according to the manufacturer's instructions. The resultant supernatant was transferred into a fresh tube, and the peptides were analyzed directly on a ClinTOF Mass Spectrometer (Bioyong Tech, Beijing, China). A three-peptide mixture (MW of 1532.8582 Da, 2464.1989 Da, and 5729.6087 Da, Cat Nos. P2613, A8346, and I6279, Sigma-Alderich) was used as internal standard. Then detected peptides were identified by linear ion trap-Orbitrap-Mass Spectrometry (LTQ-Orbitrap-MS).

Results:

An average of 105 peptide mass peaks were detected in both the NSCLC and control group. There were 16 peaks having peak frequencies above 50%, and six of them were significant different between the NSCLC and control groups (p-value < 0.05) when considering their relative peak intensity. Four peptides (MW of 1064.6 Da, 1077.0 Da, 1274.5 Da, and 1524.4 Da) were successfully identified by LC-MS/MS. Three different diagnostic models (Fishert, Linear SVM, and RBF) were established within these four peptides, to make a better combination. When two peptides were selected, the RBF model provides the best specificity and sensitivity of 95.04% and 95.72%. When three peptides were selected, the RBF model provides the best specificity and sensitivity of 98.04% and 97.72%. When all the four peptides were selected, the RBF model provides the best specificity and sensitivity of 99.94% and 99.72%, which means a diagnostic panel for identifying NSCLC and pulmonary nodule was successfully established. Further identification of these four peptides provided indicated that peptides of 1064.6 Da and 1077.0 Da were identified as fragment of FGA1, isoform1 of fibrinogen alpha chain precursor. Peptides of 1274.5 Da, and 1524.4 Da were identified as fragment of APOA1, apolipoprotein a-I precursor.

Conclusion:

Four serum peptides were identified as new biomarkers for NSCLC. A diagnostic panel of four serum peptides with 99.94% specificity and 99.72% of sensitivity was established for identifying NSCLC and pulmonary nodules by using the RBF model.

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High Resolution Accurate-mass Mass Spectrometry (HRAMS) Offers Superior Accuracy For Quantitation of Steroids and Proteins

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Background: We demonstrate the clinical laboratory application of high resolution accurate-mass mass spectrometry (HRAMS) for quantitation of steroids (17-hydoxyprogesterone (17OHPG) and androstenedione (ANST)) and a protein biomarker, insulin like growth factor-1 (IGF1). We show how HRAMS resolves interferences that are unresolved on a triple quadrupole instrument in ~5% of patient samples from our daily clinical volume for 17OHPG and ANST. Additionally we show how HRAMS identifies IGF1 variants (V-IGF1) not distinguished from wild-type IGF1 by immunoassays.

Methods:

(a)HRAMS for 17OHPG and ANST

Sample extraction involved equilibration with internal standard, protein precipitation followed by online extraction of the supernatant and chromatographic separation on a reversed-phase column. Analysis was performed on either an API 5000 in MRM mode or a Q Exactive in SIM mode (70,000 resolution). API 5000 and Q Exactive were compared. Transitions on the API 5000 were: ANST=287.2/97.1 and OHPG=331.3/109.1. On the Q Exactive the ions 287.2006 (ANST) and 331.2268 (OHPG) were added to the inclusion list. Samples with known chromatographic interferences on the API 5000 were analyzed on the Q Exactive.

(b)HRAMS for IGF1

100 µL sample is equilibrated with N15 labeled internal standard and treated with 400 µL acidified ethanol (1N HCl: Ethanol) and incubated at room temperature for 30 minutes followed by addition of 90 µL of neutralizing buffer (1.5 M trizma). The samples were then centrifuged at 3000 rpm for 10 minutes and cooled for 30 mins at -20oC. The samples are centrifuged again and analyzed by online extraction of the supernatant and chromatographic separation on a reversed-phase column. The m/z used to quantitate the wild-type IGF1 was 1093.5298. The Q-Exactive mass spectrometer was operated at a resolution of 70,000 and mass accuracy of 10 ppm was used for quantitation.

Results:

(a)A good correlation between the API 5000 (MRM) and the Q Exactive (SIM) with a slope of 0.9716 (n=162, R^2=0.9944) for ANST and a slope of 0.9567 (n=154, R^2=0.9955) for OHPG was obtained. Interassay imprecision was found to be acceptable using the Q Exactive and was 6-10% for ANST (range = 61-1932 ng/dL) and 11-12% for OHPG (range = 56-1831 ng/dL). The limit of quantitation was confirmed on the Q Exactive to be 15 ng/dL for ANST, and 40 ng/dL for OHPG. Most importantly, patient samples that showed interferences with either ANST or OHPG on the API 5000 showed no interference at the analyte retention time on the Q Exactive.

(b)HRAMS IGF1 assay compared well with the immunoassay slope of 0.9449 (n=1720, R^2=0.9573. In our validation studies for the HRAMS assay for IGF1 15 out of 2465 (0.6%) patients ~50% of the calculated IGF1-IA was V-IGF1. The V-IGF1 was found to have the same retention time as the native IGF1 and m/z difference (Δm/z) of ~4.5Da resulting in an m/z of 1097.9483. While immunoassay is unable to distinguish between the two variants, HRAMS enables identification potential novel IGF1 variants.

Conclusion:

HRAMS offers superior resolution and mass accuracy, which is useful for improving accuracy in quantitation of steroids and proteins in a clinical laboratory.

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Combining Nanobody Immunoenrichment and MALDI-TOF Mass Spectrometry to Detect and Isotype Monoclonal Immunoglobulins

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Background: Screening panels for monoclonal gammopathies include serum protein electrophoresis (SPEP), immunofixation electrophoresis (IFE) and serum free light chain quantitation (FLC). All three are recommended since together they achieve the highest sensitivity. As a standalone test, IFE has the best sensitivity for detecting monoclonal proteins (M-proteins) and provides immunoglobulin isotype information. However, IFE remains a laborious and expensive component of screening panels. Our objective was to develop a sensitive and rapid methodology to detect and isotype M-proteins that is cost-effective and automatable with the goal of reducing both the complexity and costs required to detect and monitor monoclonal gammopathies.

Methods: We developed an immunoenrichment MALDI-TOF MS assay that utilizes nanobodies (single-domain antibody fragments) to detect and isotype M-proteins. Briefly, in 96-well plate format, patient samples are incubated with 5 different nanobodies coupled to beads specific to the γ, α, and µ heavy chain domains of IgG, IgA, IgM or kappa and lambda (κ and λ) light chain domains. Beads are washed with PBS, incubated in 5% acetic acid/TCEP to release and disassociate heavy or light chains which are then spotted with matrix onto a 96-well MALDI plate. Subsequently, automated acquisition (~10 seconds/sample) is performed using a Bruker Microflex LT MALDI-TOF instrument. Mass spectra corresponding to light chains enriched by each nanobody are overlaid and M-proteins are detected and isotyped by the presence of distinct peaks within specific *m/z* regions occupied by the light chain repertoire. To compare sensitivity between IFE and MALDI-TOF MS, 14 IFE^+ patient samples were measured neat or diluted with pooled normal serum (10-, 100-, 200- and 500-fold). Blinded readers identified the dilution at which each sample became negative using both methods. Residual sera (N=124) was analyzed from patients with physician-ordered SPEP and IFE to assess concordance between IFE and MALDI-TOF considering both isotype identification and detection of M-proteins. Of these samples, 89 were IFE^+ (29 $SPEP^+/IFE^+$, 49 $SPEP^-/IFE^+$, and 11 $SPEP^-/IFE^+$ for κ or λ only) and 35 were IFE^-.

Results: In 11 of 14 patient samples tested by serial dilution, MALDI-TOF MS was more sensitive than IFE detecting ~10-fold lower concentrations of M-proteins. In the remaining 3 samples both methods performed equivalently, with agreement in isotype determination for all 14 samples. For concordance studies, MALDI-TOF MS identified M-proteins in 87 of 89 IFE^+ samples (97.8%) The two discordant samples were $SPEP^-/IFE^+$ (1 IgMλ, and 1 λ FLC). Of the 35 IFE^- samples, MALDI-TOF MS was negative for 31, identifying 4 additional samples with detectable M-proteins. In samples positive by both methods (N=87), isotyping concordance was 98.9%. In the one discordant case (IgMλ) MALDI-TOF isotyping failed to identify a monoclonal light chain in the λ spectra. In two cases MALDI-TOF identified the presence of a second clone in addition to the isotype identified by IFE suggesting biclonality.

Conclusion: This study demonstrates that detection and isotyping of M-proteins by MALDI-TOF MS is robust and exceeds the sensitivity of IFE. This method is amendable to automation, rapid and in its current format is cost-competitive with current clinical isotyping assays.

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Determination of Plasma Catecholamines by LC/MS/MS for Clinical Research

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Background:

Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is ideally suited for the rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of catecholamines (dopamine, epinephrine and norepinephrine) in plasma. This method uses a procedure by filtration on Agilent Captiva ND^{Lipids} tubes and an offline solid phase extraction (SPE) procedure for efficient sample preparation.

Methods:

An efficient solid phase extraction (SPE) sample preparation procedure was developed for the simultaneous extraction of dopamine, epinephrine and norepinephrine in plasma. Calibrators were created by spiking clean plasma with various concentrations of each analyte. The chromatographic system consists of an Agilent Pursuit pentafluorophenyl (PFP) column and a mobile phase comprised of methanol and water containing 1 mM ammonium fluoride. Deuterated internal standards were included for each analyte to ensure accurate and reproducible quantitation.

Results:

Chromatographic separation of all analytes is achieved through the use of a pentafluorophenyl column. The separation of epinephrine/normetanephrine and metanephrine/3-methoxytyramine are especially critical since these compounds share common fragments. Without proper separation by retention time, fragmentation of these compounds can cause interferences with one another and lead to inaccurate quantitation. The described analytical method achieves the required functional sensitivity and is capable of quantitating analytes over a sufficiently wide dynamic range with a single injection. All analytes displayed excellent linearity from 5 to 2500 pg/mL (0.03-16 nmol/L). All calibration curves displayed an $R^2 > 0.999$. Back-calculated accuracies for all calibrators ranged from 95% to 109% and showed inter-day CVs below 5%. Commercially-available quality control material was used to test the reproducibility of this method. Measurements were repeated in triplicate to assess intra-day reproducibility and on three separate days to assess inter-day reproducibility, and CVs were found to be below 4%.

Conclusion:

A robust method for quantifying dopamine, epinephrine and norepinephrine in plasma with excellent reproducibility and accuracy has been developed.

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Thermal cis/trans isomerization of vitamin k1 induced by electrospray ion source

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1. Background

The level of vitamin K1 in human body is important for their healthy. However, foods may contain appreciable quantities of the essentially non-active cis-isomer as a consequence of either its presence in synthetic K1 used during food supplementation, or photo-isomerization of the trans-isomer during exposure of the food to light. Since higher sensitivity and accuracy alternatives to chromatography would be suitable for the determination of geometric isomers in human plasma, the advancement of using mass spectrometry (MS) for this purpose has become more commonly. However, in-source isomerization could be a factor that affect the analysis results, thus, characterization of isomer ions in MS is of significance. In this work, ion mobility technology was adopted for isomerization investigation.

2. Methods

2.1 Conventional analysis

The liquid chromatography separation of isomers was performed by an ultra-performance liquid chromatography (UPLC) and C30 column. Fluorescence and electrospray-time of flight (ESI-TOF) were respectively adopted for isomer detection. An isocratic elution program was applied with 0.025 % formic acid (FA) and 2.5 mM ammonium formate (AF) in methanol with a 20-min run at a flow rate of 0.6 mL/min. Vitamin K1 was detected with fluorescence after post-column reduction with metallic zinc column, and excitation and emission wavelengths were set at 243 nm and 430 nm. The conditions of ESI-TOF were set as follows: the ion source was positive ESI with the capillary voltage was 3.5 kV, sampling and extraction cone voltage were 25.0 V and 4.0 V; ion source and desolvation gas temperature set at 90 °C and 300 °C; cone gas and desolvation gas flow rate were 50 L/h and 500 L/h.

2.2 Ion mobility analysis

Ions will go through the ion mobility (IM) cell that is flanked by a trap and transfer sub-cell that can function as two independent collision cells for fragmentation. The entrance to the mobility cell is preceded by a helium cell operated at high pressure to facilitate transport of ions into the ~3 mbar nitrogen pressure mobility cell. In order to realize a desired separation, key parameters including trap gas flow rate, IM gas flow rate, wave velocity and height were investigated.

3. Results and conclusions

Because the cis isomers had collision cross sections that were smaller than those of the corresponding trans ones, they were detected first followed at a later drift time by the trans isomer, and the separation of cis- and trans-isomers of vitamin K1 was demonstrated using IM with drift time separations of several milliseconds, furthermore, IM spectrum helped confirm that in-source isomerization occurred during positive ion electrospray. This in-source isomerization was found to be temperature dependent. Although cis/trans isomerization could not be eliminated during electrospray, this temperature dependence suggests that softer ionization conditions can minimize cis/trans isomerization in the ion source. Until cis/trans isomerization of vitamin K1 can be eliminated in the ion source, the slow process of LC separation of vitamin K1 isomers in matrix will still be necessary for quantitative application.

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Pre-column double derivatization method to quantify > 35 clinically relevant amino acids by HPLC-TripleTOFTM 5600

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Background: Clinically relevant amino acids are measured in physiological fluids to diagnose a number of inborn errors of metabolism. Widely used methods are HPLC with various detectors such as UV and mass spectrometry. The gold standard method for amino acid analysis is ion exchange chromatography with ninhydrin post-column derivatization. However, this method is laborious and time consuming. Many reverse phase chromatography methods have difficulty in separating isobars in one run and require extra sample preparation steps and multiple injections. Also, since many amino acids are polar, in reverse phase chromatography ion pair reagents are frequently used but cause problems in cleaning mass spectrometer. To avoid ion-pairing, amino acids are commonly derivatized to make them non-polar for reverse phase separation. Butylation is the most commonly used technique but many amino acids are refractory to butylation. To solve this problem, we developed a double derivatization method. Butanol and dansyl chloride were selected to derivatize amino acid carboxyl group and amino group, separately. TripleTOF™ 5600 (Time of Flight) in a high sensitivity and TOF MS scan positive mode were used for amino acids quantitation with high mass resolution.

Method: 50 µl patients' biological fluids (heparinized plasma, urine and CSF) were used. Isotopically labeled amino acids were used as internal standards. The general sample preparation steps were: 1) protein precipitation; 2) transfer and split of supernatant; 3) drying of supernatant and derivatization of residue with butanol and dansyl chloride; 4) reconstitution of residue and injection of the mixture.

Waters Acuity UPLC coupled to TripleTOF™ 5600 (AB Sciex) with a phenomenex column (Kinetex, C18, 2.6 µm, 100 x 3 mm) was used. Mobile phase A was 2mM ammonium formate / 0.1 % formic acid in water; Mobile phase B was acetonitrile. Flow rate was 0.5 mL/min. The column temperature was 50°C. The gradient was 2-40% B for 15 min, then 100 %B for 3.5 min and back to 2% B with total run time of 22 min.

Results: This method was validated and was able to quantify > 35 clinical relevant amino acids including glutamine, glycine, alanine, citrulline, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine and arginine. In general, the recovery ranged from 90 to 110 % and total imprecision was less than 15%. The quantitation of amino acids with isotopically labeled internal standards showed the best results. Most amino acids were doubly derivatized at carboxyl - and amino - groups except phosphoethanlomine and taurine. The isobaric compounds were well separated. The results from butylation and dansylation were generally in good agreement. The method compared well with ninhydrin derivatization HPLC method.

Conclusion: This double derivatization method is accurate, precise, sensitive and fast for the measurement of > 35 physiologically relevant amino acids.

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Measurement of aldosterone in clinical research; the quest for accuracy

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Background: Liquid chromatography tandem mass spectrometry (LC-MS/MS) is increasingly becoming the method of choice for steroid hormone measurements for clinical research, but thorough method validation is essential to ensure the quality of the method. Through the use of sample preparation, chromatographic separation and mass spectrometric detection, quantification of aldosterone by LC-MS/MS has been shown to provide greater selectivity than more traditional analytical techniques. Chromatographic separation of aldosterone from isobaric steroid species is paramount to obtaining accurate data. Since no higher order reference material is available for aldosterone in matrix, we have assessed external quality assurance samples from the UK National External Quality Assessment Service (UK NEQAS). Here we present a study of the effects of chromatographic selectivity on the analytical bias for quantification of aldosterone, followed by comparison to an established LC-MS/MS method for aldosterone analysis.

Methods: Aldosterone certified reference material in acetonitrile was purchased from Cerilliant (Round Rock, TX) to create calibrators in stripped serum purchased from Golden West Biologicals (Temecula, CA). QC material was prepared in plasma purchased from SeraLab (Haywards Heath, UK). Samples (n=35) were obtained from UK NEQAS (Birmingham, UK) to compare the analytical bias of the developed LC-MS/MS methods. Plasma samples (n=59) were analyzed using the finalized method and the results were compared to an independent LC-MS/MS method. Samples were precipitated prior to Solid Phase Extraction (SPE) on the Waters® Oasis® MAX µElution plate. Using an ACQUITY UPLC® I-Class system, samples were injected onto either a 2.1 x 50mm Waters AQCUITY UPLC BEH Phenyl column (Method 1) or a 2.1 x 100mm Waters CORTECS UPLC C_{18} column (Method 2) using a water/methanol gradient elution and quantified with a Waters Xevo® TQ-S mass spectrometer.

Results: Method 1 and 2 were shown to provide imprecision of <10%CV across the range 36-720pg/mL. Method 2 improved chromatographic resolution of aldosterone from interfering steroids in comparison to Method 1, which included baseline resolution of the 18-hydroxycorticosterone interference from aldosterone and its internal standard.Method accuracy assessment by the analysis of UK NEQAS samples through comparison to the All Laboratory Trimmed Mean (ALTM) results was performed using Method 1 and 2. The aldosterone scheme has on average 30 participating laboratories (range 26-33), with the largest group using a competitive fixed-time solid-phase radioimmunoassay (average 23 laboratories, mean %CV=9.4%), while the LC-MS/MS group comprised of only 2-3 laboratories (no %CV available). Method 2 demonstrated a greater bias (-23.1%) than Method 1 (-5.3%) when compared to the ALTM. Using Method 2, comparison with samples (n=59) previously analyzed by an independent LC-MS/MS method demonstrated good agreement, showing no significant bias ($p > 0.05$).

Conclusion: Chromatographic selectivity has been investigated for the quantification of aldosterone in plasma for clinical research. LC column selection has been found to be a critical parameter that can significantly change analytical bias for the analysis of aldosterone. This further highlights the need for a reference measurement system for aldosterone, enabling harmonization of methods across different laboratories.

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Rapid, Comprehensive and Simultaneous Determination of Inborn Errors of Metabolism using an Untargeted Metabolomics Methodology

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Background

The objective of this study is to validate the use of an untargeted metabolomics methodology to detect metabolic disturbances in fasted plasma samples for the detection of inborn errors of metabolism. Inborn Errors of Metabolisms (IEMs) are caused by defects in the enzymes that help process nutrients, causing an accumulation of toxic substances or a deficiency of substances needed for normal body function. Making a swift, accurate diagnosis of an IEM is critical in preventing developmental disorders. While there are several hundred recognized IEMs, state public health programs only screen for 40 disorders or fewer at birth and do so with an array of targeted assays. The approach described here allows for the detection of metabolic disturbances caused by IEM's beyond what is currently available with targeted assays and at a lower cost.

Methods

All samples were run over three separate chromatographic methods. Proteins were first precipitated from plasma samples via MeOH. The supernatant from the crash was divided into aliquots for each method, dried and then reconstituted in method appropriate solvents. All three methods were run on ThermoFisher Q-Exactives coupled to Waters Acquity UPLCs and included a positive ion reverse phase (RP) method, a negative ion RP method and a negative ion HILIC method. One hundred, twenty-six (126) compounds across three platforms were validated to CLSI standards including assessing precision, linearity, carryover, LOD, interference, stability and others. The Q-Exactives alternated between MS scans from 70-1000 m/z and MS^n scans and were operated at 35K resolution. Compounds were identified by match to authentic standard library based on retention index, accurate mass and fragmentation.

Results

The intra-assay precision (within run), as measured by %CV for all 126 compounds in triplicate plasma samples, was <20% except in 8 instances and the total, inter-assay precision during a multi-day trial was <20% except in 6 instances. When the signal intensities are scaled against a set of six plasma matrix controls that are run on a daily basis, the precision of the assay improves significantly. All 126 compounds showed linearity of >0.95 R^2. In addition to carryover, stability, and interference studies, an accuracy study was performed on a set of 200 pediatric plasma samples. The cohort included 130 samples from patients with 21 known IEMs and 70 samples from healthy individuals. The method correctly identified 20 of the 21 disorders.

Conclusion

Results of this proof-of-concept study demonstrate that metabolomic profiling has the potential to detect a wide range of IEMs and could represent an attractive initial screening option for other diseases with a suspected biochemical, genetic origin. Importantly, this methodology accurately identified the affected individual patients when compared to a healthy population, demonstrating the power of this methodology for personalized medicine. In this presentation, we highlight the testing carried out to validate our untargeted metabolomics methodology and present a case study of a patient that was identified with citrullinemia through biochemical signatures in multiple pathways by screening a single draw plasma sample on this metabolomics platform.

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Development of a Reference Measurement System for Urine Albumin

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Background: Urine albumin and urine albumin/creatinine ratios are used in the detection, treatment, and monitoring of chronic kidney disease. Although inter-method differences and analyte heterogeneity have been reported for urine albumin measurements, accuracy assessments of the available methods have been hindered by the lack of a reference measurement system, including reference measurement procedures and reference materials, for this clinical analyte. To address the need for a reference measurement system for urine albumin, we have developed a candidate reference measurement procedure (RMP) for the value-assignment of urine albumin in a matrix-based standard reference material.

Methods: The candidate RMP incorporates an isotopically-labeled (15N) full-length recombinant human serum albumin (15N-rHSA) material as the internal standard, which permits the absolute quantitation of albumin in human urine. A total of 11 tryptic HSA peptides with 2 transitions per peptide were selected on the basis of retention time reproducibility, peak intensity, and the degree of HSA sequence coverage. The calibrators were generated by spiking charcoal-stripped human urine with the 15N-rHSA internal standard at approximately 22.0 mg/L and the unlabeled NIST SRM 2925: Human Serum Albumin Solution (0.8443 g/L ±0.0320 g/L) with HSA concentrations ranging from 5 to 300 mg/L. The samples were digested with trypsin via a conventional digest method and the products were analyzed using LC-MS/MS (Agilent 6460 mass spectrometer coupled to the Agilent 1290 Series LC system) in the positive ion mode under multiple reaction monitoring (MRM) MS conditions.

Results: The multiplexed urine albumin assay displayed good linearity over a concentration range of 5 to 300 mg/L with r2 values >0.99 over the 23 MRM measurements. Minimal matrix effects were observed from the analysis of the HSA peptides in the buffer system (50 mM ammonium bicarbonate in water) compared to the urine (NIST SRM 3667) system, with CV values ranging from 0.1-2.3%. The inter-peptide and intra-peptide precision for 5 QC levels showed CV values <15%. To investigate the accuracy of the method, a total of 23 calibration curves were generated from the MRM measurements and used to determine the urine albumin content of 15

non-pooled patient urine samples. The combined (all 23 MRM measurements) CV values for each of the 15 patient samples ranged from 5.9-12.7%.

Conclusion: MS-based quantification of urine albumin provides both accurate and repeatable measurements at both micro- and normoalbuminuria levels, which can facilitate early diagnosis of kidney dysfunction. In addition to the quantitative advantages, we are also able to qualitatively evaluate molecular heterogeneity of endogenous urine albumin via the incorporation of multiple peptides that span the HSA sequence in the multiplexed assay. The high degree of selectivity and sensitivity of the MS-based urine albumin assay coupled with the highly purified SRM 2925 calibrator (primary reference standard) will support the value-assignment efforts of the matrix-based urine albumin secondary reference material.

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LC-MS/MS Method Optimization for the Analysis of 25-Hydroxyvitamin D and 24,25-Dihydroxyvitamin D Metabolites in Human Serum and Plasma

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Background: The quantitative analysis of 25-hydroxyvitamin D levels in serum and plasma provides an essential assessment of patient Vitamin D status and an evaluation of potential Vitamin D deficiency. We have optimized our LC-MS/MS method for the quantitation of 25-hydroxyvitamin D2/D3 and 24,25-dihydroxyvitamin D3 levels in patient serum and plasma samples. As part of this method optimization, we have incorporated the ongoing between-run analysis of internal and external quality control samples, including the testing of the NIST Standard Reference Materials SRM 972, SRM 972a, SRM 968e, and SRM 1950 human serum and plasma samples for additional accuracy and precision assessments of the 25-hydroxyvitamin D metabolites levels.

Methods: 25-hydroxyvitamin D2/D3 and 24,25-dihydroxyvitamin D3 analytical calibration curve standards and controls were prepared in normal human plasma depleted of Vitamin D metabolites. Isotopically labeled hexadeuterated 25-hydroxyvitamin D2/D3 internal standards were added to 200 uL of samples, and extracted using liquid/liquid extraction. Extracts were then dried under a stream of nitrogen, reconstituted in methanol, and transferred to autosampler vials for injection to the LC-MS/MS. Analysis was performed using an AB SCIEX API4000 LC-MS/MS system coupled with an Agilent 1100 LC and operated using APCI in the positive ion mode, with Multiple Reaction Monitoring (MRM) for quantitation of the Vitamin D metabolites.

Results: Linearity was demonstrated across the range of 1.25 -180 ng/mL for the 25-hydroxyvitamin D2/D3 metabolites, with calibration curve R2 >0.999, and across the range of 2-100 ng/mL for the 24,25-dihydroxyvitamin D3 analyte, also with calibration curve R2 >0.999. Six levels of 25-hydroxyvitamin D2/D3 controls prepared in depleted normal human plasma, and tested by two different analysts, demonstrated between-run LC-MS/MS results (n = 52) within 2.0% of target concentrations with precision (%CV) within 3.7%. LC-MS/MS testing of 24,25-dihydroxyvitamin D3 normal human plasma controls showed between-run results (n =24) within 2.5% of target concentration levels, with %CVs within 2.0%. LC-MS/MS between-run analysis of the NIST SRM materials demonstrated 25-hydroxyvitamin D metabolites results within 2.3% of target concentrations. LC-MS/MS testing of 94 patient serum and plasma samples was completed for the assessment of comparative Vitamin D metabolite concentration levels.

Conclusion: This LC-MS/MS method has been demonstrated to be an accurate and precise method for the analysis of 25-hydroxyvitamin D and 24,25-dihydroxyvitamin D3 levels in human serum and plasma samples. As an additional ongoing measure of method accuracy, precision, and specificity, we continue to analyze standard reference material and external quality assessment samples as part of our routine testing using this LC-MS/MS method.

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Development And Validation Of A High Sensitivity MicroLC-MS/MS Underivatized Method For Estradiol In Human Serum

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Background: There are considerable demands to accurately measure estradiol (E2) at very low concentrations (<5 pg/mL) in postmenopausal women, men, pediatric patients, and patients receiving breast cancer treatment. Historically, high sensitivity E2 assays used in clinical laboratories are radioimmunoassays, which may lack specificity and accuracy for measuring E2 over a wide concentration range. Most current high sensitivity LC-MS E2 methods can overcome these analytical limitations, but requires large sample volume and involves complex sample preparation with dansyl chloride derivatization. Our study aims to develop a high sensitivity, underivatized method using MicroLC-MS/MS to reliably measure E2 concentrations below 5 pg/mL using low sample volume.

Method: Estradiol-d4 (C/D/N isotope, Quebec) was used as the internal standard (IS) and calibrators were made by spiking E2 standards (Sigma, MO) into the E2-depleted serum (Golden West Biologicals, CA). 290µL of each calibrator and samples were mixed with 10µL of 2.5 ng/mL IS, and extracted with 3mL extraction buffer consisting of 90% hexane (Sigma, MO) and 10% ethyl acetate (Sigma, MO). The organic phase was evaporated under nitrogen at 37°C, and the dried residue was dissolved in 60µL of 1:1 methanol/water (v/v) prior to an injection of 5µL into the MicroLC-MS/MS. Analytes were separated on a micro-C18 column (0.5x50mm, 3µm, YMC America, PA) with a flow rate of 35µL/min and a run time of 3.5min using the Eksigent micro-LC 200 system (SCIEX, MA). The gradient started with 10% mobile phase A (0.01% ammonium hydroxide in water) and 90% mobile phase B (0.01% ammonium hydroxide in acetonitrile). The mobile phase B was increased to 98% over 1.5min and then held steady for 1 min. E2 and IS are detected by the SCIEX QTrap 6500 mass spectrometer in negative mode using the following transitions: E2: 271/145 (quantifier), 271/143 (qualifier); IS: 275/147.

Results: In this method, it is crucial to use HPLC columns with stability at the pH of 10. Our method demonstrated good linearity over a concentration range of 3– 820 pg/mL with r2 > 0.999. Total precision displayed CVs< 15% for all three QC levels (4.4 pg/mL, 49 pg/mL and 555 pg/mL) and the limit of quantitation was 3 pg/mL (CV< 20%). Method comparison studies for samples with E2 < 100 pg/mL showed: [RIA E2] = 0.92[LC-MS E2] +1.18, mean bias = -1.0 pg/mL (n= 29); [Cobas e602 E2] = 1.1[LC-MS E2]+ 3.60, mean bias = +6.0 pg/mL (n=40). Hemoglobin and bilirubin was observed to negatively interfered with this method at H-index of 370 and I-index of >9, respectively. There is no significant interference of triglycerides up to 800 mg/dL.

Conclusion: Our MicroLC-MS/MS method without derivatization, shows good analytical performance and can reliably measure E2 in blood samples with a LOQ of 3 pg/mL.

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Evaluation and Comparison of Nonderivatization and Derivatization Tandem Mass Spectrometry Methods for Analysis of Amino Acids and Acylcarnitines in Dried Blood Spot

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Introduction

Flow injection tandem mass spectrometry (FIA-MS/MS) has been frequently used to analyze amino acids (AA), acylcarnitines (AC), and succinylacetone (SUAC) in dried blood spots (DBS) for inborn errors of metabolism research. Original sample preparation techniques detect butyl esterification (i.e., derivatized) of AAs, ACs, and SUAC. However, with improved sensitivity of MS instruments, it is possible to detect AAs, ACs, and SUAC as their native free acids (i.e., nonderivatized). This simplifies analytical operation and minimizes the use of corrosive chemicals. Using quality control (QC) DBS samples enriched with different levels of multianalytes, we conducted a comprehensive study to evaluate and compare nonderivatization and derivatization tandem MS methods on a triple quadrupole mass spectrometer.

Methods

We developed a method in which AAs, ACs and SUAC were extracted in a single extraction resulting in significant reduction in labor and time. The 3.2 mm DBS punches were extracted by incubating with acetonitrile-water-formic acid mixture containing hydrazine and stable-isotope labeled internal standards. The extracts were derivatized with n-butanolic-HCl followed by evaporation and reconstitution with mobile phase. For the nonderivatization method, the extracts were simply dried and reconstituted with mobile phase. In both cases, the extracts were analyzed by Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer. Quantification of 12 AAs, 18 ACs, and SUAC was achieved using a meta-calculation software, iRC PRO™. The off-line automated tool streamlined data processing of peak area, concentration and user-defined formulas.

Results

We utilized DBS QC samples to evaluate and compare the two tandem MS methods. The QC samples contained enriched multianalytes at three concentrations: low, intermediate and high. For each method, we evaluated within-run precision by means of ten successive, independent measurement of DBS samples (n=10) and run-to-run precision by means of ten independent measurement in seven different test series

(n=70). We also compared quantitative results of multianalytes from nonderivatization and derivatization methods.

Within-run Precision

For the derivatization method, the within-run precisions (n=10) for AAs, ACs, and SUAC at three concentrations were less than 8.9% (low), 8.3% (intermediate), and 9.0% (high).

For the nonderivatization method, the within-run precisions for AAs, ACs, and SUAC were less than 7.6% (low), 7.2% (intermediate), and 9.8% (high).

Run-to-run Precision

For the derivatization method, the run-to-run precisions (n=70) for AAs, ACs, and SUAC were less than 15.0% (low), 15.6% (intermediate), and 16.1% (high).

For the nonderivatization method, the run-to-run precisions for AAs, ACs, and SUAC were less than 12.8% (low), 12.8% (intermediate), and 12.6% (high).

Method Comparison

The method differences of 12 AAs and SUAC between quantitative values resulting from butyl esters and free acid techniques at three concentrations were less than 3.8% (low), 4.8% (intermediate), and 3.2% (high). The method differences of 18 ACs were less than 14.2% (low), 11.4% (intermediate), and 10.5% (high). Therefore the two methods were highly correlated. Our data are consistent with the reported results from a comprehensive empirical analysis.

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Mass-spectrometric relative quantitation of glycated hemoglobin S

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Background: Glycated hemoglobin (reported as HbA1c) is an indicator of the average glucose level in the blood over the previous several months. The presence of hemoglobin genetic variants can affect the accuracy of some widely used HbA1c tests if the operator is unaware of the presence of genetic disorder. Hemoglobin S (HbS) is the most common hemoglobin mutant and consists of a β-chain substitution of Glu with Val at the seventh position. The aim of the study was to measure the extent of the glycation of the β chains in order to compare glycation rates for normal and mutated (β*) β-chains of heterozygous hemoglobin S (HbAS).

Methods: We analyzed 20 hemolysate samples from HbAS subjects both with and without diabetes. The diluted and desalted lysate samples were introduced into a triple quadrupole mass spectrometer (AB Sciex API-4000) at a flow rate of 50 µl/min. The instrument was operated in ES+ mode, six 10-sec scans were summed over 600-1400 m/z range. The relative quantification was performed by comparison of ion intensities of structurally similar intact globin chains in the deconvoluted spectra (β vs β*, α^g vs α, β^g vs β, β^{*g} vs β*, where g - glycated). For samples with low HbA1c, boronate affinity concentration of the glycated fraction was perfomed prior to analysis.

Results and conclusion: The ratio β*/β: 40/60 was found to be relatively stable for all samples analyzed. We found the glycation rate to be slightly higher for the β* compared to the normal β. The mass-spectrometric approach developed easily allows for relative quantitation of glycated forms of HbS and could be used to examine glycation rates for other hemoglobin variants. Further studies are needed to determine the extent to which the different glycation rates may impact clinical interpretation of HbA1c results obtained by various routine assay methods.

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Vedolizumab Quantitation in Serum using SRM And microLC-ESI-Q-TOF Mass Spectrometry

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Background: Vedolizumab is a humanized $IgG_1\kappa$ therapeutic monoclonal antibody (mAb) that inhibits T-cell migration to the gut mucosa by selectively targeting $\alpha_4\beta_7$ integrin and inhibiting its ability to bind to mucosal-addressin-cell-adhesion-molecule-1 (MAdCAM-1). Vedolizumab was recently FDA approved for the treatment of patients with moderate-to-severe ulcerative colitis and Crohn's disease who have no response or have lost response to TNF-α inhibitors. Currently there are no clinically available methods to measure vedolizumab. There is demand from the gastroenterology practice to guide therapy and improve outcomes for these patients refractory to multiple therapies. **Objective:** To develop and compare mass spectrometry (MS) methods to quantitate vedolizumab using: (1) tryptic peptides unique to the mAb by LC-MS/MS and (2) its intact light chain (iLC) accurate mass by microLC-ESI-Q-TOF/MS as a surrogate for drug concentrations. Furthermore, determine if vedolizumab concentrations correlate with C-reactive protein (CRP) concentrations. **Methods:** Vedolizumab was spiked into pooled human serum to generate a 7-point standard curve (5, 10, 25, 50, 100, 150, 250µg/mL) and control material. Proteotypic peptides unique to the heavy (HC) and light (LC) chains of vedolizumab were identified for quantification by selective reaction monitoring (SRM). Horse IgG was added to samples as an internal standard, followed by reduction, alkylation and trypsin digestion. The mixture was analyzed by LC-MS/MS (AbSciex API 5000). For iLC analysis monoclonal immunoglobulin Rapid Accurate Mass Measurement (miRAMM) was used. Briefly, infliximab was spiked into serum as internal standard and immunoglobulins were purified using Melon Gel. Light chains were released using DTT and injected on an Eksigent microLC system. Spectra were collected on an ABSciex-Triple-TOF-5600-MS. Vedolizumab iLC were quantified by summing multiple charge states corresponding to vedolizumab based on retention time and accurate mass measurements. Assay characteristics were defined by measuring precision and linearity. Subsequently, vedolizumab was measured in residual sera from patients undergoing therapy (n=110 samples from 28 unique patients) using both methods. Chart-review was conducted to correlate infusion date with vedolizumab concentration. Clinically-ordered CRP (immunoturbidimetry, reference interval (RI) ≤8mg/L) was also compared to vedolizumab concentrations. **Results:** Lower limit of quantitation (LOQ) for both HC and LC peptides was 5.0µg/mL; while LOQ for vedolizumab iLC quantitation was 10µg/mL. Both methods were linear up to 250µg/mL, n=10, R^2>0.99. Inter- (n=10 days) and intra-assay (n=10 runs) precision ranged from 3-19% for both methods using three levels of controls (10, 50, 150µg/mL). Method comparison using Passing-Bablok regression fit (x=proteotypic method) was y=0.79x + 4.78 (r=0.90). Vedolizumab concentrations, measured in patient sera by both methods in different time points of treatment went from <5–160µg/mL. Serial measurements (varying from 2-13) were available for 19 patients and trended inversely with infusion dates (R^2=0.86). Vedolizumab was also inversely correlated with CRP. When dichotomized into within (n=13) or above RI (n=24), vedolizumab measured 23±21 vs. 37±23 µg/mL (p=0.03). **Conclusions:** This study not only demonstrated that vedolizumab can be quantitated in patients by both proteotypic peptides and intact light chain MS, but more importantly, showed that those measurements may correlate with inflammation and aid in therapeutic monitoring of response to treatment.

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Quantitation of Ubiquinone (Coenzyme Q10) and Retinyl Palmitate in Serum/Plasma using Liquid Chromatography Electrospray Tandem Mass Spectrometry

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Introduction: The monitoring of vitamin levels in patients utilizing supplementation therapy is important for the prevention of potential toxicities and measurement of treatment outcomes. Vitamins play an essential role in human health and wellness. While essential, many vitamins cannot be synthesized within the body and must be obtained externally, most commonly from dietary intake. Retinyl Palmitate and Ubiquinone (Coenzyme Q10) are powerful antioxidants that are responsible for maintaining a variety of essential functions throughout the body. Coenzyme Q10 is considered an essential co-factor in the mitochondrial respiratory chain responsible for oxidative phosphorylation and plays a unique role in the electron transport chain. Retinyl Palmitate (RP) is a synthetic alternate form of vitamin A (retinol) and is widely used in nutritional supplements in its place. Vitamin A serves in multiple roles: it is important for growth and development, maintenance of the immune system and good vision.

Mindful of the numerous unique features and the idiosyncratic nature of these molecules we set out to improve their detection utilizing positive electrospray ionization tandem mass spectrometry spectroscopy (ESI- LC-MS/MS). Our aim was to improve the sensitivity and to simplify the extraction process and create a robust method suitable for the routine analysis from serum or plasma samples.

Method: Aliquots of 200 µl of standard, control and patient serum/plasma samples were spiked with Coenzyme Q10-D9 serving as an internal standard. The extraneous proteins were precipitated from the samples with ethanol and followed by the addition of hexane. Samples were analyzed on an API 3200 triple quadrupole mass spectrometertandem mass spectrophotometer (Applied Biosystems) equipped with a TurboIon Spray® source and a Shimadzu Prominence 20A HPLC system. Chromatographic separation was achieved using a LUNA 3µ PFP (2) 50 x 2.0 mm, 100Å column (Phenomenex) at a flow rate of 0.85ml/min, for a total run time of 5 minutes. The MS/MS was operated in positive electrospray and multiple reaction monitoring (MRM). Two transitions were monitored for each analyte: 880.7 ->

197.3 and 880.7 ->237.3 for Coenzyme Q10 (CoQ10), 269.4 ->93.0 and 269.4->107.0 for Retinyl Palmitate and 889.7->206.3 and 889.7 -> 189.1 for Coenzyme Q10-D9. **Results:** Validation was performed on 127 previously run samples, adhering to CLSI-derived protocols. Data analysis was performed using EP Evaluator® for the following parameters: precision (inter-, intra-, LOD/LOD), linearity, recovery, correlation, carry-over, interference and stability. CoQ10 linearity testing showed the assay was linear from 0.22 - 12.0 mg/L with a slope of 0.954 and an observed error of 6.2%. RP demonstrated linearity from 0.03 - 2.0 mg/L and a slope of 0.994 with an observed error of 8.8%. Both analytes recovered between 93.0-106% and the correlation coefficient between CoQ10 and RP comparing our method and the reference method was r=0.9205 and 0.9958 respectively. Correlation of our assay demonstrated no significant bias and all precision studies were CV% = ≤20.0%. No significant interfering substances were observed.

Conclusion: We have successfully developed and validated a robust, simple and effective method for extracting and monitoring Coenzyme Q10 and Retinyl Palmitate levels in patient samples.

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Simultaneous Determination of p-Phenylenediamine, N-Acetyl-p-phenylenediamine and N,N-Diacetyl-p-phenylenediamine in Human Urine by LC-MS/MS

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BACKGROUND: *p*-Phenylenediamine (PPD) is used in manufacture of hair dyes and skin decoration. In some developing countries, suicidal, homicidal and accidental cases by PPD were recorded. In this work, a sensitive LC-MS/MS method for determination of PPD and its metabolites *N*-acetyl-*p*-phenylenediamine (MAPPD) and *N,N*-diacetyl-*p*-phenylenediamine (DAPPD) in human urine has been developed and validated.

METHODS: PPD, MAPPD and DAPPD were extracted for urine with methylene chloride at alkaline pH. Acetanilide was used as internal standard (IS). The analytes and IS were separated on an Eclipse XDB- C18 column (150 X 4.6 mm, 5 µm) using a mobile phase of acetonitrile-1% formic acid in gradient elution. Detection was performed by LC-MS/MS using electrospray positive ionization under multiple reaction-monitoring mode. The transition ions m/z 109 → 92, m/z 151 → 92, m/z 193 → 92, and m/z 136 → 77 were selected for the quantification of PPD, MAPPD, DAPPD, and IS, respectively.

RESULTS: Calibration curves were linear in the range 10–2000 ng/mL for all analytes. The mean recoveries for PPD, MAPPD and DAPPD were 57.62, 74.19 and 50.99%, respectively. Intra- and inter-assay imprecisions were within 1.58–9.52% and 5.43–9.45% respectively for PPD, MAPPD and DAPPD. Inter-assay accuracies were within -7.43 and 7.36 for all compounds. PPD, MAPPD and DAPPD were stable in urine at – 20 ^{0} up to 24 hrs.

CONCLUSIONS: The method was successfully applied to analysis of PPD, MAPPD and DAPPD in urine samples collected from suicidal and accidental cases.

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Evaluation of the effects of 25OHD2 and 3-epi 25OHD3 on five automated 25OHD Immunoassays with comparison to a liquid chromatography tandem mass spectrometry method

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Background: Vitamin D testing is becoming increasingly important with the recognition that it is correlated with autoimmune diseases, cardiovascular diseases and cancer, and vitamin D deficiency is common in the worldwide. Recently, automated immunoassays are becoming popular to cope with the increasingly workload. However, the efficiency to separate and measure $25OHD_3$, $25OHD_2$ as well as 3-epi $25OHD_3$ was different, which may cause variation between methods.

Methods: Our objective was to evaluate the effects of $25OHD_2$ and 3-epi $25OHD_3$ on Roche, Siemens, Abbott, IDS and DiaSorin immunoassay systems with comparison to a liquid chromatography-tandem mass spectrometry (LC-MS/MS). We selected 332 serum samples from routine vitamin D assay requests, including 166 serum samples contained only $25OHD_3$, 111 serum samples contained both $25OHD_3$ and $25OHD_2$, 32 serum samples contained $25OHD_3$ and 3-epi $25OHD_3$, and analyzed with these methods.

Results: In the samples with no $25OHD_2$ or 3-epi $25OHD_3$, all the immunoassays correlated well with LC-MS/MS (r≥0.872), DiaSorin and Roche showed negative bias less than 2.5 ng/mL while Abbott and IDS showed positive bias less than 2.0 ng/mL. However, Siemens showed the biggest positive bias 12.5 (11.0-14.0) ng/mL and the poorest Kappa (0.468) while other four immunoassays showed excellent agreement with LC-MS/MS-1(Kappa>0.8)(Using 20 ng/mL as the cut-off for vitamin D deficiency). When there is $25OHD_2$ or 3-epi $25OHD_3$ in the samples, the correlation and clinical agreement decreased between all the immunoassays and LC-MS/MS. After using the regression models to transfer the cut-offs for immunoassay methods, Siemens had the biggest improvement of Kappa values (0.637) and all the immunoassays showed an acceptable agreement with LC-MS/MS.

Conclusions: Most automated immunoassays showed acceptable correlation and agreement with LC-MS/MS when there is no detectable $25OHD_2$ and 3-epi $25OHD_3$. Both $25OHD_2$ and 3-epi $25OHD_3$ affects the immunoassay methods a lot and bias existed between methods. Using one general cut-off for all the methods may be not appropriate.

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Determination of Plasma Renin Activity by LC/MS/MS for Clinical Research

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Background:

Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is ideally suited for the rapid analysis of analytes in complex matrices. A highly sensitive and specific LC/MS/MS analytical method has been developed for the determination of plasma renin activity (PRA) for clinical research. Plasma samples are incubated for 3 hours at 37°C. A sample preparation procedure by solid phase extraction (SPE) allows efficient extraction of Angiotensin I in plasma. Plasma renin activity is calculated by subtracting Angiotensin I concentration in a blank plate.

Methods:

For Generation sample, the plasma sample is incubated under controlled pH conditions for 3 hours at 37°C. The reaction is quenched and internal standard is added. The mixture is subjected to SPE extraction and analyzed by LC/MS/MS. For Zero sample, a duplicate plasma sample is immediately subjected to SPE extraction and analyzed by LC/MS/MS. Plasma Renin Activity is calculated by subtracting Angiotensin I concentration in the Zero sample from the Generation sample. The result is divided by the duration of incubation (3 hours = 10800 seconds) for a final value in ng/L/s. Angiotensin I (Proteochem) calibrators are prepared in 1% bovine serum albumin buffer pH 6 solution. Quantifier and qualifier transitions were monitored. Isotopically labelled Internal Standard (AnaSpec) was used. Bio-Rad Lyphochek controls were used. The chromatographic system consists of an Agilent Poroshell 120 SB-C18 column coupled with a guard column and a mobile phase comprised of methanol and water containing 0.2% formic acid.

Results:

The described analytical method achieves the required functional sensitivity and is capable of quantitating Angiotensin I over a sufficiently wide dynamic range. Angiotensin I displayed an excellent linearity from 0.1688 to 100 ng/mL. The calibration curve displayed an $R^2 > 0.9996$. Back-calculated accuracies ranged from 93% to 114%. Commercially-available quality control material was used to test the reproducibility of this method. Measurements were repeated in triplicate to assess intra-day reproducibility, and on three separate days to assess inter-day reproducibility, and CVs were found to be below 6%.

Conclusion:

A robust analytical method for quantifying angiotensin I in plasma by LC/MS/MS which is applied for the determination of Plasma Renin Activity has been developed. Typical method performance results are well within acceptable criteria.

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Targeted Screening of Drugs of Abuse using Laser Diode Thermal Desorption and mass spectrometry

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Background:

Toxicology laboratories generally use screening methods to obtain a semi-quantitative response for samples suspected of containing drugs of abuse. Some screening techniques are fast but less specific and generate by far too many false positive results. Using mass spectrometry combined with ultra-fast high-throughput LDTD ion source enhances specificity while maintaining and even surpassing the speed and throughput of traditional screening methods.

The Laser Diode Thermal Desorption (LDTD) ion source uses an infrared laser diode to indirectly thermally desorb neutral species of drugs of abuse molecules from urine sample extracts. These neutral species are carried into a corona discharge region, where they undergo efficient protonation and are introduced directly into the mass spectrometer. The total analysis time is under 9 seconds with no carry-over.

The objective of this experiment is to validate a simple urine extraction, the analysis method and to test different real samples using the LDTD-MS/MS. A cross validation study against the LC-MS/MS approach for the analysis of targeted drugs of abuse (PCP, ecstasy metabolite, amphetamine and methamphetamine) was done in order to evaluate the performance of the proposed alternative LDTD-MS/MS method

Methods:

A calibration curve and quality control samples were prepared in blank urine. 100 µL of calibrators, QC and patient specimens were transferred to an Eppendorf tube. 20 µL of internal standard (Amphetamine-d5, Methamphetamine-d9, PCP-d5 and MDMA-d5 at 750 ng/mL) in a mixture of Water:Methanol (1:1)) and 250 µL of sodium hydroxide (1N) were added. The mixture was vortexed. A liquid-liquid extraction was then performed by adding 250 µL of 1-Chlorobutane. Following the mixing and centrifugation at 5000 rpm for 3 min, 100 µL of the organic layer was transferred in an Eppendorf tube and 10 µL of HCl (0.01N) in methanol solution was added and mixed. 6 µL was deposited in the LazWell Plate and evaporated to dryness. The LDTD laser power was ramped to 45% in 3 seconds, and shut down after 2 seconds. Positive ionization mode was used and AB Sciex-5500 QTrap system was operated in MRM mode.

Results:

The calibration curves show excellent linearity with r higher than 0.995 between the quantification range of 4 to 800 ng/mL for Amphetamine, Methamphetamine, PCP, MDA, MDEA and MDMA. Inter-run accuracy and precision are between 98.4 to 107.7% and 2.6 to 9.3%, respectively, for every drug. No matrix effect or carryover was observed. This method was cross validated with results from a traditional LC-MS/MS method with real patient specimens. All negative samples correlated accordingly. A correlation higher then 0.99 between LC-MS/MS and LDTD-MS/MS methods for positive samples was calculated. All negative samples are detected as negative in both methods.

Conclusion:

A fast liquid-liquid extraction combined to LDTD-MS/MS technique provides a unique ultra-fast and specific mass spectrometry high-throughput method for screening. This method has demonstrated accurate, precise and specific results with a run time of 9 seconds per sample.

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Development of a LC-MS/MS assay for the detection of sulfonylurea drugs, and application of the assay in emergent hypoglycemia cases.

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Sulfonylurea agents are commonly used in the treatment of type 2 diabetes mellitus. The medications decrease blood glucose by increasing the release of endogenous insulin from the beta cells. Sulfonylureas are most common drugs that causes hypoglycemia with overdose or when ingested by nondiabetic patients. Since the consequences of hypoglycemia can be devastating, a rapid differential diagnosis and treatment is essential for patients. Therefore, detection of the presence of sulfonylurea allows for the correct identification of the underlying cause of hypoglycemia and rule out the other causes, such as insulinoma, sepsis, and hepatic disease. **Objective:** This study was designed to develop and validate a qualitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for the detection of sulfonylurea compounds in serum, and utilized the assay to screen for sulfonylurea exposure in emergent hypoglycemia cases. Analytes include tolbutamide, chlorpropamide, tolazamide, acetohexamide, gliclazide, glipizide, glyburide, and glimepiride. **Methods:** Serum samples spiked with deuterated internal standard were processed by protein precipitation using acetonitrile, followed by centrifuge and separation of the supernatant from the protein pellet. The sample was dried under nitrogen in a 37°C water bath. The drugs were reconstituted in 80% mobile phase A and 20% mobile phase B. LC-MS/MS was performed using an Agilent HPLC with an ABSciex 3200 LC-MS/MS in positive ESI mode. Compounds were identified by a combination of retention time, two MRM transitions and the product ion spectrum. Separations were performed using a Phenomenex Luna 3u C8(2) 100Å, 50 × 2.0 mm, with a gradient from 30% to 95% organic over 5.5 min (Mobile phase A: 20 mM ammonium acetate in 95:5 HPLC Honeywell Water : methanol; mobile phase B: 100% methanol). Validation of the final method included determining the lower limit of detection (LOD), linearity, within-run imprecision, between-run imprecision, matrix effect, carry over, and recovery for each analyte. **Results:** The calibration curves for each analyte exhibited consistent linearity and reproducibility in a range of 5 - 1000 ng/ml. Within run coefficient of variation ranged from 4.3% to 15% at 50 ng/ml. Between-run coefficient of variation ranged from 10% to 21% at 50 ng/ml. No significant ion suppression was detected for any of the analyte (88 - 97%). Recovery ranged from 95 to 120% with an average of 103%. No carryover was observed at toxic levels to each analyte. **Conclusion:** We have developed and validated a sensitive and specific LC-MS/MS assay for sulfonylurea detection. Since April 2014, the method has been used in thirteen emergent hypoglycemia cases, in which four cases were positive for glipizide. The results were achieved with a rapid turnaround time of 2-3 hours, and provided real-time information for the differential diagnosis by the treating team.

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Identification of Four Serum Peptides as Acute Promyelocytic Leukemia Biomarkers

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Background:

Minimal residual disease (MRD) following therapy is the most significant adverse prognostic factor in acute promyelocytic leukemia (APL). The routine MRD analysis requires the collection of bone marrow specimens, which is truly painful for the patients. In this study, we aimed to identify some candidate serum peptides biomarkers for monitoring the MRD in adult APL.

Methods:

Serum samples were obtained from 12 APL patients receiving intravenous As_2O_3 treatment and in remission. In total, 46 serum samples were divided into five groups according to treatment courses (Table 1).

Serum samples were fractionated using weak cation exchange magnetic beads and then used for ClinTOF Mass Sepctrometer (Bioyong Tech, Beijing, China) analysis. Control samples consisting of three peptides with molecular weights of 1532.8582, 2464.1989, and 5729.6087 Da (Cat. Nos. P2613, A8346, and I6279, Sigma-Alderich, St. Louis, MO, USA) were analyzed and used as external standards.**Results:**According to the group pattern in Table 1, there are three peak peptides intensities (m/z 3937.8, 3953.4, and 3964.8) differed between group 1 and 2; seven peaks (m/z 2075.5, 2872.8, 3145.2, 3153.4, 3937.8, 3953.4, and 3964.8) differed between group 1 and 3, two peaks (m/z 3953.4 and 3964.8) differed between group 1 and 4. The peak at m/z 2872.8 differed between group 1 and 5.

Further identification by LTQ-Orbitrap-MS provided the peaks were corresponded to these five peptides: 1) Ig heavy chain V-III region BUT (m/z 2075.5); 2) ribosomal RNA processing 15-like protein (m/z 2872.8); 3) filaggrin (m/z 3145.2); 4) isoform F of SON protein (m/z 3153.4); and 5) isoform 1 of histone-lysine N-methyltransferase mixed lineage leukemia 2 (m/z 3937.8).

Conclusion: Among these five identified peptide, four of which (m/z 2872.8, 3937.8, 3953.4, and 3964.8) are potential biomarkers for predicting disease relapse, monitoring MRD, and evaluating therapeutic response in APL patients.

Table 1. Description of patients in the 5 groups

Group No.	Time of therapy	No. of Patients	Gender (M:F)	Age(years)	
				Mean	SD
1	Before treatment	12	7:5	29	13.4
2	One week	10	6:4	30	12.8
3	Two week	9	7:2	29	13.9
4	Three week	10	6:4	29	13.9
5	Four week	5	2:3	31	9.3

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Use of an Animal-free Synthetic Surrogate Serum Matrix for Preparation of Calibrators and Controls in Immuno- and LC-MS Based Clinical Assays

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Background:

The objective is to determine the utility of a well-defined, stable, animal-free matrix for use as a serum (or stripped serum) substitute in clinical assays. The potential clinical benefits would include the complete absence of both clinically relevant endogenous analytes as well as any potentially harmful blood-borne pathogens. This, along with the ability to manufacture with precise lot-to-lot consistency could prove clinically useful as a blank, calibrator and control matrix as well as a sample diluent. The matrix under evaluation was prepared with 2% recombinant human serum albumin (rHSA) expressed from an animal-free rice expression system. Such a synthetic surrogate serum matrix could provide more consistently reliable clinical performance compared to animal-derived matrices for both ELISA and LC-MS based clinical assays.

Methods:

The 'synthetic surrogate serum' was tested in commercially available IVD ELISA kits to different analytes including beta-2 microglobulin and thyroglobulin. Blank and mock samples were prepared by spiking respective analyte reference materials at varying concentrations into the synthetic surrogate serum and comparing results to spiked pooled human serum, spiked stripped serum and spiked matrix provided by the kit manufacturers. The synthetic surrogate serum was also tested in direct comparison to pooled human serum in an LC-MS based clinical assay for measuring blood levels of methotrexate (MTX). Several comparisons were performed including measuring MTX spiked human serum samples and patient samples using either pooled human serum or synthetic surrogate serum calibrators.

Results:

For the ELISA kits, several concentrations of analyte were tested, spanning the range of each of the respective kits. For all analytes, measurements showed interferences when pooled human serum was utilized indicating endogenous levels of target analyte. This was also observed when stripped serum was utilized with the thyroglobulin ELISA kit. As expected, no interferences were observed in any of the kits when the synthetic surrogate serum was used. Further, synthetic surrogate serum matched the performance of the matrix provided by the kit manufacturers indicating a suitable matrix for these ELISA kits. In the LC-MS based MTX assay, comparison of pooled human serum calibrators used to calculate synthetic serum substitute calibrator concentrations was described by the Deming equation y=1.048x - 6.14 with a correlation coefficient of $R^2 = 0.9995$. Comparison of the values assigned to patient samples when using calibrators from pooled human serum or synthetic surrogate serum was described by the Deming equation y = 0.963x + 3.52 with a correlation coefficient of $R^2 > 0.999$.

Conclusion:

We have formulated a simple, animal-free matrix which has been shown to be suitable as a calibrator/blank matrix as well as a patient sample diluent. This has been shown in commercially available IVD ELISA kits as well as in an LC-MS based clinical assay. Unlike typical animal based matrices, this synthetic surrogate serum offers a reproducibly defined formulation and is completely void of any analytes of interest or potentially harmful blood-borne risk factors.

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A Simple and Sensitive Method for Quantification of 5HIAA and VMA by Liquid Chromatography-Tandem Mass Spectrometry

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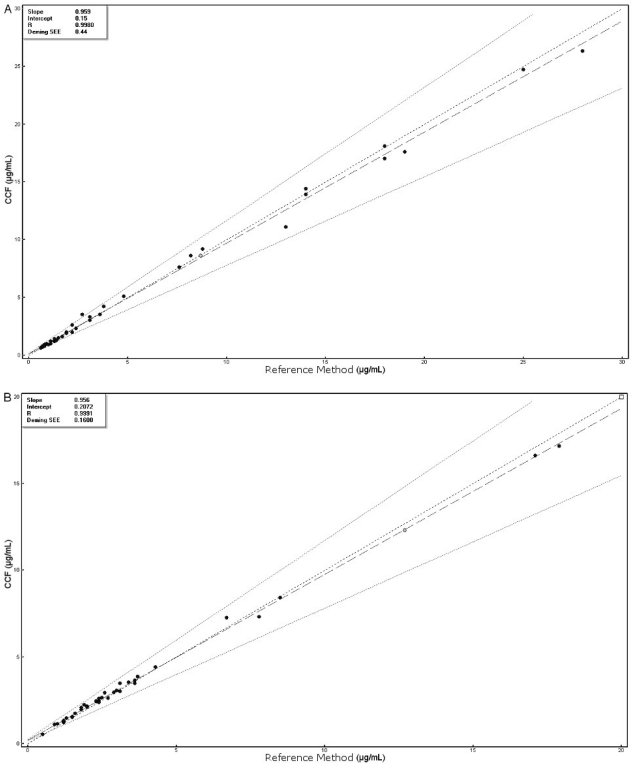
Background: 5-Hydroxyindole-3-acetic acid (5HIAA) and Vanillylmandelic acid (VMA) are important in the detection and diagnosis of carcinoid and neurogenic tumors, respectively. 5HIAA, the major metabolite of serotonin, plays a major role in the study of neurologic disorders and monitoring the progression of carcinoid disease. Measurement of urinary VMA has been beneficial in the diagnosis and follow-up of pheochromocytoma and other catecholamine secreting tumors, as VMA is present in higher concentrations than other catecholamines in urine.

Objective: To develop a simple and sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method for measurement of 5HIAA and VMA in urine. Methods: Patient urine sample (50µl) and 100µl of internal standard (IS) solution (0.5µg/mL 5HIAA-d5, and 0.5µg/mL VMA-d3 in acetonitrile) were combined and vortex mixed. Samples were centrifuged at 15500 g for 3 minutes and 50µL supernatant was mixed with 200µL water. The resulting solution (15µL) was analyzed on an LC-MS/MS system by multiple reaction monitoring (MRM) in the negative ESI mode using a Scherzo SM-C18 analytical column. Total chromatographic time was 11 minutes. Results: A mixing study determined that matrix effects were compensated for by the deuterated internal standards. No carryover was observed up to 99.0µg/mL and 97.5µg/mL for 5HIAA and VMA, respectively. Precision of the assay for both 5HIAA and VMA was shown to be within 10% CV at three concentration levels over 10 days. Linearity of the assay was determined to be 0.2 to 50µg/mL for both 5HIAA and VMA. Results from patient sample comparisons (n=40) with an independent clinical lab is shown in Figures 1A (5HIAA) and 1B (VMA). Conclusion: This LC-MS/MS method offers simple sample preparation and sensitive quantification of 5HIAA and VMA in urine and has been validated for use in our clinical lab.



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Online Solid Phase Extraction and LC/MS/MS Detection of Thyroid Hormones in Biological Fluids

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Thyroid hormones play critical roles in the regulation of biological processes such as growth, metabolism, protein synthesis, and brain development. Specifically, thyroid hormones, 3,3',5,5'-tetraiodo-L-thyronine (thyroxine or T4) and 3,3',5-triiodo-L-thyronine (T3), are essential for development and maintenance of normal physiological functions. For a clinical laboratory, measurements of total T4 and total T3, along with estimates of free T4 (FT4) and free T3 (FT3), are important for the diagnosis and monitoring of thyroid diseases. Most clinical laboratories measure thyroid hormones using immunoassays. The immunoassay-based methods offer a relatively rapid, high patient sample throughput that lends itself to automation but are significantly compromised by problems with assay interference and are perturbed by changes in protein levels that alter the free hormone availability.

Liquid chromatography mass spectrometry (LC/MS) has been reported to offer superior specificity and speed over the immunoassays for determination of thyroid hormones in biological matrices such as serum and tissues. Nevertheless, the reported sample preparation procedures, typically by liquid-liquid extraction followed by solid phase extraction (SPE), involve multiple time consuming steps, and are less compatible with automation.

In the present work, an online SPE-LC/MS method has been developed for the determination of thyroid hormones in biological fluids. The method exploits RP-Amide or C8 as the trapping column and Phenyl phase as the separation column, respectively. The preliminary experiments demonstrated that, under the optimized conditions, both RP-Amide and C8 effectively trapped the thyroid hormones extracted from spiked rabbit plasma sample which had been protein precipitated. And with both traps, sharp peak shapes were observed. However, the RP-Amide traps are advantageous over the commonly used C8 traps with higher signals and recoveries. Additionally, RP-Amide traps are more flexible with washing solvent as it is compatible with 100% aqueous mobile phases and can be used with up to 20% methanol with minimal sample loss.

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Rapid Analysis of 25-hydroxyvitamin D with Separation from its C3 Epimer

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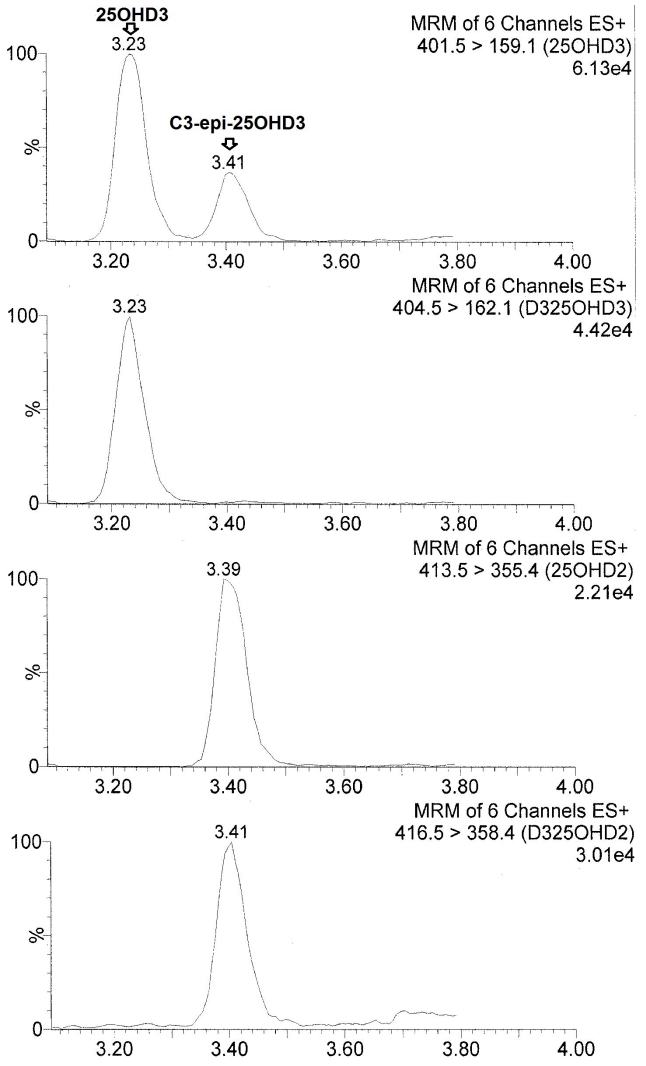
Measurement of 25-hydroxyvitamin D (25OHD) in serum/plasma is useful for the assessment of a patient's vitamin D status. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is regarded as the "gold standard" for measurement of 25OHD. However, LC-MS/MS methods are either susceptible to interference from the C3-epimer of 25OHD3 or suffer from long chromatography and/or sample preparation. **Objective:** The goal of this study was to validate a simple, fast (under 5 min) and interference-free LC-MS/MS method to measure 25OHD2 and 25OHD3 in blood. **Method:** Sample preparation involved protein precipitation followed by solid-phase extraction. Total chromatography run time was 4.8 min. Two transitions are monitored for each analyte. **Results:** The method is free from ion suppression, carryover and interference. The assay is linear from 4 to 311 ng/mL and 5 to 311 ng/mL for 25OHD2 and 25OHD3, respectively. Recovery was evaluated by measuring spiked vitamin D-stripped serum diluted to 9 different levels and ranged from 90% to 105% and 98% to 110% for 25OHD2 and 25OHD3, respectively. Precision was evaluated by repeatedly measuring 3 different levels twice a day for 5 days using spiked patient samples. Total CV was less than 7.2% for both analytes. Method comparison (n=52) was performed with an LC-MS/MS method from a reference laboratory. Deming regression analyses for 25OHD2 and 25OHD3 show slopes of 0.995 for both, intercepts of -1.63 and 0.26, SEE of 3.16 and 2.84, ranges of 6-66 ng/mL and 4-71 ng/mL, respectively. **Conclusion:** This method represents the first reported assay to separate C3-epimer in under 5 minutes and has been validated for routine clinical use. **Figure 1:** Representative method chromatograms showing from top to bottom: 1) 25OHD3 (RT: 3.23 min, 30 ng/mL), 2) D3-25OHD3, 3) 25OHD2 (30 ng/mL), 4) D3-25OHD2 and 5) Total ion chromatogram. The peak at RT 3.41 min in top panel represents the C3-epi-25OHD3 (10 ng/mL).



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The PhoTorrentTM Atmospheric Pressure Photoionization (APPI) Source Utilized for High Efficiency Photoionization of Testosterone and 25-OH Vitamin D3

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Background:

Atmospheric Pressure Photoionization (APPI) is commonly used to ionize compounds of interest for environmental research and is becoming popular in clinical research. It is an effective ionization method for many compounds which are not conducive to electrospray ionization (ESI) or atmospheric chemical ionization (APCI), leading to enhanced sensitivity and lower background for a wide range of compounds. The IONICS PhoTorrentTM APPI source is a modular assembly, easily interchangeable with the ESI or APCI hardware on a high performance dual probe ion source. This study demonstrates the characteristics of the IONICS modular PhoTorrent APPI source for two clinical compounds (Testosterone and 25-OH Vitamin D3). Performance under a range of conditions, including various total flow rates and dopant levels, was determined.

Methods:

The IONICS PhoTorrent APPI source module achieves high efficiency, broad range photoionization with the aid of a 10.6eV lamp from Syagen Technologies. The PhoTorrent APPI module was mounted onto the dual probe source of an IONICS 3Q 320 LC-MS/MS, in place of the ESI/APCI probes. Standards for Testosterone and 25-

OH Vitamin D3 were purchased from Sigma-Aldrich and prepared in concentrations ranging from 0.0001 -10 ng/mL (Testosterone) and 0.1-100 ng/mL (25-OH Vitamin D3). Standards were dissolved in 20/80 water/methanol with 0.1% formic acid. The injection volumes were 10 µL. Duplicate injections were made for each concentration. The samples were eluted using a Shimadzu Nexera UHPLC with a C18 column using a mobile phase of 20/80 water/methanol, 0.1% formic acid, 5mM ammonium acetate.

Results:

The IONICS PhoTorrent APPI source demonstrated sensitivity to as low as 100 ag/µL for Testosterone while maintaining excellent linearity (R2 = 0.9997) over the range of 0.0001-10 ng/mL. This was achieved using a total solvent flowrate for Testosterone of 300 µL/min. A dopant of toluene to 10% of the total flow rate was added to enhance photoionization.

The 25-OH Vitamin D3 achieved a sensitivity as low as 100 fg/µL while maintaining linearity (R2 = 0.9999) over the range of 0.1-100 ng/mL. The 25-OH Vitamin D3 was run with a total flowrate of 500 µL/min, again with a 10% dopant of Toluene. In general, it was observed that increasing flow rate provided sharper LC peaks, resulting in the expected increase in signal to noise ratio. Minimal variation in peak area was observed across a total flow range of 100-500 µL/min.

Conclusion:

The PhoTorrent APPI source therefore demonstrates high linearity and excellent LLOQ's, and is well suited to flow rates of 100-500 µL/min. Development of the modular PhoTorrent APPI Source assembly facilitates switching between modes, from APPI to ESI and APCI, increasing the number and range of compounds that can be analyzed effectively with minimal hardware changes.

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Determination of Monosialogangliosides in Human Plasma by a Novel UPLC/MS/MS Assay Coupled with Chemical Derivatization

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Objective

Developing a LC/MS/MS method to quantitatively monitor the plasma level of monosialogangliosides in patients with GM3 Synthase Deficiency (GSD), an inherited neurological disorder characterized by seizure and profound developmental stagnation, for clinical diagnosis and therapeutic evaluation during an ongoing clinical trial.

Clinical Relevance

Gangliosides are a large subfamily of glycosphingolipids that present abundantly on the plasma membrane of neuronal and glial cells of vertebrates. These molecules are structurally characterized by a distinctive oligosaccharide moiety attached to a ceramide portion with variable length on the fatty acid chains. Physiologically, they are believed to play critical roles in the regulation of various receptor-mediated cell signaling pathways and cellular events. Disruption in their metabolic pathways pathologically leads to the pathogenesis of numerous neurodegenerative disorders, such as Parkinson disease, Alzheimer disease, and ganglioside GM3 synthase deficiency (GSD). Therefore, a reliable LC/MS/MS method with enhanced sensitivity is urgently demanded for relevant biomedical studies.

Methodology

In this study, a novel reverse phase UPLC/MS/MS method for determination of three monosialoganglioside species, GM1, GM2, and GM3, in human plasma has been developed and validated. This assay employed DMTMM & PAEA chemical derivatization for signal enhancement and D3-labeled monosialogangliosides as internal standards (IS). The analytes and ISs were extracted from plasma using protein precipitation procedure, cleaned up with a mixture of water/methanol/chloroform, dried under nitrogen purging, and derivatized with DMTMM & PAEA. Thereafter, the samples were injected into a Shimadzu Nexera UHPLC system interfaced to an AB Scix Qtrap 5500 mass spectrometer that operating in ESI positive and Multiple Reaction Monitoring (MRM) mode to achieve highly sensitive and specific detection.

Validation

Considering the m/z from singly charged molecular ions of monosialogangliosides were mostly beyond the detection range of our Qtrap 5500 mass spectrometer, and they showed low preference to be doubly charged by both positive and negative ESI, we introduced a novel chemical derivatization with DMTMM & PAEA to increase the abundance of their doubly charged molecular ions in positive ESI. The sensitivity of monosialoganglioside species in positive ESI was observed to be enhanced for 15-20 times after derivatization. In addition, more than 15 different components were chromatographically resolved from each other within an 11min run. Moreover, calibration curves ranging from 10~2000, 10~2000, and 80~16000 ng/ml with correlation coefficients of 0.9981, 0.9989, and 0.9977 were established for measurements of monosialogangliosides GM1, GM2, and GM3, respectively. Thereafter, we validated this quantitative assay based on the FDA guideline for extraction recovery, precision, accuracy, stability, and matrix effect. The extraction recovery was found to be above 80% for each monosialoganglioside species using our sample preparation strategy. The relative percent error and coefficient of variation from measurements were below 11 and 11% for each monosialoganglioside species. The loss from derivatized analytes during storage was revealed to be insignificant (<10%) under studied conditions. Matrix effect from plasma was observed to be minor (<20%) on analytes during the analysis.

Conclusion

In summary, we developed and validated a novel quantitative assay for determination of monosialoganglioside species in human plasma using LC/MS and chemical derivatization.

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A Novel Quantitative LC-MS/MS Method for Salivary Cortisol

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BACKGROUND Cortisol is a steroid enzymatically derived from cholesterol mainly in the adrenal gland and is important in glucose metabolism and stress response. The cortisol concentrations undergo a cyclic diurnal variation with the lowest concentrations at midnight and the highest in the morning. However, in Cushing's syndrome the diurnal variation is lost and midnight concentrations are similar to morning concentrations. As such, midnight salivary cortisol is a good diagnostic biomarker of Cushing's syndrome. The preferred method for measuring salivary steroids is LC-MS/MS due to its high specificity and sensitivity. The objective of this work was to establish a quick and sensitive method for measuring salivary cortisol. **METHOD** Saliva (200 µL) collected using a Salivette device (Sarstedt, Newton, NC) was mixed with internal standard (50 µL; d_4-cortisol 5 ng/mL in 1:1 methanol:water) and vortex mixed, followed by addition of 1 mL of methyl-*tert* butyl ether. The mixture stood at room temperature for an hour then was vortex mixed for 30 sec, and centrifuged at 2,000 g for 3 minutes. The organic layer (900 µL) was transferred into a glass tube and dried under a flow of nitrogen at 35ºC. The residue was reconstituted with 1:1 methanol:water (200 µL) and injected (25 µL) onto an Accucore C18 analytical column (3.0 x 50 mm, 2.6 micron; Thermo Fisher Scientific, Waltham, MA) in a Prelude LC system coupled with a TSQ Quantiva mass spectrometer (Thermo Fisher Scientific). The mass spectrometer with heated electrospray ionization was set in the positive ionization and multiple reaction monitoring mode. The transitions were 363.3->121.2 and 363.3->267.1 for cortisol and 367.4->121.1 and 367.4->273.1 for d_4-cortisol. Quantification was based on peak area ratio of cortisol to the internal standard. **RESULTS** The analytical cycle time was 4.25 minutes per injection. This method was free from matrix effect or

interference. It was linear from 0.3 to 15.6 ng/mL with analytical recovery of 95-118%. No significant carryover was observed from samples with concentrations up to 47.5 ng/mL. The total CV was 14.8%, 7.3%, and 6.6% at 0.7 ng/mL, 10.7 ng/mL, and 21.6 ng/mL respectively. Comparison (n=33) with another LC-MS/MS method offered by an independent clinical laboratory gave a Deming regression correlation coefficient (R) of 0.925, slope of 1.04, an intercept of -0.002, and a standard error of estimate of 0.86. The mean bias between these two methods was 3.6%. **CONCLUSIONS** This fast LC-MS/MS method is sensitive and accurate making it suitable for measuring salivary cortisol in a clinical lab.

A-275

Workflow Study of a New HPLC, a New Tandem MS and a New Data Processing Software for General Clinical Use

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Background: There is a growing presence of LC-tandem MS in clinical laboratories because this analytical technique can provide definitive identification and quantitation of target compounds using chromatographic separations and specific transitions of tandem MS from precursor ions to product ions. Users can use LC-tandem MS to develop their own tests, use a smaller amount of samples, and measure several analytes in a single run. In addition to choosing proper MS ionizations and LC chromatographic separations for analysis of target compounds (analytical phase), clinical laboratories often encounter challenges with sample clean-up (pre-analytical phase) and data processing (post-analytical phase) when facing the demand to provide test results in a timely manner with increasing numbers of incoming samples. This

study is to focus on the workflows of the pre-analytical phase (sample clean-up and delivery to MS detection), analytical phase (LC separation and MS analysis) and post-analytical phase (data processing) of three Class I medical devices for general clinical use.

Methods: The HPLC instrument consists of two separate channels, both of which include an online sample cleanup Turboflow column for removing sample matrix, and an analytical column for chromatographic separation. This HPLC is capable of cross-channel sequencing by only introducing the portion of the chromatogram of target compounds into the mass spectrometer therefore doubling the sample throughput. This HPLC can operate in two different workflows: (1) TX mode for sample matrices that require on-line sample cleanup and (2) LX mode for less complex sample matrices that can use dilute-and-shoot technique bypassing Turboflow Column. The Tandem MS has a selected reaction monitoring scan. The workflows of Tandem MS include HESI and APCI ionization modes with polarity switching. The data processing software provides three levels of user permissions for technicians, supervisors, and lab directors. This software has built-in flexibility in assigning roles and responsibilities, and audit trail function is provided for streamlining record keeping.

Results and Conclusion: The ability to run tests overnight, unattended was conducted using the example compound Alprazolam in synthetic serum using TX workflow with HESI positive ion mode. A total of 2000 crashed synthetic serum samples spiked with Alprazolam and isotopically-labeled internal standard were analyzed continuously for 100 hours, with an additional 44 QC samples inserted at intervals during the same 100 hours. Cross-channel RSD's (n=2000) of retention time, concentration and ion ratio (m/z 274 to m/z 281) of Alprazolam were observed at <2%. The precision studies of between-instruments (n=120, 5 replicates, 4 runs, 2 channels, 3 units) of different ionization modes on HESI probe and APCI probe were conducted using four example compounds in polarity switching mode (+/-) in either HPLC TX workflow, or HPLC LX workflow . HESI (LX workflow with synthetic urine sample matrix): Reserpine(+); Chloramphenicol (-); APCI (TX workflow with synthetic serum sample matrix): Testosterone (+); Estradiol (-). The corresponding between-instruments RSD's of concentrations were < 4% for Reserpine and Testosterone, and <10% for Chloramphenicol and Estradiol. The software streamlined the post-analytical phase of processing data and helped ensure data integrity.

For in vitro diagnostic use.

A-276

Development and application of a rapid and simple multiplexed UPLC-MS/MS method to measure triazole antifungals and metabolites

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Background: Triazole antifungals have become central to the treatment of invasive fungal infections, most notably in immunosuppressed populations such as those receiving bone marrow transplants. However, interactions with other drugs as well as environmental, genetic and disease-specific factors have led to wide variability in circulating drug levels leading to both decreased efficacy, or conversely, increased toxicity. As a result, there is growing clinical demand for therapeutic drug monitoring of these antifungals along with their metabolites. Here, we present a simple and rapid stable isotope dilution liquid chromatography tandem mass spectrometry method to measure fluconazole, voriconazole, voriconazole-N-oxide, posaconazole, itraconazole, and hydroxyitraconazole in serum.

Methods: Briefly, analytes were extracted from 10 µL of serum by simple protein precipitation with acetonitrile in the presence of deuterated stable isotope internal standards. Chromatographic separation was achieved using a two solvent gradient elution UPLC method with a reversed phase C18 column. Mass spectrometric analysis was performed using electrospray ionization (ESI) and collision-induced dissociation tandem mass spectrometry.

Results: Total run time was 3 minutes, with each analyte showing a high degree of linearity ($r^2 > 0.99$) from 0.01 µg/mL to 10 µg/mL (0.1 µg/mL to 100 µg/mL for fluconazole), accuracy and precision from sub- to supra-therapeutic levels (DEV and CV < 15%), as well as a high degree of correlation between our measurements and those obtained from a national reference laboratory. The validated method was then applied to clinical serum samples and showed a substantial variability in the relative serum concentrations of voriconazole to voriconazole-N-oxide and between itraconazole and hydroxyitraconazole, suggesting significant inter-patient variability in drug metabolism. Additionally, the method was also developed and applied to clinical cerebrospinal fluid specimens.

Conclusion: A simple and rapid LC-MS method to monitor multiple triazole antifungals and metabolites from serum and cerebrospinal fluid will provide a common platform for therapeutic drug monitoring. This will allow for a better understanding of antifungal metabolism and distribution, and subsequently better clinical management for a growing and vulnerable population of immunosuppressed patients.

A-277

Serum methylmalonic acid quantitation by LC-MS/MS for monitoring methylmalonic acidemia

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Background: Methylmalonic acidemia is an inherited metabolic disorder that is monitored and managed with serum methylmalonic acid (MMA) concentration and acylcarnitine and urine organic acid profile analysis. Due to the heterogeneous etiology for methylmalonic acidemia, an analytical assay for serum MMA levels requires a large clinical reportable range (0.1 - 1000 µM). Current literature describes multiple mass spectrometry method using MMA quantification to monitor Vitamin B12 deficiency, with a smaller emphasis on the challenges of quantifying MMA for methylmalonic acidemia. Here, we aim to develop a simple liquid chromatography tandem mass spectrometry (LC-MS/MS) method to quantify serum MMA across a wide dynamic range using small sample volumes for monitoring pediatric patients with MMA.

Methods: Extraction procedure was optimized to reduce extraction time and sample volume. 50 uL of patient serum was mixed with d3-MMA and 4.6 M formic acid. After 5 minutes pre-treatment, sample was loaded onto Biotage 96-well ISOLUTE SLE+ 200 supported liquid extraction plate. MMA and d3-MMA were then eluted using methyl tert-butyl ether (MTBE). Eluted MTBE was evaporated under nitrogen at 50 ºC, reconstituted in 100 µL of water, and then 10 µL was injected into the Waters Alliance 2795 HPLC coupled with triple quadruple tandem mass spectrometer (Waters QuattroMicro). Separation of MMA from endogenous isobaric succinic acid was achieved on Phenomenex Gemini C18: 100 x 3.00 mm x 3 µm by gradient method at 0.6 mL/min started with 85% mobile phase A (water) and 15% mobile phase B (methanol with 0.1% formic acid and 2 mM ammonium acetate) and ended with 95% mobile phase B. Electrospray ionization was set at negative mode and multiple reaction monitoring was used to monitor MMA (117 to 73 and 117 to 55) and d3-MMA (120.1 to 76 and 120.1 to 58). The sample-to-sample run time was 7 minutes. Controls and calibrators were made in-house by spiking Seracon II stripped serum with MMA.

Results: To accommodate serum MMA measurement across several orders of magnitude from pediatric patients, assay development was focused on carryover, linearity, and sample volume. Carryover was minimized through needle wash studies, and no significant carryover was observed up to 500 µM after maximizing the number of needle wash cycles attainable for a 7 minute run using 40% methanol. The assay was shown to be linear between 0.1 - 500 µM (R^2 = 0.999). Extracted patient samples diluted 8 fold with water had acceptable agreement with straight samples (N=18, 0.15 - 500 µM), suggesting that sample volume can be reduced to 20 µL. Intra-assay imprecision was determined to be 1.9 % coefficient of variation (N = 3, 1.3 µM). LC-MS/MS method comparison with a reference lab yielded a Deming regression slope of 1.088, intercept of -1.388, and R^2 of 0.999 (N = 18, range 0.1-500 µM).

Conclusion: With fast turnaround time, small sample volume, excellent chromatographic separation, improved selectivity through ion ratio monitoring, and a wide dynamics range, we have shown a promising new method for measuring serum MMA levels by LC-MS/MS to monitor and manage pediatric populations with methylmalonic acidemia.

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Method development for the determination of 17α-hydroxyprogesterone in human serum by isotope dilution liquid chromatography tandem mass spectrometry

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Background: Hormones are an important class of clinical diagnostic markers. Accurate measurement of hormone levels is necessary for proper diagnosis and treatment of many diseases. 17α-Hydroxyprogesterone (17OHP) is a metabolic precursor of cortisol; elevated levels of 17OHP are indicative of congenital adrenal hyperplasia. Quantification of 17OHP in serum has traditionally employed immunoassay methods, which suffer from poor antibody specificity. These methods are subject to interferences by other similar steroids, which result in falsely elevated

levels. Normal level of 17OHP : Female: 0.1-12 ng/mL; Male: 0.3 to 2.5 ng/mL. We developed ID LC/MS/MS as another method for the determination of 17OHP in serum

Methods: Human serum samples were obtained from male and female human serum. 17OHP-d_8 was used as an internal standard. 0.5 mL of serum sample was taken into an amber vial. An appropriate amount of isotope standard solution was spiked into the sample vial to make a 1:1 weight ratio. After equilibration for 1 hour, 5 mL of CH_2Cl_2 was added to sample. Sample was shaken for 5 min and centrifuged at 3,000 rpm for 5 min. CH_2Cl_2 phase was separated and dried under nitrogen stream. Then sample was dissolved with 100 µL of 95% ethanol. Aliquot of the ethanol solution was filtered and then analyzed by LC/MS/MS. The LC column was Biphenyl and kept at 40 ℃ during the chromatographic run. The mobile phase was gradient of A (0.1% formic acid in acetonitrile) and B (0.1% formic acid in water), and the flow rate was 0.25 mL/min

Results: Two levels of 17OHP in male and female serum were determined. The optimum solvent for the extraction of 17OHP in serum was CH_2Cl_2 among many other solvents. The optimum LC conditions were gradient of A (0.1% formic acid in acetonitrile) and B (0.1% formic acid in water); 0 (30% A)-21 min (50% A)-31 min (70% A)-32 min (100% A)-37 min (100% A)-38 min (30 % A)-43 min (30% A). 17OHP and 17OHP-d_8 were monitored at mass transfer m/z 331.3/97.3 and 339.3/100.3 respectively. The level of 17OHP in male serum showed nearly 0.7 ng/mL and that of female showed about 1.8 ng/mL. These results showed some difference compared to commercial immunoassay methods.

Conclusion:

17OHP is an indicative of congenital adrenal hyperplasia and very trace level in human serum. An optimized ID LC/MS/MS method was proposed as another method for the determination of 17OHP in serum. Extraction of 17OHP from serum matrix and HPLC separation from interferences were successfully established by this method. This method can be proposed as an accurate method for the development of certified reference material.

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Determination of Urine Caffeine and Caffeine Metabolites by use of Polarity-Switching LC-MS/MS

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Background: Caffeine is a psychoactive stimulant commonly found in beverages such as coffee, tea, soft drinks, and energy drinks. It is a dietary compound of considerable health interest and has been studied as a risk factor for many diseases and conditions. We used an LC-MS/MS method for measuring urine caffeine and its metabolites in a representative sample of the US population (National Health and Nutrition Examination Survey NHANES] 2009-2010) in part to explore the potential use of these biomarkers in assessing dietary caffeine exposure. While LC-MS/MS was well suited for this application, it had one intrinsic limitation. Caffeine and its xanthine metabolites were best detected with positive mode ionization, whereas uric acids typically performed best with negative mode ionization. In the absence of the ability to perform analyses in both ionization modes in the same injection, we had to analyze each sample twice to obtain optimal results. Employing a new generation tandem mass spectrometer we explored the possibility to use polarity switching to measure these compounds in the same chromatographic injection.

Methods: Urine samples were diluted, amended with stable isotope labeled internal standards, alkalinized to convert specific metabolites to stable forms, and acidified and filtered prior to analysis. A UHPLC system with a solid core C_{18} column and a binary water/methanol gradient (0.05% formic acid) was used to chromatographically separate the analytes. Detection was performed on a tandem quadrupole MS/MS. Quantitation, confirmation, and internal standard MS/MS transitions were identified and optimized for all analytes. Polarity switching was performed at 20 ms. Analytes were quantified by interpolation against an 11-point calibration curve covering the concentration range expected in a population setting (0.005-600 µmol/L depending on the analyte).

Results: The performance of our polarity switching LC-MS/MS method was equivalent to or exceeded that of its predecessor. Limits of detection were 0.003-0.1 µM depending on the analyte. Between run imprecision (CV) determined over 30 days at 3 QC pool concentrations was 5-10% for 14 of 15 analytes. Spike recoveries were typically ±10% of being quantitative (100%). A comparison of our new and existing methods (180-sample convenience set) showed that results were highly correlated (Pearson $r \geq 0.9985$, $P < 0.0001$) and bias was low (Bland-Altman bias -2.3-3.6%; weighted Deming regression slope 0.95-1.04). The ability to measure all compounds in a single run resulted in a 45% reduction in analysis time, reduced solvent consumption, increased column lifetime, and simplified data review, thus improving overall sample throughput while reducing analysis costs.

Conclusion: Polarity switching permitted the analysis of all analytes in a single chromatographic injection while simultaneously using both ionization modes. This is an improvement upon existing LC-MS/MS methods in which separate chromatographic runs for each ionization mode were necessary because polarity switching was either not possible or could not be performed sufficiently fast because of instrumental limitations. Our new method is being used for the NHANES as well as other biomonitoring studies.

A-280

Measurement of 24-h urine free cortisol using ID-LC/MS

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Background:

The urinary free cortisol (UFC) has generally been determined by immunoassay. However, immunoassay is known to be nonspecific with cross reaction and inaccuracy. Therefore we developed candidate reference method for urinary free cortisol using isotope dilution-liquid chromatography mass spectrometry (ID-LC/MS) and evaluated the method.

Methods:

After solid-phase extraction, the cortisol and internal standard (cortisol d4) detected in the multiple-reaction monitoring mode using an positive electrospray ionization; cortisol m/z 363->121, cortisol d4 m/z 367->121. Intra-assay imprecision and inter-assay imprecision were evaluated with three different concentrations of QC samples. Matrix effect, stability, lower limit of detection and lower limit of quantification were also evaluated. In addition, we compared UFC values of forty-five 24-h urine specimens measured by our ID-LC/MS with those measured by ARUP lab using ID-LC/MS and those measured by DxI chemiluminescent immunoassay in our laboratory. Cross-reactivity of prednisolone was also evaluated.

Results:

Intra-assay CVs (n = 8) were 1.49-3.88% and inter-assay CVs (n = 20) were 3.15-5.90%. The limit of detection and limit of quantification of UFC were 0.5 ng/mL and 1.0 ng/mL, respectively. Significant matrix effect was not observed. The immunoassay showed large positive proportional and constant bias compared with our ID-LC/MS ($y = 4.5455x + 11.898$, $r = 0.665$). In addition, there was positive proportional bias of UFC by our method comparing with those by ID-LC/MS of ARUP lab but showed good correlation ($y = 1.37x + 0.19$, $r = 0.999$). Cross-reactivity of prednisolone with cortisol by our ID-LC/MS method was 0.9%, which was significantly lower than 19.3% by chemiluminescent immunoassay (Table 1).

Conclusion:

UFC determination by ID-LC/MS showed good precision, sensitivity, and low cross-reactivity with prednisolone, indicating use of ID-LC/MS is necessary for patients taking synthetic steroid hormones. However, there was also some bias between different LC/MS methods, which should be standardized by use of common standard material for calibration.

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Towards a "Random Access" Model for Clinical LC-MS/MS

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Background: Advances in analytical performance and high-throughput capability have vastly improved the scope of clinical LC-MS/MS. One application where LC-MS/MS continues to lag behind many alternative platforms is that of "random-access" functionality. This largely limits LC-MS/MS to batch analyses. Here, we describe a working LC-MS/MS model incorporating routine batch methods for vitamin D and immunosuppressant drugs (ISDs) operating in parallel with a third method, busulfan, requiring a "random-access" type approach.

Methods: LC-MS/MS was performed using a TLX-2 online sample preparation liquid chromatography (SPLC) system (Thermo-Scientific, Franklin, MA) coupled to an ABSciex 5500 Q-Trap mass spectrometer (ABSciex, Concord, Ontario, Canada). Batch methods for vitamin D

and ISDs were performed on separate SPLC multiplexing channels, 1 and 2 respectively. Each channel operates with a separate sample clean-up (TurboFlow) column and analytical LC column combination in series multiplexed into a single MS/MS. Channel 1 uses a solvent system composed of 0.1% formic acid in water and methanol. Channel 2 uses 15 mM ammonium acetate/0.1% formic acid in water and

methanol. The third method within this model is for the anti-neoplastic drug busulfan. Therapeutic drug monitoring for busulfan requires pre-dose blood draw (trough level) and re-draw at 6 hour intervals thereafter over a period of

up to 96 h. Busulfan analysis cannot be carried out in batch mode; it is

imperative that analysis is performed on sample receipt as plasma levels are required to guide acute changes in dosing. Plasma samples for busulfan were cleaned up by methanolic protein crash with isotopic dilution (busulfan-d8) followed by online SPLC on either channel.

Results: MS/MS for busulfan on channel 1 was monitored using the molecular ion transition *m/z* 247.3>151.1. MS/MS for busulfan on

channel 2 was monitored using the ammonium adduct precursor ion transition (*m/z* 264.2>151.1). Total analysis time on either channel was less than 15 min per sample. Comparison between the two busulfan channels demonstrated that they are interchangeable. Deming

regression (n = 41) for channel 1 versus channel 2 was Channel 1 = 1.04 * Channel 2 - 0.33; r = 0.998. Deming regression between our bi-channel busulfan data (LC-MS/MS) and reference method (GC-MS) data was GC-MS = 1.03 * LC-MS/MS - 0.24; r = 0.998; n = 36. Cross-center (n = 22) verification of our method demonstrated a mean bias of 0.0% (bias range: -10.2% to 12.4%) against the all-method mean across the concentration range 0.88 - 12.14 µmol/L (n = 6).

Conclusion: This model successfully combines batch and "random-access" type methods on a single multiplexing LC-MS/MS platform. Increased speed and throughput is achieved by running busulfan on both channels interchangeably. It also offers robustness allowing continued analysis in the event of a hardware problem on one SPLC channel. This approach offers simplicity, with fixed solvents on each channel and no column switching required. Since all three methods operate on APCI positive mode, a single probe is used throughout. We have demonstrated a working LC-MS/MS model for successfully combining batch and "random-access" type methods for use in the routine clinical laboratory.

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Analysis of Bile Acid Profiles by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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Objective: Primary bile acids are synthesized in the liver and conjugated to either glycine or taurine prior to excretion in the bile, where they serve many important physiological functions such as cholesterol homeostasis and lipid digestion. Within the intestine, bacterial enzymes metabolize conjugated, primary bile acids into numerous forms. In this work we focus on bile acid profiling by LC-MS/MS, utilizing multiple reaction monitoring (MRM) mode, and in patients with necrotizing enterocolitis (NEC).

Method: Bile acid extraction from serum or plasma was performed by methanol precipitation, 20 minute room temperature incubation and centrifugation. After centrifugation, the supernatant was dried under nitrogen. The residue was dissolved in methanol and 10 µl were injected for LC-MS/MS analysis. Bile acid extraction from stool samples was achieved by homogenization, lyophilization, and addition of 0.1M NaOH. Samples were then incubated for one hour at 60°C. Water was added and samples were homogenized. Following centrifugation, bile acids were isolated using solid phase extraction (C18 cartridges, Waters). Resultant sample was dried under nitrogen, dissolved in methanol, and injected (10 µl) for LC-MS/MS analysis.

LC-MS/MS analysis was performed on a Shimadzu HPLC system connected to a 4000 Qtrap (ABSCIEX) mass spectrometer in MRM and negative ion mode. Separation was performed on a Kinetex C18 column (150×4.60 mm, 2.6 µm ID, Phenomenex). The mobile phase A consisted of 5 mM ammonium acetate in water and mobile phase B consisted of 5 mM ammonium acetate in methanol. Bile acids were eluted over a 12 minute gradient. The gradient started at 80% B, increased to 93% at 7.5 minutes, then 98% at 7.6 minutes. After a 2.4 minute hold, the gradient was decreased to 80% for the final 2.2 minutes.

Results: The linearity was up to 12.5 µM for 14 metabolites, and 25 µM for 5 additional metabolites with correlation coefficient from 0.9933-0.9996. The limit of quantitation was 0.05 µM for all 19 bile acids. The recoveries were 99.4-105% for Tauro Ursodeoxycholic acid (TUDCA), 95.3-110% for Taurocholic acid (TCA), 99.7-105% for Hyodeoxycholic acid (HDCA), 93.4-105% for Taurochenodeoxycholic acid (TCDCA), and 96-105% for Lithocholic acid (LCA). The intra-assay precision CV was 2.8-14.4% for 0.2 µM and 4.1-13.4% for 7 µM. The total precision was evaluated during a 22 week period (one run/week). The CV was 9.7% -19.7% at a concentration of 7.0 µM, 11% -21% at 14 µM. This method was compared with a commercial LC-MS/MS method for three major bile acids. The correlation coefficients were 0.9984 for Cholic acid (CA), 0.9994 for Chenodeoxycholic acid (CDCA), and 0.9989 for Deoxycholic acid (DCA), respectively. Differences in the bile acids profiles were observed in patient's samples collected after surgery compared to samples collected after disease treatment.

Conclusion: We have implemented an LC-MS/MS method for the separation and detection of nineteen bile acids in plasma/serum and stool samples within a twelve minute analysis time. Absolute quantification of bile aids in both normal pediatric population and patient samples is currently underway to determine normal pediatric reference intervals and changes in the bile acid levels associated with NEC.

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A combined method for Liquid chromatography-tandem mass spectrometry (LC-MS/MS) measurement of aldosterone/plasma renin activity ratio

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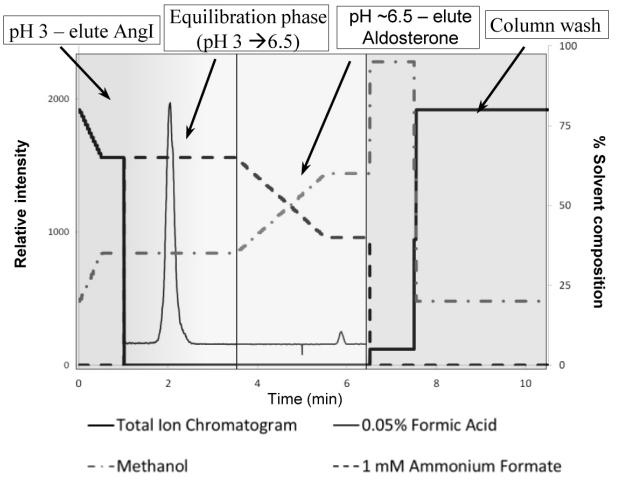
Background: The ratio of aldosterone to plasma renin activity (PRA) is used to help diagnose and evaluate hyperaldosteronism. Aldosterone/PRA methods by LC-MS/MS provide benefits in sensitivity and specificity; however, differences in the chemical behavior and ionization efficiency between the renin product angiotensin I (angI), and aldosterone have until now prevented simultaneous determination of these analytes. This study therefore presents a novel combined LC-MS/MS method for determination of the aldosterone/PRA ratio.

Methods: AngI and aldosterone were quantified from EDTA plasma or stripped serum spiked with isotopically-coded internal standards (IS) and subjected to solid-phase extraction with Waters HLB extraction cartridges. AngI was generated for 1 hour at 37°C in 27.5 mmol/L maleic acid buffer with 0.5 mmol/L AEBSF and chromatographic separation of angI and aldosterone employed an Agilent 1260 quaternary pump with a Phenomenex EVO C18 column (150 mm × 3.0 mm, 5 µm) at 800 µL/min. Analysis was on an Agilent 6460 triple quadrupole mass spectrometer operating in dynamic multiple reaction monitoring mode. The solvent gradient employed 0.05% formic acid, methanol, and 1 mM ammonium formate. Figure 1 displays an example chromatogram with the gradient conditions, and total run time was 10.5 minutes.

Results: Modulation of the solvent gradient as well as pH allowed detection of angI and aldosterone by LC-MS. AngI is detected in positive mode by b5 and y8 ions, while aldosterone was detected in negative mode using transitions 359.4 ◊ 331.4/189.1. AngI is eluted at approximately 2 minutes, while aldosterone is eluted at 5.8 minutes. Detection limits are comparable to immunoassays for each analyte (1 - 50 ng/mL angI, 50 - 2000 pmol/L aldosterone).

Conclusion: By employing a quaternary pump and a three solvent system, we demonstrate a novel LC-MS/MS method for combined analysis of aldosterone and PRA, and for improved workflow in the clinical laboratory.



A-284

A Simple and Robust Nano Liquid Chromatography-Mass Spectrometry Method for the Analysis of Hemoglobin Variants

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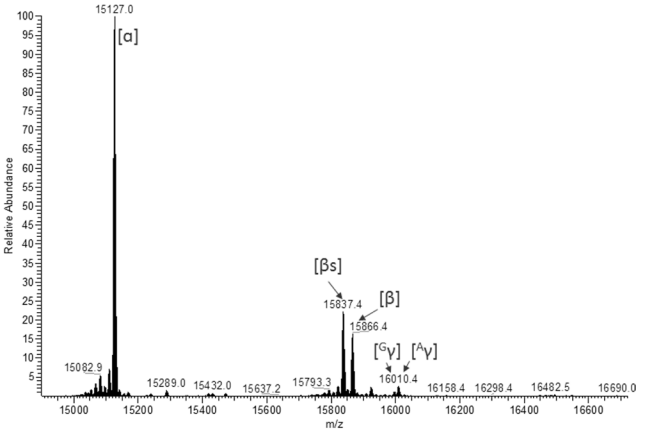
Background: Hemoglobinopathies are a group of disorders affecting red blood cells with abnormal hemoglobin and are the most common inherited disorders. Gel electrophoresis and chromatography assays are the most common techniques used for hemoglobin analysis. In some instances the results from these methods can be ambiguous due to the co-migration of bands in gel and co-elution of different variants on chromatography. The objective of this work was to develop a simple and robust nano liquid chromatography-mass spectrometry (nLC-MS) method for the analysis of intact hemoglobin chains to identify abnormal hemoglobin variants. **Method:** Whole blood (10 µl) and 50 µL of extraction buffer (1:1 mix of 0.2% formic acid in deionized water and 0.2% formic acid in acetonitrile) were vortex mixed and centrifuged. Supernatant (25 µL) was mixed with 75 µL of 0.2% formic acid in deionized water and extracted using a ZipTip (0.2 µL of C18 resin). The ZipTip procedure is as follows: wash with 0.2% formic acid in acetonitrile, condition with 1:1 (0.2% formic acid in deionized water:0.2% formic acid in acetonitrile), then 0.2% formic acid in deionized water. Load sample, wash with 0.2% formic acid in deionized water. Elute with 0.2% formic acid in acetonitrile. The extracted hemoglobin solution was analyzed on an nLC-MS coupled with a Q-Exactive high resolution MS. The raw spectra were deconvoluted in Xcalibur to identify hemoglobin variants (Figure 1). **Results:** This nLC-MS method was successful in identifying abnormal hemoglobin variants in patient samples (n=29) previously identified using alternate methods. **Conclusion:** This simple and robust nLC-MS method for the identification of hemoglobin variants provides accurate results.



A-285

Sensitive Measurement of Free T3 and Free T4 in Serum by LC-MS/MS, using a Simple Ultrafiltration Sample Preparation Procedure

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Background:

The measurement of free thyroxine (FT4) and free 3,3′,5-triiodothyronine (FT3) in serum are important because these values can be used to help researchers assess various states of thyroid function in men, women, and children. Currently most measurements of FT3 and FT4 are performed by equilibrium dialysis immunoassay methods, which may suffer from a lack of specificity. Measurement by LC/MS/MS has the potential to increase the accuracy of these measurements; however sensitivity has posed a challenge due to the very low levels of circulating FT3 and FT4 in serum.

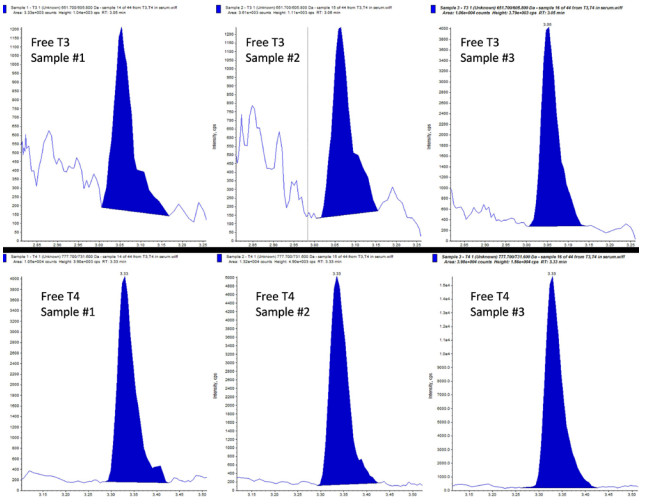
Methods:

Here we present a sensitive method using the AB SCIEX QTRAP® 6500 system, employing a simple and rapid ultrafiltration sample preparation to isolate the free T3 and free T4 prior to analysis by LC/MS/MS. Liquid chromatography separation was accomplished using a Phenomenex Kinetex C18 column (2.6um, 3.0 x 50mm) at a flow rate of 500uL/minute, and mass spectrometric detection was performed in positive MRM mode, using electrospray ionization.

Results:

Unlike earlier attempts to analyze FT3 and FT4 by LC/MS/MS, this sensitive method requires a relatively small injection volume of only 50uL of the final sample, derived from an original sample volume of 400uL serum. We have performed a comparison study using a cohort of samples that have been previously analyzed by immunoassay, and an excellent correlation has been observed. The method LOQ was <0.5 pg/mL for both FT3 and FT4. Replicate injections of the calibration standards demonstrated excellent linearity, accuracy and precision over the concentration range from 0.5 pg/mL to 100 pg/mL of FT3 and FT4. As this method requires an injection volume of only 50uL of the prepared sample, we believe this represents the most sensitive method reported in the scientific literature.

Representative chromatograms are shown in Figure 1, demonstrating both low and high concentrations of FT3 and FT4 in serum samples.



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Development of a serum based exosomal biomarker signature to distinguish indolent and aggressive prostate cancer

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Background: Current tumor markers for prostate cancer lack specificity and are not able to accurately identify those with aggressive disease who require immediate treatment. Exosomes are cell membrane-derived vesicles found in numerous noninvasive bodily fluids such as serum, saliva, urine, and amniotic fluids. They have been demonstrated to carry specific proteins, lipids, mRNA, and microRNAs. Thus, exosomes can potentially harbor cancer biomarkers. Despite the vast information that exosomes can provide, the isolation of pure exosomes is still a challenge in the field. The current methods of isolating exosomes are time-consuming, laborious, and non-specific with most procedures isolating a heterogeneous population of microvesicles.

Objectives: The goal of this study was to develop a more targeted and reproducible method for exosome isolation. We used a combination of centrifugation, and affinity enrichment to devise a quick and robust method for this process. Using such procedure, we wanted to delineate a biomarker signature to distinguish prostate cancer patients with aggressive disease and those from benign controls.

Method: We used affinity reagents to exosomal membrane proteins (CD63/CD9/hsp70) coupled with ultracentrifugation to isolate exosomes from serum of prostate cancer patients with aggressive disease and those from benign disease controls. The isolated exosomes were subject to downstream proteomics analysis using quadrupole and ion-trap mass spectrometers. The identified peptides were further analyzed using gene ontology algorithms, allowing us to map the identified peptides onto cellular signal transduction processes.

Results: In total, over 800 proteins were identified from our serum samples. Of these, a protein signature of ~40 proteins was able to distinguish aggressive disease from benign controls. Gene ontology studies demonstrate that these proteins function in pathways related to growth & proliferation (mTOR), cytoskeleton (alpha-actinin), extracellular matrix (alpha sarcoglycan), and altered signaling (centrasomal protein of 152kD).

Conclusion: Using our optimized serum exosome isolation procedure coupled to downstream proteomics analysis, we were successful in delineating a protein biomarker signature to separate aggressive prostate cancer from benign disease. This signature, or its components, may be useful as an adjunct to prostate biopsy, in appropriate triage of patients with elevated PSA.

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Quantitative Determination of D/L-Methamphetamine in Urine by Liquid Chromatography-Tandem Mass Spectrometry

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Background

The D-methamphetamine isomer possesses the well-known psychostimulant effects of the drug, while the L-methamphetamine has no effect on the central nervous system. Long-term use of D-methamphetamine is associated with depression and suicide as well as serious heart disease and violent behaviors. Consumption of over-the-counter and prescription medications such as Vick's inhaler and Desoxyn respectively may yield positive methamphetamine results. Chiral separation of D and L stereoisomers can help determine if the source was putatively licit or illicit. Cyclodextrins are commonly utilized as chiral selectors in capillary electrophoresis methods but these methods are not common methodologies implemented in forensic laboratories. In this study, a LC-MS/MS method for separation and quantification of methamphetamine stereoisomers derivatized with Nα-(2,4-Dinitro-5-fluorophenyl)-L-alaninamide (Marfey's reagent) is presented. The validated method was applied to the identification of methamphetamine stereoisomers in human urine samples.

Methods

Urine samples were prepared by solid phase extraction using a Strata-X™ cartridge in a 96 deep-well SPE plate. Analytes were eluted from the cartridge with methanol followed by evaporation to dryness under stream of nitrogen at 45 ºC. Samples were reconstituted in a solution of sodium bicarbonate and the Marfey's reagent (Sigma-Aldrich) was added. The derivatization reaction was performed at 56 ºC for 1 h. After quenching the reaction with HCl and centrifuging the mixture, 5 µL of sample were injected into the LC-MS/MS system. Chromatographic separation was performed on a Kinetex C18, 100x2.1 mm, 5 um column under isocratic conditions with 45% water (A) and 55% methanol (B) for 5 min. Prior to the isocratic conditions a short gradient of 10% to 55% B over 1 min was run. Mass spectral data were obtained in positive electrospray mode using an Agilent 6400 Series Triple Quadrupole LC/MS Systems. Derivatized methamphetamine MRM transitions monitored were 402.1 → 284.0, 118.9 and methamphetamine MRM transitions monitored were 150.1 → 119.1, 91.1. Six levels of the calibration curve were prepared with the measurement range from 50-5000 ng/mL.

Results

The incubation time and Marfey's reagent concentration added for derivatization were optimized. Preliminary data showed a good separation of the derivatized methamphetamine stereoisomers in less than 5 min. The method has a limit of quantitation in all matrices of 50 ng/mL. Imprecision and accuracy for the 3 quality controls tested met all acceptable criteria. The average precision and accuracy values were 8% and 90% respectively. The average recovery of the derivatized methamphetamine versus non-derivatized methamphetamine was 92%. The validated method was applied to the identification and quantification of methamphetamine stereoisomers in human urine samples. Although the derivatization procedure in the present study may increase the sample preparation duration, the conventional reverse-phase separation on a C18 column was achieved in less than 5 min. Additionally, a higher sensitivity was achieved in all matrices tested compared to other chiral separation methods.

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A Fast and Simple LC-MS/MS Quantitative Method for Benzodiazepines in Urine

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BACKGROUND Benzodiazepines (BZD) were introduced in the early 1960s and are an important class of psychotropic drugs that are used often in clinical practice as tranquilizers, sleep inducers, antiepileptics, hypnotics, anticonvulsants, anxiolytics, and muscle relaxants. Due to the tranquilizing effects of BZD, they are often used for suicide attempts, abused recreationally, and employed for drug facilitated sexual assault. Therefore, they are frequently encountered during emergency toxicological screenings, drug abuse testing, and forensic medical examinations. This method was developed for rapid and accurate measurement of BZD compounds in urine for pain management services. **METHODS** BZDs and/or metabolites (Lorazepam, Temazepam, 7-Amino-Clonazepam, Hydroxyalprazolam, Hydroxytriazolam, Nordiazepam, Oxazepam) were included in this method. Urine sample (50 µL) was mixed with an enzyme solution (50 µL; 10,000 U/mL *H. rufescens* β-glucuronidase in 1M sodium acetate, pH 4.5) and an internal standard mix (50 µL). The resulting mixture was vortex mixed for 15 seconds, followed by incubation at 60°C for 30 minutes. The sample was then centrifuged at 17000 g for 3 minutes. The supernatant was mixed with 250 µL methanol, vortex mixed for 15 seconds, and centrifuged for 10 minutes at 17000 g. The supernatant (250 µL) was mixed with 250 µL water and 25 µL of the mixture was injected onto an Accucore C18 column (3.0 x 50 mm, 2.6 micron; Thermo Fisher Scientific, Waltham, MA) in a TLX2 system coupled with a TSQ Ultra mass spectrometer (Thermo Fisher Scientific). The mass spectrometer was set at positive heated electrospray ionization and multiple reaction monitoring mode. Quantification was based on

peak area ratio of the individual analyte to the corresponding internal standard. Two transitions were monitored for all the compounds while one for each internal standard. **RESULTS** The chromatography was 4.35 minutes per injection. This method was free from matrix effect or interference. It was linear from 60-9500 ng/mL with analytical recovery ranging from 80-120% for all compounds. No significant carryover was observed from samples with concentrations up to 10,000 ng/mL. The total coefficients of variation (CV) ranged from 2.9%-9.4% for the tested concentrations of 100 ng/mL, 2550 ng/mL, and 5000 ng/mL. **CONCLUSION** This LC-MS/MS method requires simple sample preparation and offers precise and accurate measurement of the BDZ. It was validated to test urine samples for pain management service.

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Recently Postmenopausal Women Have Elevated Urinary 8-iso-Prostaglandin F2α (8-iso-PGF2α) Assayed by LC-MS/MS after Solid Phase Extraction

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Background: the adverse effects of menopause are reported to include alterations in lipid profile, body mass index, insulin levels and also increased risk of hypertension, cardiovascular diseases, osteoporosis, diabetes mellitus, cancer and other degenerative changes in postmenopausal females. It has been correlated with the increased production of free radicals after menopause. Oxidative stress is reported to increase after menopause, suggesting that the decrease in sex hormones occurring at the time of menopause could predispose women to higher levels of reactive oxygen species (ROS). Recently, F2-isoprostanes are shown to be reliably measured and used as the most reliable markers of oxidative stress status. Plasma or tissue 8-iso-PGF2α concentrations provide a "snap-shot" assessment of oxidative stress. The potential for interferences arising from serum or plasma during sample preparation makes the measurement of urinary concentrations that show no diurnal variations and less subject to interferences provide a better assessment of oxidative stress.

Objective: the objective of the study was to determine the association between the status of oxidative stress and menopause in healthy recently postmenopausal women using urinary 8-iso-PGF2α as a marker for identifying oxidative stress. Hypothesis of an important antioxidant role for estrogens would be reflected in strong inverse correlations with urinary 8-iso-PGF2α concentration.

Methods: urinary 8-isoprostaglandin F2α (8-iso-PGF2α) and estradiol were measured in 21 young women and 23 recently postmenopausal women. The subjects were healthy, were not smokers, and were not taking any medications or vitamins. Blood pressure, serum cholesterol, and glucose did not differ between the groups. Solid phase extraction columns (C18) were used for the extraction of 8-iso-PGF2α using a single solid phase extraction (SPE) cartridge. Chromatographic separation was achieved using C18 analytical column with 0.1% formic acid in water and a mixture of methanol: acetonitrile under isocratic conditions, and detection by negative mode selected reaction monitoring (SRM) mass spectrometry (MS), using an 8-iso-PGF2α standard to identify specific chromatographic peaks. The quadrupoles were set to detect the negatively charged molecular ions [M-H]− and a high mass fragment of 8-iso-PGF2α at m/z 353→193 amu.

Results: The limits of detection and quantitation for 8-iso-PGF2α were 12.3 and 31.6 pg/mL, respectively. The intra- and interassay imprecisions were 4.1% and 6.7%, respectively. 8-iso-PGF2α (238±41 [postmenopausal] versus 154±36 [non menopausal women] pg/mg creatinine, $P<0.05$) were higher in postmenopausal. Levels of 8-iso-PGF2α showed significant negative correlation with estradiol levels in postmenopausal women ($r = 0.57$, $P<0.01$). No such correlation was found in non

menopausal women (r= 0.29, P> 0.05). Associations were adjusted for age, body mass index (BMI), physical activity.

Conclusion: This study supports an association between the status of oxidative stress and menopause in healthy recently postmenopausal and that endogenous E2 play a role to modify oxidative stress by decreasing urinary 8-iso-PGF2α concentrations as assayed by LC-MS/MS.

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Development and Validation of Metanephrines and Catecholamines in urine by LC-MS/MS

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BACKGROUND: Urinary determination of biogenic amines excretion is used to diagnose phaeochromocytoma. Liquid chromatography-tandem mass spectrometry has high analytical specificity and can allows rapid testing for identification of these disorders.

OBJECTIVE: Development a simple, rapid and sensitive LC-MS/MS method to determination of biogenic amines metanephrines and catecholamines in urine using deuterated internal standard (IS).

RESULTS: 1000 µL of urine samples containing the internal standard were treated by liquid-liquid extraction using ethyl acetate. Chromatographic separation was performed on a BDS HYPERSIL C18 column (125 mm x 3 mm, 3 µm) and mobile phase water:methanol (98:2, v/v) with 0.25% of formic acid at 200 µL/min. Detection was performed on a triple quadrupole mass spectrometer with electrospray ionization (ESI+). The method has a chromatographic running time of approximately 8 min. The precision value was less than 7.0% for all analytes. The average of recovery was 92-108% for all analytes.

CONCLUSIONS: The LC-MS/MS method has been developed successfully for the quantitative analysis of these biogenic aminas and has applied for clinical analysis

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A Rapid and Sensitive LC-MS/MS Method for the Analysis of Free Thyroid Hormones

F. Carroll. *Restek Corporation, Bellefonte, PA*

Accurate and sensitive measurement of low levels of free hormones is necessary to assess thyroid function for both veterinary and human clinicians. Reverse tri-iodothyronine (rT3) is an inactive form resulting from T4 biotransformation. Since rT3 functions as the feedback inhibitor of thyroid hormone production, the measurement of rT3 can be an important diagnostic marker in clinical implication. The intent of this application was to develop an LC-MS/MS method for the analysis of thyroid hormones (T3, rT3, and T4) at the free form levels using the highly efficient and selective Raptor™ Biphenyl column. The clinical applicability of the method was demonstrated by analyzing the fortified thyroid hormone in phosphate buffer saline (PBS) containing 4% human albumin.

Human albumin dissolved in PBS solution was used to prepare calibration standards ranging from 2 to 400 pg/mL. Standard solutions were spiked with internal standard, T4-13C6, and mixed with acetonitrile in a glass vial. An aliquot of ethyl acetate was added, stirred, and then centrifuged. The organic phase was removed and placed into a glass vial, and evaporated to dryness under a stream of nitrogen. The dried extract was reconstituted in a 30:70 water:methanol solution and injected on to the Raptor™ Biphenyl column (100x2.1mm, 2.7µm) for the analysis on a Waters ACQUITY UPLC® I-Class System coupled with a Waters Xevo® TQ-S mass spectrometer using electrospray ionization in positive ion mode.

Good linearity (1/x weighted) was obtained for all three forms of thyroid hormones with coefficients of variation (r2) > 0.990 from 2 to 400 pg/mL (for T3) or 5 to 400 pg/mL (for T4 and rT3). The %deviation was < 15%. Simultaneous analysis of all 3 analytes was performed with a fast 3.5 minute total run time for each injection with complete separation of T3 and rT3 isobars. It was demonstrated that the Raptor™ Biphenyl column is excellent for rapid and sensitive analysis of thyroid hormones. With the established method, as low as 2 pg/mL (T3) or 5 pg/mL (T4 and rT3) of thyroid hormones can be accurately determined with less than 3.5 minutes of analysis time. The analytical method is thus applicable to the clinical analysis of free thyroid hormone at low pg/mL levels.

A-292

The Analysis of Vitamin D and Metabolites in Plasma by LC-MS/MS

F. Carroll. *Restek Corporation, Bellefonte, PA*

Vitamin D deficiency has been linked to an increased risk for many chronic diseases including diabetes, heart disease, autoimmune diseases, and some cancers. Vitamin D exists in two forms, vitamin D2 and vitamin D3. Each undergoes metabolism to form 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3. For accurate determination of vitamin D levels in the blood, it is important to distinguish between these metabolites and to separate them from major matrix interferences. In this method, the Raptor™ ARC-18 column combines the speed of superficially porous particles (SPP) with the resolution of highly selective USLC® technology to produce a simple and rugged method for the determination of vitamin D metabolites in serum and plasma.

The suitability of ARC-18 column for the analysis of vitamin D metabolites was first demonstrated for the analysis of neat standard solution containing fat-soluble vitamin D and metabolites. The ARC-18 was then used to analyze the 25-hydroxyvitamin D metabolites of fortified and plasma (Rat). The quantitation was performed with calibrated standards prepared in 4% human albumin PBS solution. Three levels of fortified metabolite concentration were measured with acceptable accuracy and precision.

The metabolites were fortified into Rat plasma and extracted with a liquid-liquid extraction method. Plasma (150mL) was mixed with 0.2M ZnSO4 (150mL) in a 2-mL glass HPLC vial. Added 300mL of methanol containing 25ng/mL of d6-25(OH)D3 (internal standard) and vortex mixed (10secs). Added 750mL of hexane, mixed for 30secs, and then centrifuged for 10mins at 4300rpm. The hexane layer (~650mL) was removed and placed into the micro-vial and evaporated to dryness under nitrogen at 55°C. The dried extract was reconstituted with 75mL of 15:85 water:methanol solution and injected (5 mL) for analysis.

The calibration standards were prepared in 4% human albumin in PBS solution and subjected to the same extraction procedure for the fortified plasma samples. A good linearity was obtained for both 25(OH)D2 and 25(OH)D3 from 1 to 150ng/mL (with 1/x weighting). The r was ~0.999 and the %deviation was ≤10%. The quantitation results of 3 levels of fortified plasma samples showed method accuracy from the %recovery of within 8% of the nominal concentration for all QC levels. The %RSD was from 0.3-10.7 indicating good method precision.

Separating fat-soluble vitamins by LC can be time consuming. The Raptor™ ARC-18 column, however, can analyze these difficult compounds using reversed-phased chromatography (RPC) in less time than traditional columns to increase productivity. Plus, in the bioanalytical arena, the ARC-18 can quantitate the metabolites of vitamin D using the same column and mobile phases.

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Comparison of Tacrolimus Quantification using the Waters MassTrak LC-MS/MS assay with the Abbott Architect Immunoassay

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* Imir Metushi and Alec Seitman have contributed equally to this abstract.

Background: Tacrolimus is a widely prescribed immunosuppressant for organ transplantation. However, due to its narrow therapeutic window it requires periodic therapeutic drug monitoring. Immunoassays which use monoclonal antibodies are widely used to monitor tacrolimus concentrations. Tacrolimus is metabolized by the CYP3A subfamily of enzymes giving rise to metabolites which are structurally similar to the parent drug that can cross react with the immunoassay. Methods: We verified performance claims of the Waters MassTrak assay and performed a patient comparison relative to the Abbott tacrolimus immunoassay on the Architect platform. We performed linearity studies by using pooled blood from patient samples measured in quadruplicates at 9 concentrations: 0.95, 4.9, 8.8, 12.7, 16.7, 20.6, 24.5, 28.5, and 32.4 ng/mL. Precision studies were performed by using three concentrations of pooled patient blood samples; low (0.5 ng/mL), medium (15 ng/mL) and high (30 ng/mL). A total of 40 patient blood specimens were run on both the MassTrak and the architect immunoassay over 20 days. In order to cover the dynamic range of the assay, we choose to divide the samples into four groups: 10 samples from each of the four ranges (0-6 ng/mL; > 6-13 ng/mL; > 13-21 ng/mL and > 21 ng/mL). Results: The Waters MassTrak assay was linear from 1 to 32.4 ng/mL with an R2 = 0.997. Precision studies of the MassTrack assay at three different concentrations over the measured analytical range were (mean ± CV): 0.6 ng/mL ± 20%, 16.0 ng/mL ± 5.4%, 31.2 ng/mL ± 5.8%. Matrix effect studies were conducted to determine whether blood could suppress ionization of tacrolimus; comparison of signal intensity between pretreated

blood samples and solvent samples revealed a difference of less than 10% for both tacrolimus and the internal standard (ascomycin). Patient comparison data between the MasTrak LC-MS/MS and Abbott immunoassay showed excellent correlation with an R2 = 0.97. However, the MassTrak method produced concentrations for tacrolimus that were consistently lower than the immunoassay with a negative bias ranging from 0 to 37% ($p < 0.0001$; via two-tailed paired t-test test). Conclusion: For the first time we report a direct comparison of the Waters MassTrak assay with the Abbott immunoassay for quantifying tacrolimus. Overall, the MassTrak LC-MS/MS assay was linear over the clinically relevant range of 1 to 32.4 ng/mL. Tacrolimus measurements in patient blood samples correlated well with the immunoassay (R2 = 0.97), however, the immunoassay values were consistently higher than those measured using the MassTrak method. We suspect that the higher measured concentrations in the immunoassay method are likely due to cross reactivity with tacrolimus metabolites by the antibody used in this assays.

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Magnetic bead-based serum peptidome profiling for identifying circulating biomarkers in esophageal squamous cell carcinoma

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Background: Esophageal squamous cell carcinoma (ESCC) is one of the most common malignant neoplasm worldwide. Patients are often diagnosed at advanced stages with poor prognosis due to the absence of obvious early symptoms. Human serum contains a mixture of proteins and peptides from various bodily tissues. Serum peptidome analysis thus provides a noninvasive window into human physiology and pathology. There is an urgent need to develop novel serological biomarkers for ESCC.

Methods: We performed A two-stage serum peptide profiling in 477 unique samples using weak cationic exchange magnetic beads coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to identify potential ESCC-associated peptides in the circulation.

Results: Three significantly differentially expressed peaks were used to establish a genetic algorithm model for ESCC. The sensitivity and specificity of the model were 97% and 95.92% in the training set, and 97.03% and 100% in the validation set, respectively. It was significantly better than the other well studied serum proteins such as squamous cell carcinoma antigen (SCC-Ag) and cytokeratin 19 fragment 21-1 (Cyfra 21-1). Notably, the diagnostic capability of this model was superior for early stage ESCC with a sensitivity of 96.94% and a specificity of 100%. The three diagnostic peptides were identified additionally and one of them, TSP1 was significantly overexpressed in tumor tissues and associated with lymph node metastasis and poor prognosis by immunohistochemical staining.

Conclusion: The novel diagnostic model may benefit the clinical applications for diagnosis and monitoring of ESCC. And the tumor-associated peptides in the circulation will deepen our understanding of tumorigenesis and serve as potential serological biomarkers for esophageal cancer.

A-295

Stability of 5-Hydroxyindole-3-acetic Acid and Vanillylmandelic Acid in Urine with Unadjusted pH at Various Laboratory Storage Conditions

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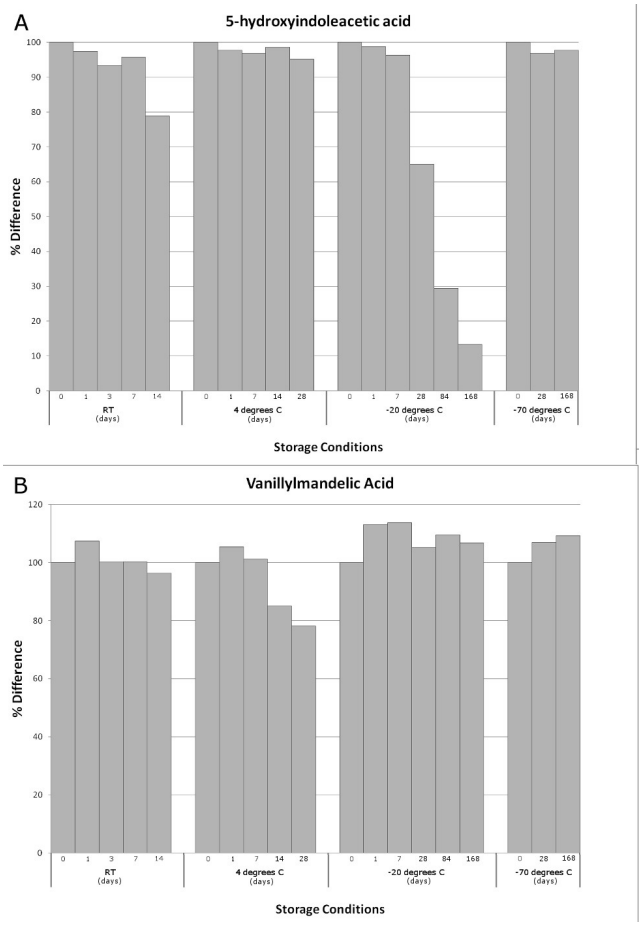
Background: Quantification of 5-hydroxyindole-3-acetic acid (5HIAA) and vanillylmandelic acid (VMA) in urine is used for the diagnosis and treatment follow-up of carcinoid and neuroblastic tumors, respectively. Several major reference laboratories provide acceptable storage conditions for 5HIAA and VMA in urine on their online directories. Many labs require acidification to improve stability. However, the stability of each analyte at various laboratory storage conditions is under represented in the literature. The objective of this study was to examine the short and long-term stability of 5HIAA and VMA in pH-unadjusted urine at room temperature (RT), 4°C, -20°C and -70°C.

Methods: Pooled patient urine was spiked with 5HIAA and VMA at 3.5 mg/L and 4.0 mg/L, respectively. The measured pH of the unadjusted urine pool was 6.2. Aliquots of the spiked urine were made for each storage condition and baseline samples were analyzed immediately. At defined time intervals for RT (1, 3, 7 and 14 days), 4°C (1, 7, 14, and 28 days), -20°C (1, 7, 28, 84 and 168 days) and -70°C (28 and 168 days) aliquots were removed and stored frozen at -70°C. By the end of this study all samples were thawed and analyzed in a single batch by an LC-MS/MS method.

Results: The stability of 5HIAA and VMA for RT was 7 and 14 days, respectively. While 5HIAA was stable for 28 days VMA was stable for only 14 days at 4°C. Interestingly, when samples were frozen at -20°C, the stability of 5HIAA regressed to 7 days, whereas VMA remained stable for 168 days. Both analytes were found to be stable for the entire study period (168 days) at -70°C.

Conclusion: 5HIAA and VMA in urine have adequate stability for routine clinical laboratories without adjusting the pH.



A-297

8-Oxoguanosine as a potential biomarker of aging: a higher level in children and elderly

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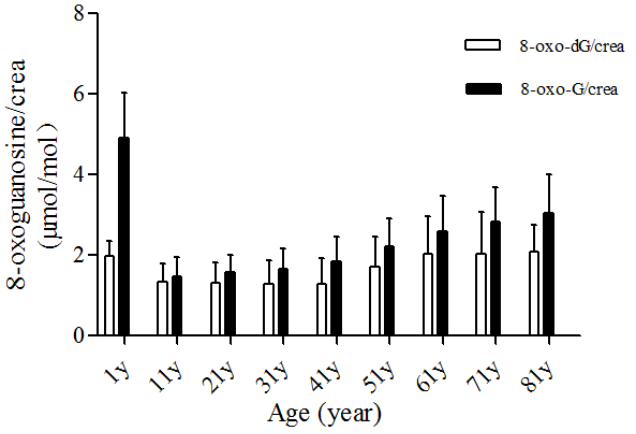
Background:A molecular biomarker of physiologic, as opposed to chronologic, age is needed in clinical medicine.Free radicals cause oxidative damage to macromolecules and lead to cell senescence. 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxo-dG) and 8-oxo-7, 8-dihydroguanosine (8-oxo-G) are two main nucleic acid oxidative adducts derived from DNA and RNA, respectively. Whether the two biomarkers could be the effectors of human aging is not well studied.

Methods: Participants undergoing routine health check-ups in West China Hospital were recruited.Children participants were from Huaxi kindergarten. Totally, 1228 healthy Chinese (from 2 to 90 years old) were selected and divided to 9 groups according to the age (1y represents participants from 1 to 10 years old, etc).Levels of 8-oxo-dG and 8-oxo-G in spot urines were analyzed using a UPLC-MS/MS system.

Urinary creatinine, serum glucose and lipids, like TG,CHOL,LDL and HDL were measured using clinical methods.

Results: The changes of two oxidized guanosine with aging was U-shaped(Fig.1). Participants younger than 10y and older than 81y had the highest oxidized guanosine levels. The value of 8-oxo-dG/crea(μmol/mol) in 1y group was 1.97±0.37, it decreased to 1.32±0.46 in 11y group, then increased gradually with aging, and reached 2.07±0.69 in 81y group. The same tendency was found in 8-oxo-G/crea,the concentration was 4.91±1.13 in 1y group, then reduced to 1.47±0.47 in 11y group and sharply increased back to 3.05±0.95 in 81y group. The reason for the high oxidized status in children and elderly adults may be the immaturity and degeneration of the repair mechanisms, respectively . It was noted the level of 8-oxo-G was much higher (≈2 fold) than 8-oxo-dG and correlated better with aging. A weak correlation was found between serum glucose, lipids and aging.

Conclusion: Measurement of 8-oxo-dG and 8-oxo-G is easily conducted. Increased 8-oxoG may be a potential biomarker to determine a person's physiologic age and identify individuals at high risk of developing age-associated disease.



A-298

Ultra-Fast quantitative analysis of Immunosuppressants in Dried Blood Spots using Laser Diode Thermal Desorption coupled to tandem mass spectrometry

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Background:

Immunosuppressant drugs are used as a proliferation signal inhibitor in organ transplants. Dried blood spots (DBS) on paper become a desirable method of sample collection. This technique reduces the steps for sample collection and shipping. To optimize the dosing regimen, concentration results from a sample are rapidly needed. The ultra-fast Laser Diode Thermal Desorption (LDTD) technology combined to a tandem mass spectrometer system is used for rapid turnaround time of sample results.

The Laser Diode Thermal Desorption (LDTD) ion source uses an infrared laser diode to indirectly thermally desorb neutral species of Immunosuppressant molecules from a dried sample extract. These neutral species are carried into a corona discharge region, where they undergo efficient protonation and are introduced directly into the mass spectrometer. Total analysis time is under 9 seconds with no carry-over.

The objective of this experiment is to validate the DBS extraction conditions, the analysis method and test different real patient samples using the LDTD-MS/MS. A cross validation study against the LC-MS/MS approach for the analysis of Immunosuppressants (Cyclosporin A, Tacrolimus, Sirolimus and Everolimus) was performed in order to evaluate the performance of the proposed alternative LDTD-MS/MS method.

Methods:

Lyophilized calibrators for Cyclosporin A, Tacrolimus, Sirolimus and Everolimus as well as Quality Control material were obtained from Chromsystem and UTAK. 25 µL of calibrators, QC and patient specimens are spotted on a Whatman 903 card and dried at room temperature (protected from light) for at least 2 hours. Six DBS punches of 3 mm were transferred in a glass tube. 100 µL of water was added and the tube was transferred to an ultrasonic bath for 10 minutes. 100 µL of internal standard (Ascomycin (5 ng/mL), Cyclosporin A-d4 (250 ng/mL), Ramycin-d3 (3 ng/mL) and Everolimus-d4 (5 ng/mL) in a mixture of ZnSO4 (1M):Methanol (20:80)) was added. The mixture was vortex-mixed. A liquid-liquid extraction was then performed by adding 200 µL methyl-tert-butyl ether (MTBE). After vortexing and centrifugation, 45 µL of the organic layer was transferred in a tube and 5 µL of desorption solution was added and mixed. 4 µL was deposited in the LazWell Plate and evaporated to dryness. The LDTD laser power was ramped to 80% in 6 seconds, and shut down after 2 seconds. Positive ionization mode was used, and the AB Sciex 5500 QTrap system was operated in MRM mode.

Results:

The calibration curves show excellent linearity with r > 0.995 between the quantification ranges of the Chromsystem standard. Intra-run accuracy and precision between 86.4 % and 107.5 % and 0.7 to 18.3%, respectively, were calculated. All QC values meet acceptance criteria of 15%. No matrix effect or carryover was observed. This method was cross validated with results from a traditional LC-MS/MS method with real patient specimens. All negative samples correlated accordingly.

Conclusion:

DBS provides an easier way for sample transport management and is an ideal match to the unique advantages provided by the LDTD technology ultra-fast analysis of Immunosuppressant drugs. This method has demonstrated accurate, precise and stable results with a run time of 9 seconds per sample.

A-299

Reference Intervals for Midnight and Morning Cortisol in Saliva Measured by an LC-MS/MS Method

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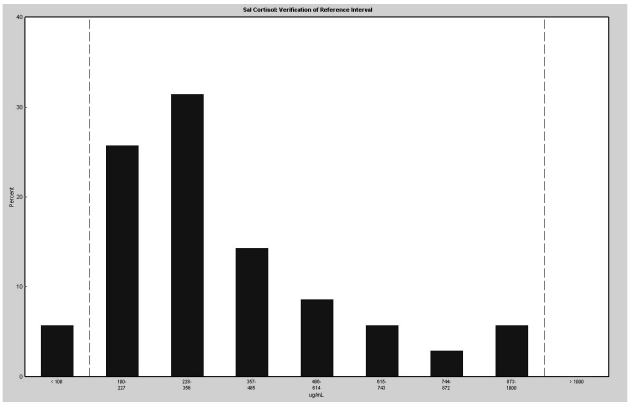
BACKGROUND Cortisol is a steroid enzymatically derived from cholesterol mainly in the adrenal gland. The cortisol concentrations undergo a cyclic diurnal variation with the lowest concentrations at midnight and the highest in the morning. Cushing's syndrome is the result of excessive secretion of glucocorticoids, primarily cortisol, and presents as a complex metabolic disorder. In Cushing's syndrome, the diurnal variation of cortisol is lost and midnight concentrations are similar to the morning concentrations. Therefore, measuring midnight levels of cortisol is important for the diagnosis of Cushing's syndrome. Salivary concentrations (typically <1000 µg/mL) correlate well with free serum concentrations (<1600 µg/mL) and do not require blood draws. There are different published reference intervals for both morning and midnight cortisol levels in saliva. In this work, we aimed to verify the reference intervals for morning and midnight salivary cortisol measured by an LC-MS/MS method. **METHODS** Collection of saliva samples was approved by the Institutional Review Board. In brief, saliva was collected in Salivette (n = 41) from healthy adults (22 females), aged 19-71 y (45±14). The exclusion criteria were: working night shift, taking any systemic steroid medications, or using any topical steroid cream. AM samples were collected between 6-8AM and midnight samples were collected between 11PM and midnight. The Salivettes were centrifuged and the samples were frozen at -70ºC until analysis. Statistics were performed using EP Evaluator Release 9 (Data Innovations, South Burlington, VT). **RESULTS** The morning cortisol results displayed a slight positive skew in the distribution for this reference population (Figure 1). The reference interval for (n=35) AM salivary

cortisol using a transformed parametric method provided an overall range of 100 to 1000 µg/mL, while the midnight range (n=41) was <100 µg/mL by nonparametric method (central 95%). **CONCLUSION** The reference range determined in this healthy adult population was not gender dependent and showed known diurnal variation.



A-300

Determination of Chromium, Manganese and Nickel in Human Serum by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

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In this study, a method to determine the trace elements Chromium, Manganese and Nickel in serum by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was developed and validated. These trace elements are very important for physiological function but, in high concentrations they may cause toxic effects to humans and in low concentrations, can cause developmental impairment.

The Chromium's clinical importance is due to its compounds in Glucose Tolerance Factor (GTF) as insulin cofactor, increasing the membrane's fluidity to promote the insulin binding toits receptor. It may be found in oil, asparagus, beer, mushroom, plum, whole grains, meat, offal and vegetables.

Manganese may cause toxic effects at high concentrations, such as respiratory diseases and nervous system disorders. On the other hand, its lack may result in growth disorders by bone frailty, osteoporosis, acromegaly, multiple sclerosis, and ovarian or testicular degeneration. It may be found in grains, nuts, peppers and fruits. It is also present in igneous rocks, fertilizer industries, metallurgical industries, ceramics, glass, batteries and gasoline.

Dermal exposure to Nickel may result in dermatitis and exposures by inhalation causes respiratory diseases. Nickel is considered carcinogenic (composite) and possibly carcinogenic (metal) by IARC. It may be found in beans, cabbage, lettuce, spinach and some fruits such as figs, pineapple, plum andraspberry. It is present in the steel, battery, liquid effluents steel, oil refinery and fertilizer plants, paper and pulp.

Therefore, a rapid, sensitive and accurate ICP-MS method was developed and validated for Simultaneous determination of 61Ni (HEHe), 52Cr (HEHe) e 55Mn (No Gas) in human serum. 500 µL of serum samples and calibrators standards were prepared by dilution (1:10, v/v) in an acidic solution consisting of nitric acid (0.1%) and triton-X 100 (0.05%). Germanium (72Ge) and Scandium (45Sc) were added as internal standard (IS).

The method was validated in terms of lower limit of quantification (LLOQ), linearity, precision, accuracy and comparability with Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Instrumental linearity was verified in the range of 0.01-100.0 µgL-1 for Chromium, 0.1-100.0 µgL-1 for Manganese and 0.5-100.0 µgL-1 for Nickel. The medium range of recovery over an interval of 0.5-100.0µgL-1 for the Chromium was 99.9-106.4%, for Manganese it was 95.7-102.3% and for Nickelit was 100.2-103.9%.

The intra and inter-day precision were less than 3.5% for Chromium, 7.2% for Manganese and 11.7% for Nickel.

In conclusion, the ICP-MS method has been developed successfully for the quantitative analysis and biomonitoring of these trace elements.

A-301

A quantitative LC-MS/MS determination of serum 17-hydroxyprogesterone utilized for the diagnosis of congential adrenal hyperplasia

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Background: Inherited genetic errors in steroid biosynthesis lead to hormone imbalances within affected individuals, which can propagate into life-threatening diseases. Cogential adrenal hyperplasia (CAH) has severe manifestations in 1 in 14 000 persons in the United States of America, resulting in salt wasting crisis in neonates as well as incorrect gender assignment in virtualized females, while mild forms of CAH are observed in less than 1 in 100 individuals in the USA, leading to sinus and pulmonary infections, orthostatic syncope and shortened stature. Ninety percent o f diagnosis cases are caused by a mutation in the gene that expresses the cytochrome P450 enzyme 21-hydroxylase. This mutation leads to elevated levels of 17-hydroxyprogesterone and the manifestation of CAH. Liquid Chromatography-tandem mass spectrometry (LC-MS/MS) allows for the rapid quantification of those steroids involved in the diagnosis of CAH, while using a lower volume of blood than radioimmunassays; important for the neonatal patient population targeted for testing. The developed liquid chromatography tandem mass spectrometry method for the quantification of 17-hydroxyprogesterone will provide physicians with information to assist their diagnosis and treatment of CAH.

Methods: A low volume and sensitive LC-MS/MS method has been developed for the quantification of 17-hydroxyprogesterone in 100 µL of serum. Sample preparation for both analytes relied upon a methyl *tert*-butyl ether liquid-liquid extraction, while chromatographic separation of analytes was carried out using an Agilent 1290 liquid chromatographic separations module (Santa Clara, CA, USA) and Agilent Poroshell C18 column (2.1 x 100 mm, 2.7 µ) with a Methanol and 5 mM ammonium formate gradient over 6 minutes. Identification of both analytes relied upon an Agilent 6460 triple quadrupole mass spectrometer using multiple reaction monitoring for quantification; 17-hydroxyprogesterone (331.0 ◊ 97.1/109.1 [H-085, Cerilliant, Round Rock, Texas, USA]) and 17-hydroxyprogesterone-d8 (339.2 ◊ 100.0/112.1 [H-096, Cerilliant]).

Results: The separation of steroid molecules by LC ensures the minimization of any interferences, which is critical in achieving accurate quantitative results. In separating these analytes, a lower limit of quantification (LLOQ) of 100 ng/L was achieved, as well as an upper linear range of 250000 ng/L. Across this linear range all calibration curves displayed excellent linearity of $R2 > 0.999$ and satisfied accuracy and precision requirements of ±10% for calibrators and quality control samples. In achieving this linear range, the diagnosis of CAH in patients will be enabled without the need for sample dilution or reanalysis.

Conclusion: The achieved linear range of 100-250000 ng/L and specificity provided by LC-MS/MS will ensure the proper diagnosis of patients afflicted with CAH, while reducing the need for sample dilution or reanalysis.

A-302

Opiate Hydrolysis by Recombinant Beta-Glucuronidases: Comparison of Commercially Available Enzymes for Pain Panels

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Background: For many drugs and xenobiotics glucuronidation is the major metabolic pathway of detoxification and only trace amounts of many parent compounds are normally present in urine. Therefore, hydrolysis of glucuronides is routinely employed for improved detection and accurate quantification of drug metabolites by HPLC-MS/MS. Enzyme hydrolysis is the preferred method to retain compound identification but can be costly, time-consuming, and is a major contributor to fouling of the column and detector. Recently introduced recombinant options from IMCS and Kura Biotec potentially provide much cleaner alternatives to the crude enzyme preparations typically employed and also allow reduced incubation times even with traditionally recalcitrant substrates. In addition, unwanted side activities present in crude preparations, such as deacetylase converting 6-MAM to morphine, are avoided entirely with recombinant versions. The assessment of a several novel recombinant β-glucuronidase for rapid hydrolysis is presented versus the most commonly utilized crude β-glucuronidase from Abalone.

Methods: Glucuronide controls for buprenorphine, codeine, lorazepam, norbuprenorphine, and (+)-11-Nor-Δ9-THC-9-carboxylic acid were treated with the recombinant β-glucuronidases and the crude enzyme. Hydrolysis efficiency was

assessed over a range of temperatures from 70°C to room temperature (RT) and 5 minutes to 2 hrs incubation times using the recommended optimum pH and buffer conditions. Hydrolysis of 6-MAM as an internal control to assess side activities was also monitored under these conditions. In addition, specific activity was assessed for all enzyme preparations utilizing glucuronide reporter substrates (absorbance, fluorescent, and MS) under the determined optimum hydrolysis conditions.

Results: The unique potential of the recombinant β-glucuronidases was demonstrated with extensive hydrolysis on traditionally recalcitrant targets, reduced hydrolysis time for the entire panel, and drastically reduced organic loading on the HPLC-MS/MS system. Additionally, the requisite step of centrifugation to remove the cell pellet after hydrolysis with the crude enzyme preparations is eliminated for the recombinant enzymes. This further streamlines the workflow and makes the method easily adapted to robotic preparation in microplate formats.

Conclusion: As more of these novel β-glucuronidases become commercially available the opportunity for commercial laboratories to streamline workflow, improve turnaround time, and reduce cost (i.e. extended column life, reduced MS downtime) will only increase the impetus for the entire industry to move away from crude enzyme preparations traditionally employed for these hydrolysis reactions.

A-303

LCMSMS quantitative analysis of Organic Acids in urine

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Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. Urine organic acids are used to exclude the possibility that a person has an inborn error of metabolism, usually one of the organic acidemias. Initially 60 urinary organic acids were analyzed in urine. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of urinary organic acids by QQQ. Various sample preparation techniques that includes dilute and shoot and liquid-liquid extraction (LLE) were evaluated along with derivatization and compared based on their ease of use, analyte recovery and post-extraction cleanliness as well as different mass spectrometer platforms. The described method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure urinary Organic Acids.

Methods: An Agilent 6460 tandem mass spectrometer with Jet Stream technology in negative Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 ul of serum were used for the analysis of urinary organic acids. Various columns were evaluated and an Agilent Poroshell 120 Bonus-RP 150 x 2.1 mm, 2.7 um with a water:methanol mixture containing 0.4% formic acid gradient achieved baseline chromatographic separation in a 20 minute run time for. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in negative mode underivatized and positive mode derivatized and accuracy of the method was verified using in-house controls and urine samples.

Results: Good linearity and reproducibility were obtained with the concentration range from 0.1 ug/ml to 100 ug/ml for the respective urinary organic acids with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.1 to 0.5 ug/ml. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations.

Conclusion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of urinary organic acids. The sample preparation techniques are quick and easily applied for high throughput analysis.

A-304

Combining High Resolution Mass Spectrometry and In Silico Structure Searches to Identify Compounds Contributing to False Positive Immunoassays

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Objective: Capture antibody-based assays are susceptible to off-target binding thus increasing false positives. We employed two techniques, high-resolution mass spectrometry (HRMS) and an *in silico* structure search, to identify compounds likely to cause false positive results in urine specimens that were positive by amphetamine (AMP) or MDMA immunoassays but failed to confirm by tandem mass spectrometry.

Methods: 100 false positive (FP) immunoassay specimens for AMP and/or MDMA were diluted five-fold and analyzed by a 1260 liquid chromatograph coupled to an Agilent 6230 time of flight mass spectrometer (TOF-MS). Full scan data from 100-1000 m/z were collected and signals with $>10^6$ counts were analyzed using MassHunter software and the Personal Compound Database Library (Agilent Technologies) to establish a library of chemical formulas from the obtained high resolution masses. Criteria for a positive identification were retention time ±0.15 minutes, mass error ±10ppm, and match score greater than 70. Separately, Scifinder (Chemical Abstracts) was used as an *in silico* structure search to generate a library of compounds that are structurally similar to amphetamine, MDMA, or compounds known to cross react with AMP and MDMA immunoassays. Initial 'hits' included thousands of organic compounds that were filtered based on frequency of citation in the literature as well as medicinal or biochemical characteristics. Chemical formulas and exact masses of 145 structures were then compared against masses identified by TOF-MS. To assess if compounds identified in the TOF-MS data or structure-based searches had cross-reactivity with the AMP or MDMA immunoassays, available verified standards (32 compounds total) were purchased and prepared in synthetic urine at half-log concentrations between 0.1 and 100ug/mL and analyzed by a Beckman AU5810 instrument with Siemens Emit II Plus reagent packs.

Results: Compounds known to have cross-reactivity with the immunoassays were identified in the structure-based search, validating the approach. In comparing chemical formulas and exact masses of 145 structures (of 20 chemical formulas) against masses identified by TOF-MS (area counts >1 $x10^6$), 10 mass matches correlating to 38 compounds represented in 67 specimens were identified. Of the 32 compounds tested by immunoassay, cross-reactivity was observed with 13 compounds by MDMA and 8 compounds by amphetamine immunoassays.

Discussion: Urine analysis by HRMS correlates accurate mass to chemical formulae but provides little information regarding compound structure. Utilizing structural data of target antigens and known-cross reacting compounds, one can search compound databases to correlate HRMS-derived chemical formulas with structural analogues. While this study demonstrates the utility of this approach, urine contains numerous metabolites that can also contribute to immunoassay FPs that may not be identified based on available compound libraries.

Conclusion: Because antibody specificity correlates with antigen structure, *in silico* structure based searches in combination with HRMS methods, facilitate identification of potential compounds that contribute to antibody cross-reactivity.

A-305

Analytical Quantitation of Tenofovir and Emtricitabine in Human Hair using LC-MS/MS for the Exploration of Hair as a Long-Term Adherence Marker

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Background:

There are a number of limitations associated

with self-reporting in assessing adherence to HIV antiretrovirals. Plasma and peripheral blood mononuclear measurements are reflective of short-term drug exposure. Hair and dried-blood spots (DBS) provide a more long-term exposure assessment. The objective of this study is to develop and validate a quantitation method for tenofovir/emtricitabine in hair using

LC-MS/MS and to access the utility of hair as a marker of long-term adherence, compared to DBS, in HIV-negative men enrolled in pre-exposure prophylaxis studies (iPrEx).

Methods:

Human hair was analyzed for tenofovir/emtricitabine using a Shimadzu LC-20AD HPLC system coupled to a Micromass Quattro Ultima triple quadrupole mass spectrometer using electrospray positive ionization. Multiple reaction monitoring analyses was performed on a reverse phase column (Synergi POLAR-RP, 4 µm, 4.6 x 150 mm). Flow rate was1 ml/min

utilizing a gradient system of aqueous to 45% methanol both containing ammonium acetate (5 mM), 0.06% trifluoroacetic acid, and 3 mg/L ammonium phosphate for 6 minutes. Sample preparation consisted of

the incubation of hair (5 mg) in a 50% aqueous methanol solution containing 1% trifluoroacetic acid, 0.5% hydrazine, and tenofovir-d6 and emtricitabine-^{12}C-

$^{15}N_2$ (internal standards) at 37°C overnight in a shaking water bath. Following centrifugation, the supernatant was then evaporated to dryness, and reconstituted with mobile phase for analysis by LC-MS/MS. The linear concentration ranged from 0.00200 to 0.400 and 0.0200 to 4.00 ng/mg of hair for tenofovir and emtricitabine, respectively and the lower limit of quantitation were equivalent to the lowest point of the standard curve. Precision and accuracy were determined through the analysis of quality control samples at 3 concentrations (0.320, 0.0400, and 0.00600 ng/ml hair (tenofovir) and 3.20, 0.400, and 0.0600 ng/ml hair (emtricitabine)). Precision, defined by the coefficient of variation (CV), ranged from 6.40% to 13.5% (tenofovir) and 2.45% to 5.16% (emtricitabine). Accuracy, defined by relative error (RE), ranged from -2.25% to +1.00% (tenofovir) and -0.250% to +4.06% (emtricitabine). Extraction efficiency was determined to be 91.3% (tenofovir) and 99.1% (emtricitabine). No carry over peaks were observed and no matrix effect (CV for matrix factor (MF) of tenofovir, emtricitabine, internal standards, and IS-normalized MF does not exceed 15%) was observed in six different lots of blank hair. **:** iPrEx Open Label Extension (OLE) enrollment consisted of 1603 HIV-negative men. Visits were performed at baseline and weeks 4, 8, 12, 24, 36, 48, 60, and 72. Plasma and DBS were collected at 4 and 8 weeks and every subsequent 12 weeks, while hair samples were collected every 12 weeks.

Results:

Tenofovir hair concentrations and tenofovir-diphosphate in DBS were strongly correlated (Spearman's correlation coefficient r 0.734, $p<0.001$), as were tenofovir hair levels and emtricitabine-triphosphate concentrations in DBS (r 0.781, $p<0.001$). However, a stronger correlation between emtricitabine hair levels with tenofovir-diphosphate in DBS exists compared to that with emtricitabine-triphosphate in DBS (r 0.587, $p<0.001$).

Conclusion:

Previous studies have shown that DBS are predictive of efficacy and the strong correlation between hair and DBS suggest that hair concentrations could be predictive as well.

A-306

Development and Validation of Dilute and Shoot- Flow injection Tandem Mass Spectrometric method for the Detection and Quantification of Phenobarbital in Human Urine

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Background: A new, sensitive and specific Dilute and Shoot- Flow injection Tandem Mass Spectrometric method for the identification and quantification of Phenobarbital in urine was developed and validated according to the US FDA and SAMHSA guidelines for the first time. Although, a variety of approaches have been employed to identify both licit and illicit drugs for urine drug testing analysis such as immunoassays, gas chromatography- mass spectrometry and high performance liquid chromatography-mass spectrometry, but also inherent several limitations such as (i) Immunoassays lacks specificity, susceptible to cross reactivity and can only be used for screening, (ii) GC-MS analysis necessitates complex derivatization of the sample. LC-MS/MS is now recognized as a gold standard technique for quantitative screening of multiple drugs, it requires an expensive HPLC column for separation, long run time, excess solvent loss, and elaborated sample preparation procedures like solid phase extraction or liquid- liquid extraction, resulting low throughput workflows which is the current rate limiting step and increases the turn-around time of the analysis. Faster methods are highly preferred in clinical settings, since majority of the patients will be tested positive for pain drugs, indicating adherence to agree upon treatment plan.

Method: Our devised method involved simple dilution of Phenobarbital spiked urine, fortified with Phenobarbital-d_5 (Internal Standard) followed by flow injection using 5mM Ammonium acetate / 70% Acetonitrile as a carrier solvent, bypassing the column compartment and detected by mass spectrometer employing negative ESI-MRM mode. Quantification and confirmation of Phenobarbital at LLOQ level (5ng/ml) was possible in this method by using MRM channels (signal/noise: 29 and 10.5) respectively.

Results: The validated method was found to be linear with the dynamic range of 5- 200ng/ml and the $r^2 > 0.9998$ was achieved. The precision (%CV) and accuracy (%RE) results for intra and inter assay at 3 QC levels (12.5, 45, 160ng/ml) and LLOQ (5ng/ml) were > 3.0% and 5.0% respectively. It is worth mentioning that, this method is devoid of significant matrix (absolute and relative) effects, where % CV's were found to be 7 and 6.2 respectively, at three QC levels and LLOQ. Phenobarbital in urine at two QC levels (12.5, and 160) was subjected to stability studies conditions: Bench top (8hr) at room temperature, freeze thaw (3 cycles), and 2 months at -20°C, excellent stability of Phenobarbital was found with % recovery of 98-100.5%.

Conclusion: The key components of this method are: Simple dilution and FI-MS/MS quantification of phenobarbital in urine with 2 minutes run time (analyte peak obtained at 0.7 minutes), enabling higher throughput analysis for urine drug testing, without compromising the data integrity when compared to traditional LC/MS/MS protocols. Nevertheless, no change in the signal intensity or sensitivity was found even after 1200 injections proving that this method was robust. Hence, the acquired results proved that this novel method is simple and scalable and has the capacity to process ~1500 samples/day, and can lead to the transfer of existing methodologies to the newer robust platforms, permitting the development of more sensitive and rapid techniques recommended for clinical and toxicological studies.

Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Nutrition/Trace Metals/Vitamins

A-307

Development of a Reference Material for the Determination of Vitamin B_{12} in Human Serum

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Background: The National Institute of Standards and Technology (NIST) provides a variety of Standard Reference Materials® (SRMs) for the analysis of nutrient levels in clinical matrices. One nutrient of interest is vitamin B_{12}, which is involved in DNA synthesis and red blood cell formation. Vitamin B_{12} deficiency can lead to anemia and permanent nerve damage. Vitamin B_{12} in serum is measured by a variety of analytical techniques, including microbiological, radioisotopic, and chemiluminescence assays. Concerns remain regarding the comparability and accuracy of these various methods, which supports the development of an accuracy control reference material. While the current SRM 1955 Homocysteine and Folate in Frozen Human Serum possesses reference values for vitamin B_{12} based on radioassay measurements at the Centers for Disease Control and Prevention (CDC), no material specifically designed for vitamin B_{12} determination in serum has been available to date. In addition, the SRM 1955 stock with be exhausted within a year and the replacement material is not slated for vitamin B_{12} value assignment. NIST, in collaboration with the National Institutes of Health-Office of Dietary Supplements (NIH-ODS), has developed a new material to fill this void.

Methods: Candidate SRM 3951 Vitamin B_{12} in Human Serum was designed to have three concentration levels of vitamin B_{12}: nominally, 100 pg/mL, 200 pg/mL, and 450 pg/mL. To produce candidate SRM 3951, a vendor pooled normal human serum to achieve the highest level. Since all donor serum screened for this project contained >200 pg/mL vitamin B_{12}, the remaining mid- and low-level materials were obtained by diluting higher-level serum with charcoal-stripped serum. All serum levels were packaged as 1-mL aliquots in glass vials and stored as fresh frozen serum.

Results: NIST intends to utilize measurement data from multiple methods in order to assign vitamin B_{12} values, as total cyanocobalamin, to all three levels of material. Duplicate analyses from six vials measured over three separate days by electrochemiluminescence immunoassay at the CDC resulted in mean values of 57.8 pg/mL (%CV=14 %), 155.1 pg/mL (%CV=5.3 %), and 383.7 pg/mL (%CV=2.6 %) for the low-, mid-, and high-level materials, respectively. In addition, NIST is developing an isotope-dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) method in order to provide a second, independent method for the determination of total vitamin B_{12}. The recent availability of ^{13}C-labeled cyanocobalamin has enabled the development of this method, which involves the spiking of serum samples at an approximately 1:1 mass ratio of labeled to unlabeled target analyte, followed by chemical conversion of various vitamin B_{12} metabolites to cyanocobalamin, affinity clean-up, and ID-LC-MS/MS analysis. The NIST method will also be utilized to establish homogeneity across the batch of candidate SRM 3951.

Conclusion: Candidate SRM 3951 will provide the first accuracy control reference material from NIST specifically designed with three unique levels of vitamin B_{12}, which will provide a valuable tool for *in vitro* diagnostics manufacturers, as well as other end-users, for validating their calibrators and methods. This, in turn, will help to ensure that clinical vitamin B_{12} measurements are accurate and that patients with deficiencies are treated appropriately.

A-308

The role of oxidized metabolites of linoleic acid in alcohol-mediated hepatic inflammation and injury in a mouse model of alcoholic liver disease

I. Kirpich[1], H. Liu[1], A. Feldstein[2], C. McClain[1]. *[1]University of Louisville, Louisville, KY, [2]University of California, San Diego, CA*

Background/Aim: Dietary fat is an important determinant of alcoholic liver disease (ALD). It has been documented that experimental ALD is exacerbated by a high polyunsaturated fat diet rich in linoleic acid. Recent publications have shown that experimental and clinical alcohol-induced liver steatosis and injury were associated with elevated oxidized linoleic acid metabolites (OXLAMs), specifically 9- and 13-hydroxy-octadecadienoic acids (9- and 13-HODEs). OXLAMs are endogenous ligands for the Transient Receptor Potential Vanilloid 1 (TRPV1), a non-selective channel with high permeability for Ca2+. We postulate that bioactive OXLAMs play a critical role in the development and progression of alcohol-mediated hepatic inflammation and injury via OXLAM-TRPV1-mediated mechanism. The aim of the study was to evaluate the role of OXLAMs and OXLAM-TRPV1 signaling in an experimental animal model of ALD.

Methods: C57BL/6 wild type (WT) and Trpv1 knock out (Trpv1-/-) male mice were fed a Lieber-DeCarli diet containing 5% ethanol (v/v) for 10 days, followed by a single dose of ethanol (5 g/kg body weight) by gavage (chronic-binge model). Liver steatosis was evaluated by histopathology and hepatic triglyceride accumulation measurement. Liver injury was assessed by plasma ALT activity; hepatocyte cell death was determined by hepatic caspase-3 activity and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay. Expression of genes associated with liver inflammation was analyzed by qRT-PCR. Plasma OXLAM levels were determined by LC/ESI/MS/MS and stable isotope dilution GC-MS methods. In vitro studies using HepG2 cells were performed to evaluate OXLAM/TRPV1 signaling.

Results: Chronic-binge alcohol administration resulted in a marked increase in plasma OXLAM levels, specifically 9-HODE and 13-HODE, in parallel with up-regulation of hepatic Trpv1 in WT animals. These effects were associated with hepatic steatosis, inflammation and injury. Genetic depletion of Trpv1 did not blunt hepatic steatosis caused by EtOH, but ameliorated hepatic injury as assessed by ALT levels (354.7+54.0 U/L in WT vs 130.6+30.5 U/L in Trpv1-/-, $p<0.05$). Trpv1 deficiency protected from chronic-binge alcohol-induced hepatocyte death assessed by caspase-3 activity and TUNEL staining. Trpv1 deficiency also prevented the increase in pro-inflammatory cytokine and chemokine expression, including Tnf-α, IL-6, Mip-2, Mcp1. Moreover, Trpv1 depletion markedly blunted EtOH-mediated induction of plasminogen activator inhibitor-1 (Pai-1), an important mediator of alcohol-induced hepatic inflammation, via fibrin accumulation. EtOH-induced up-regulation of pro-inflammatory cytokines in WT but not in Trpv1-/- animals was in parallel with the activation of hepatic NF-kB and extracellular-signal-regulated kinase (ERK) pathways. One of the possible mechanism(s) underlying hepatic inflammation in our model might relate to the nature of TRPV1, as a channel with high permeability for Ca 2+. Thus, exposure of hepatocytes to 9-HODE and 13-HODE in vitro resulted in activation of TRPV1 signal transduction with increased intracellular Ca2+ levels, suggesting that OXLAM/TRPV1/Ca2+ signaling may be a potentially relevant pathway contributing to ALD.

Conclusions: This study indicates for the first time that the TRPV1 receptor pathway may be involved in the hepatic inflammatory response in an experimental animal model of ALD. TRPV1-OXLAM interactions appear to play a significant role in hepatic inflammation/injury, further supporting an important role for dietary lipids in ALD.

A-309

Assessment of excess cancer risk associated with exposure to some airborne trace elements

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Backgrounds: Airborne particulate matter (PM), especially fine particles, could be closely related to health problems in a typical urban environment. PM may consist of toxic trace elements that considered potential health risk factors due to their carcinogenic properties. Of these trace elements, cadmium (Cd), chromium (Cr), arsenic (As), beryllium (Be) and nickel (Ni) were classified as probable human carcinogens (group B1) in accordance to USEPA, 2011. Makkah is the holy city in Saudi Arabia with a total population of 1.7 million, although it has limited industrial activities, it has unique characteristics every year, over 2.3 million of pilgrims stay in Makkah during Hajj period which increase transportation pollution problems creating unspecified amount of trace elements pollution in air. Objective: This study aimed to determine the excess cancer risk (ECR) of population associated to the inhalation exposure of five heavy metals (Cd, Cr, As, Be, Ni) in ambient air of Makkah. Materials and Methods: The study was conducted in Arafat Area, east of Makkah (latitude 21° 21' 1 N, longitude 39° 58' 1 E), representing one of the holy places highly crowded in Hajj season. Meteorological data were recorded and air samples were collected using mini volume sampler (Airmetrics, USA) at 10m height on a 47mm Teflon filter at 16.6 l/min flow rate for 24 hrs once in a week for 6 months during summer and autumn 2014, in accordance to USEPA standards method (29/2000). Trace elements content of the collected PM10 samples were analysed using ICP-MS 7300 (Perkin Elmer, USA) in reference to a standard solution of trace elements using a protocol certified by (US-NIST). The recovery yields of trace elements were higher than 95% with

detection limits ≤3 ng/m3 for all trace metals. Since trace elements carcinogenicity risk were unknown in Makkah, determination of ECR due to inhalation exposure to each metal was calculated in accordance to unit risk factor (URF) presented in US-EPA-IRIS. Results: Mean atmospheric concentrations of Cd, Cr, As, Be and Ni were 0.098, 0.008, 0.016, 0.03 and 0.012 µg/m-3 respectively in summer months. While in autumn, the mean concentrations were 0.06, 0.006, 0.01, 0.002 and 0.01 µg/m-3, respectively. The ECRs for Arafat area, were found to be (1.08×10-4, 7.21×10-4, 4×10-6, 4.6×10-6 and 2.4×10-6) for Cd, Cr, As, Be and Ni respectively, exceeding USEPA's level of acceptable inhalation risk (1.0x10-6) for each element. Conclusions: Higher concentrations of atmospheric trace elements in summer are probably due to high temperature as well as high wind speed, a common phenomenon in Makkah, which increases atmospheric turbulence leading to a greater amount of re-suspension of dust from roadside and blowing sand particles. The findings of this study can be addressed as reference for authorities' regulations and to develop air quality management strategy for protecting the public health. However, larger prospective studies are warranted to explore the health effects of long-term exposure to ambient air trace elements. Acknowledgments: This work was supported by King Abdulaziz City for Science and Technology (KACST) under National Science, Technology and Innovation Plan (NSTIP), KSA.

A-310

Total antioxidant status, magnesium and calcium levels in seminal plasma of infertile men

E. R. Ekong, E. W. Ohobu. *University of Calabar, Calabar, Nigeria*

Background: Poor-quality semen may result from testicular production of abnormal spermatozoa, post-testicular damage to spermatozoa in the epididymis or the ejaculate from abnormal accessory gland secretions.

Objectives: This study focused on assessment of the levels of seminal plasma total antioxidant, magnesium and calcium and their influences on sperm quality of infertile men.

Material and methods: A total of 120 subjects were recruited for this study, forty nine (49) were apparently healthy fertile men who served as control while seventy one (71) were infertile men further classified into oligospermic (n=33), asthenospermic (n=30) and azoospermic (n=8) based on the WHO classification. Informed consent was given by all participants in this study. Total antioxidants capacity (TAC) measurement was done by a kit from REL Assay Diagnostics, Turkey. Seminal plasma magnesium (Mg) was determined using a kit from Teco Diagnostics, Anaheim, Netherland while calcium (Ca) was estimated using a kit obtained from Giese Diagnostics, Rome, Italy.

Results: The percentage motility (69.80±7.29%) and sperm count (84.40±65.08 x106/ml) of the normospermic group was significantly higher ($p<0.05$) than those of asthenospermic (30.50±10.45% & 7.93±5.47 x106/ml), oligospermic (33.33±13.84% & 7.93±5.38 x106/ml), and azoospermic (0.00% and 0.00 x106/ml) cohorts. Also, the TAC (0.56±0.20mmol trolox equiv./l), Mg (0.91±0.47mmol/l) and Ca (3.17±8.29mmol/l) levels of the normospermic group were significantly higher (<0.05) than those of the asthenospermic (TAC =0.29±0.17mmol trolox equiv./l, Mg=0.33±0.57mmol/l, Ca=1.95±4.98mmol/l), Oligospermic (TAC=0.31±0.18mmol trolox equiv./l, Mg=0.34±0.56mmol/l, Ca=1.92±4.93mmol/l) and Azoospermic (TAC=0.28±0.23mmol trolox equiv/l, Mg=0.41±0.85mmol/l, Ca=2.73±3.25mmol/l).

Discussion: This study shows that reactive oxygen species and abnormal seminal plasma calcium affect sperm qualities associated with fertility. While low calcium may be responsible for loss of motility and unsuccessful acrosome reaction, supraphysiological levels may propagate chemical sterilization. This may be as a result of the anti-gonadal and oxidative stress inducing effect of hypercalcemia and the ensuing suppression of testicular status.

Conclusion: Normal secretory status of the accessory reproductive glands is critical to male fertility; also, exogenous sources of calcium and magnesium such as Calcium Chloride and Magnesium Sulfate in municipal water treatment may be a culprit of male infertility due to their suppressive effect on testicular status.

A-311

Comparison Study of PAML's VDSP Certified LC/MS/MS Vitamin D Assay and the VDSP Certified Siemens Centaur Vitamin D Assay

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Objective:

The primary objective of this study was to compare two methods that are certified under the United States Centers for Disease Control (CDC) Vitamin D Standardization Certification Program (VDSCP). The methods are PAML's certified LC-MS/MS Vitamin D assay and the Siemens Healthcare Diagnostics ADVIA Centaur Vitamin D Total assay (Centaur).

Methods:

A total of 116 samples were analyzed by both methods in this study. The sample set included 39 samples obtained through the CDC, that are single donor serum materials independent of the VDSCP, with values ranging from 9.6-67.2 ng/mL. An additional 10 native serum samples were spiked with 25(OH)vitamin D_3 (D_3) and provided by Siemens. The remainder of the samples were PAML patient residuals, with total 25(OH)vitamin D values ranging from 9-84 ng/mL. All samples were stored frozen at -70°C. In preparation for analysis, the samples were thawed, vortexed, and aliquotted. The analysis by both assays was conducted by the respective clinical departments. The resulting data were compiled and analyzed using method comparison regression analysis and principle component analysis (PCA).

Results:

The study method comparison results are summarized in the table. The additional PCA results show a high influence of 25(OH)vitamin D_2 (D_2) in the second PC, and D_2 and the Centaur results are negatively correlated in the third PC.

Conclusions:

The data shows a negative bias of the Centaur compared to the LC-MS/MS. The PCA shows that the D_2 is influencing the data set, however the regression analysis does not show a marked difference between samples with D_3 only and samples that contain D_2. One source of potential bias is the D_3 epimer which co-elutes with D_3 in the LC-MS/MS method. The epimer does not cross-react in the Centaur assay. Additional studies are underway to determine if bias is due to individual measurements by the two assays.

Data Sets	Corr. Coeff.	Deming Slope	Deming Intercept	Average Bias
Full Study (N=116)	0.9555	0.882	-1.909	-6.137
CDC Sample Set (N=39)	0.8903	1.033	-3.706	-2.799
Samples with D_3 only (N=67)	0.9653	0.870	-0.199	-4.737
Samples with D_2 (N=42)	0.9158	0.923	-5.445	-8.123

A-312

The first decade of a global external quality assurance program for serum-based nutritional biomarkers

B. M. H. Toombs, R. L. Schleicher, M. Zhang, C. M. Pfeiffer. *Centers for Disease Control and Prevention, Atlanta, GA*

*Background***:** The VITamin A Laboratory-External Quality Assurance program (VITAL-EQA) was established in 2003 to ensure the existence of laboratories around the globe that are proficient in quantitative analysis of serum retinol, particularly those labs taking part in national nutrition surveys. The program, administered by the U.S. Centers for Disease Control and Prevention, has since expanded to include vitamin D metabolites, inflammation and iron indicators, and B vitamins.

Methods: Twice each year, an average of 19 labs test serum samples in duplicate over the course of 3 days and estimate concentrations of nutritional biomarkers. Results are submitted to CDC, reports are issued, and labs are given guidance as requested or needed.

Results: The method used to determine the performance of each lab was updated in the 5th year to place an emphasis on imprecision and bias based on biological variation rather than scoring against acceptable and target ranges generated from consensus and CDC data, respectively. Using vitamin A as an example, based on biological variation, labs need a CV <2.4%, <4.8%, and <7.1% to achieve an optimum, desirable, or minimum imprecision performance rating, respectively. For bias, deviation from

the target value needs to be <±4%, <±8%, and <±12% for an optimum, desirable, or minimum bias rating, respectively. Of the labs that participated in at least 2 rounds during the last 5 years of the program, 24% had an acceptable bias (minimum or better) for all 3 levels of vitamin A tested in both their first and last rounds. In their first round of participation, more than 1/3 of labs (38%) had an unacceptable bias for all 3 levels. Of this subgroup of labs, nearly 2/3 moved to having an acceptable bias in their last round (27% for all 3 levels, 27% for 2 of the 3 levels, and 9% for 1 level), while the remainder (36%) still had an unacceptable bias for all 3 levels. The program has grown from 11 to 21 participating labs, and from 2 to 7 nutritional indicators. As of the last round, the number of labs reporting biomarker data were as follows: 18 retinol, 5 25-hydroxyvitamin D, 7 C-reactive protein, 9 ferritin, 6 soluble transferrin receptor, 7 vitamin B12, and 7 folate.

Conclusions: The number of labs benefitting from this program has nearly doubled since its inception. More labs are added each year, and the performance rating method continues to evolve to better determine the quality of the data and aid the regions serviced by each lab.

A-313

Rapid and Simultaneous Quantitative Determination of Vitamin A, D and E by UPLC-UV

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Objective: To validate and implement a rapid, simple, and sensitive Ultra Performance Liquid Chromatography (UPLC) method for the simultaneous determination of Vitamin A (retinol), 25-OH-vitamin D2, 25-OH-vitamin D3 and Vitamin E-α (α-tocopherol) and Vitamin E-γ (γ- tocopherol) in human serum/plasma for routine clinical use at the Children's Hospital Los Angeles.

Method: Tocopherol Acetate (100 µL) as the internal standard was added to 500 µL of serum/plasma. After a 5 minute incubation at room temperature, 800 µL of M/I mixture (Methonol: 2-propanol, 9:1 v:v) was added and vortexed for 15 seconds, followed by 4 ml of hexane, vortexed for 4 minutes and then centrifuged at 1500 rpm for 2 minutes. The top phase was transferred to a 12 X 75 mm glass tube, and dried under N2. The pellet was then reconstituted with 150 µL of methanol, and filtered out with a 0.2 µm syringe filter. 4 µl of filtrate was injected into Waters UPLC Acquity system.

Retinol, 25-OH-vitamin D2, 25-OH-vitamin D3, α-tocopherol and γ-tocopherol and Tocopherol Acetate were analyzed simultaneously using the UPLC with UV detector at 265 mm. Separation was achieved with Water's UPLC BEH C18 1.7 µm, 150 x 2.1 mm column at 32 °C. The method incorporates gradient elution with mobile phase A (0.1% formic acid in 85% acetonitrile and 15% water) and mobile phase B (0.1% formic acid in 96% acetonitrile and 4% methanol).

Results: The linearity was up to 6.66 µg/ml for retinol, 200 mg/L for 25-OH-vitamin D3, 200 mg/L for 25-OH-vitamin D2, 190 mg/L for α-tocopherol and 42 mg/L for γ-tocopherol,

respectively. The limit of quantitation for retinol, 25-OH-vitamin D3, 25-OH-vitamin D2, α-tocopherol and γ-tocopherol were as follows: 0.025 µg/ml, 4.2 mg/L, 4.2 mg/L, 0.28 mg/L and 0.7 mg/L respectively. The recoveries were 102-110% for retinol, 114-119% for 25-OH-vitamin D2, 109-117% for 25-OH-vitamin D3, 98-101% for α-tocopherol and 99-117% for γ-tocopherol. The inter-assay precision CV was 8% for retinol, 9.9% for 25-OH-vitamin D2, 6.9% for 25-OH-vitamin D3, 11% for α-tocopherol and 9.5% for γ-tocopherol. The intra-assay precision CV was 2.2% for retinol, 6.7% for 25-OH-vitamin-D2, 3.7% for 25-OH-vitamin-D3, 0.8% for α-tocopherol and 1.3% for γ-tocopherol. This UPLC method was compared with a LC/MS-MS method. The correlation coefficients (R) were 0.97 for retinol, 0.98 for total 25-OH-vitamin D, 0.97 and 0.96 for α-tocopherol and γ-tocopherol. Reference intervals were established for retinol and α-tocopherol and γ-tocopherol based on the healthy pediatric population seen at Children's Hospital Los Angeles. For retinol, 17y: 0.22-0.96 µg/ml. For α-tocopherol, 17y: 5.38-19.08 mg/L. For γ-tocopherol, 17y 0.33-4.19 mg/L.

Conclusion: Because of its low cost, short analysis time (8 min) and excellent chromatographic reproducibility, this UPLC method is suitable for simultaneous analysis of these three vitamins in human serum/plasma. This new method will allow laboratories to meet the increasing demands for monitoring of fat-soluble micronutrients, and can be easily adopted and scaled for high-throughput clinical practice if more expensive LC/MS-MS is not available.

A-314

Amalgam fillings, fish consumption and urbanization are associated with high urinary excretion of mercury in healthy individuals.

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Background: Mercury (Hg) heavy metal pollution is a serious environmental hazard all over the world. Bioaccumulation of Hg is associated with neurotoxicity and damaging effects on several cellular components such as membranes, proteins, and DNA. This study was performed to assess concentration of Hg in urine samples of otherwise healthy individuals and exposure to commonly known predisposing factors.

Methods: This study was performed in clinical chemistry department at King Khalid University, Riyadh between Jan. 2013 and March 2014. A total of 246 otherwise healthy individuals comprising of 153 (62.2%) males and 93 (37.8%) females with a mean age of 37±11 years were included in the study. Along with the demographic details information regarding consumption of fish, having amalgam tooth fillings, residence in urban or rural areas and smoking habit was also recorded at time of collection of random urine samples. Hg levels in urine were determined by inductively coupled plasma mass spectrometry.

Results: The median concentration of Hg [7.8 µg/g creatinine Interquartile range; (IQR) 4.55] in urine samples of 95/246 individuals with one or more amalgam fillings was significantly higher (p=0.00001) than 151/246 individuals with no amalgam filling (1.8 µg/g creatinine; IQR: 1.96). Participants consuming fish meals more than once a week (108/246) had higher urinary levels of Hg (median 4.04 µg/g creatinine; IQR: 3.44; p=0.0001) compared to those (138/246) consuming less than one fish meal per week (median 1.92 µg/g creatinine; IRQ: 2.97). Similarly individuals (174/246) residing in urban areas had higher urine concentration of Hg (median 3.64 µg/g creatinine; IQR: 3.3) compared to individuals (72/246) residing in rural areas (median 2.65 µg/g creatinine; IQR: 2.98; p=0.006). No difference in urinary Hg concentration was detected between smokers (114/246) consuming more than ten cigarettes a day (median 2.4 µg/g creatinine; IQR: 3.29) or less than ten cigarettes (132/246) a day (median 2.6 µg/g creatinine IQR: 3.1).

Conclusion: Amalgam fillings, frequent consumption of fish and residing in urban areas are significant predisposition to bioaccumulation of Hg.

A-315

Introducing Candidate Standard Reference Material (SRM) 2378 Fatty Acids in Human Serum

B. A. Benner, M. M. Schantz, K. E. Sharpless. *NIST, Gaithersburg, MD*

Objective: To characterize the fatty acid composition of a new SRM that consists of serum acquired from donors who were or were not taking dietary supplements that contained fish oil or flaxseed oil.

Relevance: Dietary fatty acids can be both beneficial and detrimental depending on the degree and type of saturation. Healthcare providers, the Centers for Disease Control and Prevention (CDC), and other organizations monitor fatty acid content of human plasma and serum as an indicator of health status and diet. Both the CDC and the National Institutes of Health - Office of Dietary Supplements are interested in serum fatty acids because they may be predictive of coronary heart disease or cancer. Candidate SRM 2378 is for use as a quality assurance sample in the analysis of serum for fatty acids, and consists of three different pools of serum acquired by a contracted laboratory from healthy donors who had been taking fish oil dietary supplements (at least 1000 mg per day) for at least one month (level 1 material), from healthy donors who had been taking flaxseed oil dietary supplements (at least 1000 mg per day) for at least one month (level 2 material), and from healthy donors eating "normal" diets without taking dietary supplements containing oils (level 3 material).

Methodology: Mass fractions of fatty acids in SRM 2378 were determined using four different extraction procedures, including saponification in methanolic KOH, treatment with sodium methoxide in methanol, hydrolysis using sequential addition of acetonitrile:hydrochloric acid and methanol:sodium hydroxide in the presence of heat, and microwave-assisted digestions using acetonitrile:hydrochloric acid. The fatty acids were esterified using sulfuric acid in methanol and pentafluorobenzyl bromide for subsequent measurement by GC with flame ionization detection (FID) and GC with electron capture ionization (negative chemical ionization) detection, respectively. SRM 1950 Metabolites in Human Plasma was employed for quality control of the methods used in this study.

Results: Significant differences in mass fractions of specific fatty acids were observed among the different levels representing different supplementations (flaxseed oil and

fish oil) or no supplementation. For example, the concentration of α-linolenic acid, a major component of flaxseed oil, was observed at two times the concentration of the level 3 material in both the level 2 and level 1 materials [(32.5 ± 4.1) µg/g and (31.5 ± 1.3) µg/g, respectively versus 17.0 ± 0.1) µg/g]. In addition, the level 1 material (fish oil supplemented) was enriched with four times the levels of eicosapentaenoic acid [(84 ± 11) µg/g versus (20.7 ± 0.8) µg/g and (18.9 ± 2.2) µg/g] and two times the concentrations of docosahexaenoic acid [(104 ± 5) µg/g versus (55.4 ± 2.3) µg/g and (54.9 ± 2.4) µg/g] observed in the level 2 and 3 materials.

Conclusions: The enrichments in specific fatty acids observed in the level 2 and level 1 materials of SRM 2378 with respect to the level 3 material (no supplementation) are consistent with donors supplementing with flaxseed oil and fish oil, respectively.

A-316

Trace element levels in Korean pregnant women and their associations with pregnancy outcomes

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Background:

Maternal trace element levels can have multiple impacts on health outcomes in mothers and their offspring. The aim of this study was to investigate the trace element status [cobalt (Co), copper (Cu), zinc (Zn), and selenium (Se)] in Korean pregnant women to assess their effects on pregnancy related outcomes: gestational diabetes, preeclampsia, gestational age at delivery, delivery with emergent Caesarean section, baby weight at birth (including assessments of low birth weight infants and those small for gestational age), children born with congenital abnormalities, prelabor rupture of the fetal membranes (PROM), preterm birth, and preterm PROM.

Methods:

This prospective study is based on 243 Korean pregnant women recruited at a referral hospital from April 2012 to January 2013. We investigated serum micronutrient status according to demographics, seasons, and obstetric characteristics together with the assessment of pregnancy and infant outcomes.

Results:

The median (interquartile range) serum trace element concentrations of all participants were as follows: 0.39 µg/L (0.29-0.53 µg/L) for Co, 165.0 µg/dL (144.0-187.0 µg/dL) for Cu, 57.0 µg/dL (50.0-64.0 µg/dL) for Zn, and 94.0 µg/L (87.0-101.0 µg/L) for Se. The serum concentrations of all four trace elements were significantly different over all three trimesters ($P < 0.05$). The overall prevalence of an excess or deficiency of the trace elements were as follows: 3.7% (n = 9) for Co excess, 70.0% (n = 170) for Cu excess, 76.1% (n = 185) for Zn deficiency, and 2.1% (n = 5) for Se deficiency. There was no significant association between maternal serum trace element status and pregnancy outcomes, except for Zn deficiency and the rate of emergent Caesarean sections (adjusted odds ratio 4.59, $P = 0.04$) and the risk of preeclampsia with Cu excess (adjusted odds ratio 12.37, $P = 0.03$).

Conclusion:

Our results suggest that maternal Zn and Cu status during pregnancy may influence the risk of emergent Caesarean sections and preeclampsia. Further research about the long-term consequences of trace element status during pregnancy is warranted.

A-317

Iodine status among the school children in Eastern region of Nepal

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Introduction: Iodine deficiency (ID) is one of the leading causes of preventable mental retardation in the world. About 19.4% population of Nepal are iodine deficient. It affects to the developing brain of fetus and neonates in particular. The geographical situation and poor accessibility to adequately iodized salt are known to be the major causes of iodine deficiency in Nepal as determined by Urinary iodine concentration (UIC) reflecting current iodine intake and dietary salt iodine content determining the level of iodine consumption at the household level.

Objective: To assess the iodine status of the school children in Eastern region of Nepal by estimating UIC and salt iodine content (SIC).

Materials and methods: A cross sectional study was conducted in fourteen eastern districts of Eastern region of Nepal; which represented eight districts from hilly region and six districts from plain region from January 2008 to January 2013. A total of 5099 primary school children (6-13 years) were enrolled in the study and salt (n=4946) and urine samples (n=5010) were collected for the assessment of iodine status. Spot urine samples (5-10 ml) were collected in tightly screwed plastic vials, and transferred to the laboratory, maintaining a cold chain in an ice cool bag, and was stored in refrigerator at 2-8ºC until analysis. UIC was estimated by ammonium-persulphate digestion microplate (APDM) method. Urinary iodine controls L1, L2 (Seronormtm, Billingstad, Norway) were analyzed to obtain intra-assay CVs (L1=7.4%, L2=3.3%) and inter assay CVs (L1=23.5%, L2=11.26%), respectively. External quality control was done by participating in Ensuring the Quality of Urinary Iodine Procedures (EQUIP) program by Center for Disease Control and Prevention (CDC), Atlanta, USA.SIC was estimated by semi-quantitative rapid test kit using standard color chart.

Results: In our study, the proportion of male (51%) and female school children (49%) was nearly equal. The median Interquartile range UIC of all the school children was 234.88 (145.28-356.50)µg/L indicating adequate iodine nutrition. However, 738 (14.7%) children were still suffering from some degree of iodine deficiency. Median UIC was significantly higher in school children from plains (p=0.045), male sex (p=0.001), those who consumed packet salt (p=0.001) and salt with >15 ppm iodine (p=0.001).

Our study showed that 715 (14.5%) of school consumed open salt whereas 4233 (85.5%) consumed packet salt. The proportion of children consuming open salt was significantly higher in hill as compared to plains (p=0.001). Majority 4224 (85.4%) of the school children consumed adequately iodized salt (>15 ppm) but 642 (13.0%) consumed inadequately iodized salt (<15 ppm) and yet 80 (1.6%) children consumed salt with no iodine (0 ppm). The proportion of children consuming inadequate iodized salt (<15 ppm) is significantly more in hill (p=0.001). Approximately 65% of open salt were inadequately iodized with 7.8% having no iodine at all but only 0.6% of packet salts were inadequately iodized (p=0.001).

Conclusion: Iodine status of the school children is improving but continuous monitoring of the population is necessary for the sustainable elimination of iodine deficiency from the country and prevention of iodine induced diseases.

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Clinical utility of serum folate measurement in tertiary care patients Argument for revising reference range for serum folate from 3.0ng/mL to 13ng/mL

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Clinical utility of Serum folate:

Importance: The reference range for serum folate levels needs to be established and clinical utility of folate measurement assessed.

Objective: Asses the need for folate testing and determine reference level for serum folate.

Design: Serum folate levels in 5313 samples from 4448 patients, at two tertiary care medical centers were examined. Clinical data reviewed for patient characteristics in general and for evidence of corrective action in patients with serum folate values <5.5ng/mL. Clinical parameters in 128 patients with serum folate levels <5.5ng/mL and 128 patients with levels >25.7ng/mL at one of the medical centers were analyzed.

Setting: Medical School affiliated, tertiary care, safety net hospitals in Georgia and Missouri.

Participants: All samples and patients tested in July 2013 through June 2014 were included.

Results: The prevalence of serum folate levels, in patients, <3.0, <4.0, <5.5 and <13ng/mL was 0.58, 1.55, 4.9, and 43.21% respectively. Patients with serum folate levels <5.5ng/mL had lower serum albumin and hemoglobin as compared to those with serum folate >25.7ng/mL. In 82 of the 128 (64%) patients with serum folate >25.7ng/mL the sample was collected after supplementation with folic acid or multivitamins. Of the 20 patients with serum folate <3.4ng/mL there was evidence of

supplementation in half. Of the 128 patients with serum <5.5ng/mL documentation of supplementation was present in 28.9%.

Conclusions: Serum folate levels are below the current "normal" level of 3.0ng/mL in a larger proportion of patients seen at tertiary care hospitals than that reported for ambulatory patients. In patients with subnormal serum folate levels, corrective action by supplementation is lacking in at least half of the patients. Since serum folate levels ≥13.0ng/mL are needed for optimal prevention of neural tube defects in the embryo/fetus and we propose that normal serum folate level should be designated to be ≥13.0ng/mL.

Table

	Georgia		Missouri		Total	
	Samples	Patients	Samples	Patients	Samples	Patients
Serum folate	2696	2075	2617	2373	5313	4448
<3.0ng/mL	15	14	12	12	0.51%	0.58%
<3.4ng/mL	21	20	18	18	0.73%	0.85%
<4.0ng/mL	41	36	34	33	1.41%	1.55%
<5.5ng/mL	153	128	100	90	4.76%	4.90%
<7.0ng/mL	304	260	202	184	9.52%	9.98%
<13ng/mL	1202	1014	967	908	40.82%	43.21%

A-319

Antioxidant Status of Subjects with Metabolic Syndrome in Port Harcourt, Nigeria

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Abstract

Background

Increased free radical production and thus oxidative stress have been implicated in the pathogenesis of metabolic syndrome. These cause depletion of defences against free radical damage which comprise antioxidant enzymes and vitamins like vitamins C and E, resulting in low plasma levels. This may aggravate impaired insulin action and endothelial dysfunction and predispose to diabetes and cardiovascular disease.

Aim/Objective

This study was designed to determine if the plasma total antioxidant status and vitamins C and E levels are lower in metabolic syndrome subjects living in Port Harcourt compared to healthy controls.

Subjects and Methods

Blood pressure, waist circumference, concentrations of plasma glucose (mmol/L), lipid profile (mmol/L), total antioxidant status (TAS, mmol/L), vitamin C (µmol/L) and vitamin E (µmol/L) were determined in 100 subjects who fulfilled the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III criteria for metabolic syndrome and 100 age- and sex-matched controls.

Statistical Analysis Used: Statistical Package for Social Sciences (SPSS) version 11.0

Results

The mean plasma TAS (1.23 ± 0.28mmol/L), vitamin C (27.5 ± 7.4µmol/L) and vitamin E (16.9 ± 4.9µmol/L) of metabolic syndrome subjects were significantly lower (P=0.0001 for all) than that of controls (1.58 ± 0.28mmol/L, 44.3 ± 7.3µmol/L, 30.8 ± 6.1µmol/L respectively).

Conclusion

The reduced TAS, vitamins C and E in metabolic syndrome subjects compared to controls may be due to increased oxidative stress resulting from an imbalance between antioxidant defences and increased free radical production. Increased intake of adequate dietary antioxidants and supplementation could be beneficial in preventing or delaying the consequences of metabolic syndrome.

Key Words: metabolic syndrome, total antioxidant status, vitamin E, Vitamin C.

Table 1: Antioxidant Levels of Subjects and Controls

Antioxidant	Subjects(N=100) Mean ± SD	Controls(N=100) Mean ± SD	P-value
Vitamin C (µmol/L)	27.5 ± 7.36	44.3 ± 7.30	0.0001*
Vitamin E (µmol/L)	16.9 ± 4.86	30.8 ± 6.12	0.0001*
Total Antioxidant Status (mmol/L)	1.2 ± 0.28	1.6 ± 0.28	0.0001*

* Statistically significant (P<0.05)

A-320

Vitamin D status in Korean children and adolescents: continuously increasing prevalence of vitamin D deficiency with advancing age

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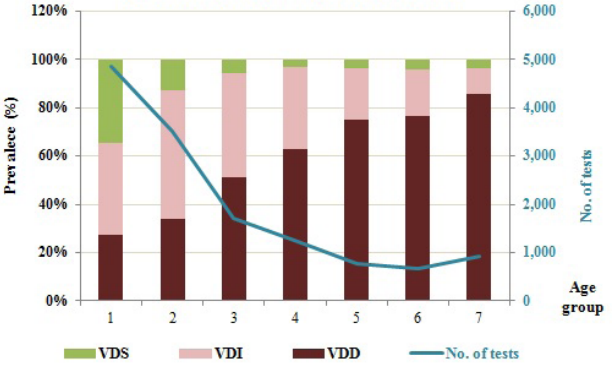
Background: The objective of this study was to assess the prevalence of vitamin D deficiency (VDD) and vitamin D insufficiency (VDI) across the age range of 0 to 20 in Korean children and adolescents using recent nationwide data.

Methods: The authors retrospectively analyzed the 25-hydroxyvitamin D (25(OH)D) results of 13,728 children and adolescents (6,755 boys, 6,973 girls) aged from 0 to 20 years. The tests were requested to Seoul Medical Science Institute from 332 medical institutions nationwide in South Korea between January 2014 and December 2014. Serum 25(OH)D was measured by chemiluminescence immunoassay (LIAISON system, DiaSorin, Italy). Participants were divided into 7 age groups (1, age <3 years; 2, 3-5 years; 3, 6-8 years; 4, 9-11 years; 5, 12-14 years; 6, 15-17 years; 7, 18-20 years). Prevalence of VDD and VDI according to gender and age groups were analyzed. VDD and VDI were defined by serum 25(OH)D <20 ng/mL and 20.0-29.9 ng/mL respectively.

Results: When the concentrations of 25(OH)D were categorized according to the above criteria, overall 44.3% and 38.5% of subjects were defined as VDD and VDI respectively. The mean concentration of 25(OH)D in girls was significantly lower than that of boys (20.9 ng/mL vs 22.7 ng/mL, p <0.0001). Concentrations of 25(OH)D were negatively correlated with age groups (r=-0.4185, p<0.0001). The prevalence of VDD according to age group was continuously increased with advancing age groups, reaching the highest VDD rate (85.8%,799/931) in the age group 7.

Conclusion: Our result shows that the prevalence of VDD is continuously increasing with advancing age in South Korean children and adolescents. In spite of very high prevalence of VDD in older children, 25(OH)D assays are requested much less than younger children. Therefore, more attention regarding vitamin D status is needed, and efforts for improving vitamin D status are required especially for older children and adolescents in South Korea.

Fig. 1. Prevalence of 25-hydroxyvitamin D (25(OH)D) deficiency and insufficiency in different age groups



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Ramadan Fasting Reduces Serum Fetuin A And High Sensitive C Reactive Protein Levels

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Fasting during Ramadan is a religious act practiced by many Muslims worldwide. The aim of this study was to evaluate the effects of Ramadan fasting on body composition, Fetuin A, high sensitive C- reactive protein and tumor necrosis factor alpha in some Egyptian volunteers. Subjects and methods: This study was conducted on 40 Egyptian volunteers (20 females and 20 males) divided into 2 age groups: below or equal to 35 years and above 35 years. Data was collected twice: one day before Ramadan and on the 28th day of Ramadan fasting. The following parameters were measured :a) body composition was determined by bioelectric impedance using

InBody-220; the fat mass, skeletal muscle mass, total body water and minerals. b) Body Mass Index (BMI). c) The waist circumference. d) blood samples were taken for determination of; serum Fetuin A by (ELISA technique), serum TNF-α by (ELISA technique) and High sensitive C reactive protein by (ELISA technique) Results: After Ramadan fasting, there was a significant decrease in the body weight of males > 35 years, the skeletal muscle mass of females ≤ 35 years and the body mass index of females >35 years. Furthermore, in most of the studied groups, there was a significant decrease in the level of fetuin A and high sensitive C reactive protein. However, no significant changes occurred in the level of tumor necrosis factor alpha after Ramadan in any group. Conclusion: Ramadan fasting has a potential anti-inflammatory effect manifested by lowering the levels of both fetuin A and Hs-CRP, irrespective to body fat percentage which did not significantly change in any of the studied groups.

Correlations between the different studied parameters before and after Ramadan fasting:			
	Before Ramadan	After Ramadan	Mean difference
	r p	r p	r p
Fetuin A versus Hs-CRP	0.718* <0.001	0.395* 0.012	0.535* <0.001
Fetuin A versus TNF-α	0.196 0.225	0.080 0.625	0.048 0.770
TNF-α versus Hs-CRP	-0.043 0.793	-0.119 0.466	0.213 0.187

A-322

Simultaneous Determination of Retinol and α-Tocopherol in Serum by Ultra Performance Liquid Chromatography

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Clinical interest in the evaluation of vitamin A and vitamin E nutriture has increased in recent years, mainly owing to the possible roles of retinol β-carotene, and α-tocopherol in decreasing the risk of cardiovascular disease and cancer.

The aim of this study was validate a simple, rapid, sensitive and cheap method for the simultaneous determination of retinol and α-tocopherol in serum using a Ultra Performance Liquid Chromatography with UV-VIS detection by comparison with a reference method.

The reference method was developed by ChromSystems®. The method was previously validated in the laboratory to confirm that the analytical procedure employed was suitable for the intended use.

The extraction procedure is based on protein precipitation and liquid-liquid extraction, in which 100 µL of serum were mixed with 450 µL of a solution of ethanol/n-butanol(50:50, v/v) (precipitant solution) and submitted a vortex for 30 seconds, followed by centrifugation at 14.000 rpm for 10 minutes. 400 µL of the clear supernatants were transferred to vials and 10 µL were injected into the UPLC equipment.

Retinol and α-tocopherol were separated by isocratic mobile phase containing a mixture of methanol:water (95:5 v/v). The flow rate was 400µL/min, using an Acquity UPLC BEH C18 1.7µm 2.1x50 mm column kept under thermostatization at 30 °C. The eluate absorbance was monitored at 325 nm until 0.9 min then turn to 295 nm until the end of the chromatographic running to quantify Retinol and α-tocopherol, respectively. The chromatographic running time was approximately 2.8 minutes.

A comparison test was carried out with 87samples, two levels of commercial controls and a commercial calibrator standard extracted and analyzed usint the two methods in order to verify the performance of the new test.

The correlation coefficient was 0.990 and 0,976 for retinol and α-tocopherol, respectively. The regression line (Passing-Bablok) showed In house = 1.14ChromSystem-0.0157 for retinol and In house = 1.21 ChromSystem-2.0628 for α-tocopherol. The recovery was calculated taking as the true value the value found in the reference method.We obtained an average recovery of 110.6% and 101.69% and the coefficient of variation was 7.1% and 4.0% for to retinol and α-tocopherol, respectively. The samples tested by both methods, showed an absolute agreement of 93.3% for retinol and 97.8% for α-tocopherol to clinical classification of patients.

With the data obtained and the statistical analysis, we can conclude that the methods tested are equivalent and produce concordant results.

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Redox Modulatory Factors In Human Breast Milk

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Background:

In this study, we aimed to investigate the difference in total oxidant status (TOS), total antioxidant status (TAS) and nitric oxide (NO) concentrations in human breast milk, according to the gestational age (preterm, late preterm, term) and lactation days after delivery (3rd, 7th, and 28th day)

Methods:

26 mothers were included in this study (9 preterm, 8 late preterm and 9 term). Breast milk was collected from participants on 3rd, 7th and 28th days. Milk samples were stored at -80℃. NO analysis was performed by a spectrophotometric method using the Griess Reagent.TOS and TAS analysis were performed by a spectrophotometric method using commercial kits provided by the Rel Assay Company.

Results:

There was a significant difference between the TAS, TOS and NO levels of the groups of lactation days (p=0.480, 0.290, 0.426 respectively), without considering the gestational age. According the posthoc Tukey comparisons of NO levels between 3rd and 7th days, 3rd and 28th days; significant difference was seen (p values <0.001 and 0.028 respectively). There was also a significant difference between 3rd and 28th, 7th and 28th days considering the TAS results (p=0.007, 0.012 respectively). And TOS result revealed a significant difference between 3rd and 7th days (P=0.022). TAS, TOS and NO levels of milk samples of the three groups, according to the gestational age, showed no significant difference. TAS and NO levels were found to be decreased during lactation.

Conclusion:

This study showed that the redox modulatory factors of human breast milk changed during lactation. However, gestational age on delivery did not affect the human breast milk with this aspect.

Table 1: TAS, TOS and NO levels in human breast milk samples on 3rd, 7th and 28th days of lactation

	3rd day(Mean ± SD)	7th day(Mean ± SD)	28th day(Mean ± SD)
TAS (mmol Trolox Equiv./L)	0.944 ± 0.254	0.928 ± 0.272	0.810 ± 0.221
TOS (µmol H2O2 Equiv./L)	0.673 ± 0.588	1.196 ± 1.121	1.096 ± 0.744
NO (mM)	0.382 ± 0.098	0.322 ± 0.092	0.282 ± 0.089

A-324

Performance and Certification of the ADVIA Centaur Vitamin D Total Assay

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Background: Vitamin D helps regulate calcium in the development and maintenance of healthy bones. The National Institutes of Health Office of Dietary Supplements created the Vitamin D Standardization Program (VDSP) to establish a standard for accurate and comparable results for the detection of 25(OH)D across laboratories. The Centers for Disease Control (CDC) provides assay manufacturers and laboratories with the Vitamin D Standardization-Certification Program (VDSCP) to help assess assay calibration.

Methods: Between January and December 2013, the Centers for Disease Controls (CDC) provided 40 blinded 25(OH)D samples in the Vitamin D Standardization-Certification Program (VDSCP), in which a set of 10 samples was evaluated each quarter against the CDC and University of Ghent Vitamin D_2 and D_3 Reference Measurement Procedure (RMP). Samples were tested blindly in replicates of four over 2 days, two replicates per day. Additional supplemental samples were also evaluated, including the four standards from the National Institute of Standards and Technology (NIST).

Results: The ADVIA Centaur® Vitamin D Total assay met the criteria for VDSCP certification. The mean bias to the reference method was 0.3%, within the acceptable bias of ±5.0%. The assay's imprecision of 5.5% was also within the acceptable range

of ≤10.0%. A linear regression of the blinded samples demonstrates a slope of 1.01 and an intercept of -1.89nmol/L. The ADVIA Centaur Vitamin D Total assay also shows an acceptable bias with the NIST Standard Reference Material (SRM) 972a vitamin D metabolite samples.

Conclusion: The VDSCP certification for the ADVIA Centaur Vitamin D Total assay establishes an acceptable alignment to a harmonized testing standard for 25(OH)D. The ADVIA Centaur Vitamin D Total assay provides laboratories with a standardized and automated means for quickly and efficiently testing patients' 25(OH)D levels.

A-325

The impact plastic tube EDTA K2 metal free in tha analysis of trace metals

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Introduction:

The trace metal determinations are performed either to evaluate the deficiency or excess of these elements in the body. Thus, pre-analytical losses and elevated values due to contamination should be evaluated. Contamination sources include: clothing and skin of the patient; collection materials such as needles, anticoagulants, covers, gel separator and tubes; material was particulated in the laboratory. The loss may be attributed to the surface adsorption of metals from the sample collection tubes or due to the presence of anticoagulants and a complex formation. There are on the market a limited number of free metal tube suppliers. The most common material was glass, but due to biosecurity issues its use was discontinued.

Purpose:

This study aimed to evaluate the performance of plastic tube metal-free with anticoagulant EDTA K2, in the determination of trace metals - Al, Cu, Zn and Se compared to glass tubes.

Materials and methods:

Samples from volunteer donors were collected in tubes BD Vacutainer® glass dry. The material was centrifuged and aliquoted into identical tube. The same procedure was carried out using BD Vacutainer ™ plastic tubes K2 EDTA. All tubes are metal-free.

The equipment used for the analysis of Al and Se was an atomic absorption spectrometer AAnalyst 800, Perkin Elmer with graphite oven with correction by Zeeman Effect. For Cu and Zn, we used the same equipment in the flame module. The samples were diluted only with Triton X® in 0.1% HNO3. Calibration curves were prepared from primary standard ICP Multi Element Standard Solution VI Certipur®.

Results

The data for all elements were evaluated for correlation of results using EP Evaluator software. The possible loss or contamination generated by the use of Tubes BD Vacutainer® EDTA K2, metal-free was investigated. The number of analysis was 53 for Al; 42 for Se; 36 for Cu and 36 for Zn.

The correlation coefficients were: Al = 0.9903; Se = 0.9551; Cu = 0.9772 and Zn = 0.8265. The slopes and intercepts were: Al 0.859 / 0.995; Se 0.897 / 9.58; Cu 1,082 / -9.5 and Zn 1.183 / -0.077. The bias was found -2.07 to Al; -0.52 to Se; -0.1 to Cu and 0.042 for Zn.

Discussion and Conclusion:

Among the metals analyzed, Al is what attracts more attention, by the contamination of sample facility. Factor noticed in a lesser extent in Zn. But the performance of the test tube was satisfactory in this regard. We did not observe losses by adsorption.

Found proper correlation in all metals, noting that the plastic tube with EDTA anticoagulant use K2 metal free proved to be compatible with the standard required by our Quality System.

A-326

Iodine Status and Thyroid Function in Weaning Infants of Eastern Region of Nepal

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Background: Iodine is one of the most important micronutrients required for normal growth of children. Insufficient iodine in children causes impaired mental function and delayed physical development.

Objectives: To assess the iodine status and thyroid function in children of age 6 to 24 months in five districts (Sunsari, Morang, Dhankuta, Terathum and Jhapa) of eastern Nepal.

Materials and Methods: This community based cross-sectional study was done in period of July 2012 to July 2013. Four hundred and eighty eight children were enrolled. Urinary Iodine Concentration (UIC), serum Thyroglobulin (Tg), free T_4 and TSH were estimated using standard commercial ELISA kits. Similarly, their household salt was analyzed for iodine content using Rapid Test Kits. Written consent was obtained from their guardians. Data were analyzed using SPSS version 20, p-value <0.05 was considered significant. Internal (Seronorm, Norway) and external quality control (CDC, Atlanta, USA) were applied for UIC estimation.

Results: The median UIC in children was 409.43 (312.65, 493.04)µg/L suggesting sufficient iodine nutrition (>100 µg/L) in those groups. Twenty three children (4.9%) had UIC insufficient (<100 µg/L). Eighty one (16.59%) have high Tg (>40ng/mL). Among those children, median UIC was 409.19 (310.70, 492.28)µg/L.The median TSH and mean free T_4 was 3.15(2.08, 4.50) mIU/L and 1.31±0.34 ng/dL, respectively. The median UIC (p=0.0001), median Tg (p=0.004) and mean free T_4 (p=0.001) were significantly different between districts. There were significant positive and negative correlation between TSH and Tg (r=0.138, p=0.002) and TSH and free T_4 (r=-0.125, p=0.006) respectively. Thyroid status of children was 90.4% euthyroid; 7.8% subclinical hypothyroidism; 1.0% subclinical hyperthyroidism; 0.6% hypothyroidism and 0.2% hyperthyroidism. In household of 107 children, 4 (3.7%) have salt iodine < 15 ppm.

Conclusion: There is high value of median UIC which might be causing high prevalence of increased level of Tg.

Key words: UIC, Thyroglobulin, iodine status

A-327

Can the folate microbiologic assay be harmonized across laboratories?

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Background: There is lack of agreement among methods to measure serum and whole blood folate. The fully automated protein binding assays performed on clinical analyzers offer high sample throughput and generally good precision, but they have questionable accuracy and may suffer from lot-to-lot variation. Chromatography-based methods have high specificity, sensitivity, and precision, but they are expensive and require complex sample preparation. The microbiologic assay is considered a gold standard method, because it fairly equally measures all biologically active forms of folate, needs only a small sample volume, and can be performed in a low-resource laboratory. However, the microbiologic assay has not yet been harmonized and use of different calibrators or microorganisms can lead to different results. We will investigate whether providing different laboratories the same "microbiologic assay kit" leads to comparable serum and whole blood folate results.

Methods: We plan to recruit laboratories that are proficient in carrying out the folate microbiologic assay and are interested in participating in a Round Robin study. We will provide them with a "microbiologic assay kit" consisting of calibrator, microorganism inoculum, and unknown serum and whole blood lysate samples to be analyzed over 2 days each, first using the laboratory's reagents, and then using the CDC kit. The laboratories will use their own protocol, instrumentation, consumables, and basic reagents throughout the Round Robin study. We will compare results among laboratories and assess whether the harmonized results using the CDC kit are more comparable than the non-harmonized results.

Results: We created sufficient amounts of 5-methyltetrahydrofolate calibrator, *Lactobacillus rhamnosus* microorganism inoculum, and 20 pools each of serum and whole blood hemolysate. The folate concentrations in these pools span the range

of results found in a population exposed to folic acid fortified foods: 9-90 nmol/L for serum folate and 170-600 nmol/L for whole blood folate. This will allow the laboratories to use higher sample dilutions compared to when samples are from a folate-deficient population, minimizing any potential of interferences or inhibition effects. We tested the homogeneity of these pools and found it to be adequate (≤10% variability for serum and whole blood folate). We invited 15 laboratories to participate in the Round Robin study. Eight laboratories have agreed to participate. The Round Robin study will be carried out in the spring of 2015 and results will be available by the summer.

Conclusions: The microbiologic assay is a reliable and practical method that could be used in the low-resource setting to assess the folate status of the population. We expect that comparable folate results can be generated as long as laboratories use the same calibrator and microorganism inoculum. If that is the case, a global network of resource laboratories could be established to facilitate the implementation of nutrition surveys that assess whether an optimal blood folate concentration is achieved in women of reproductive age for prevention of neural tube defects.

A-328

Associations between Vitamin D Status and Serum Cardiovascular Risk Markers in an Adult Brazilian Population.

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Background: Vitamin D is essential for bone mineralization, but a growing body of evidence points at a broader role; vitamin D deficiency has been found to be associated with mortality and several diseases ranging from cardiovascular disease to autoimmune diseases and liver diseases. Low serum 25-hydroxyvitamin D (25OHD) levels have been associated with increased prevalence of cardiovascular diseases. A possible relation between serum cardiovascular risk markers and 25OHD might explain this association. The objective of this study was to evaluate the association between the serum 25OHD levels and cardiovascular risk markers in a population from Rio de Janeiro State in Brazil. **Methods:** This was a cross-sectional study in a sample of patients who performed the dosage of 25OHD in a private reference laboratory in Brazil over a period of one year (January to December 2014). Data on laboratory tests and demographic variables were available from a database of the local Laboratory Information System. We analyzed samples of both genders aged ≥ 20 years who tested for 25OHD (Chemiluminescence, Abbott) and also for total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides (TG) (Enzymatic Colorimetric, Roche), apolipoprotein ApoA-1 and ApoB (Nephelometry, Siemens) and homocysteine (Chemiluminescence, Abbott). Vitamin D deficiency was defined as serum concentration below 20.0 ng/mL, values between 20.0 and 30.0 ng/mL were considered insufficiency and those above 30.0 up to 100.0 ng/mL corresponded to the optimal levels. **Results:** Laboratory tests were equally distributed throughout the different seasons of the year (332,565 samples). 77 percent of the studied population was female and the mean age was 53 ± 18 years. Serum 25OHD ranged between 3.2 and 99.8 ng/mL, with a mean of 29.4 ± 10.4 ng/mL. Vitamin D deficiency, insufficiency and normal were observed in 16.6%, 42.0%, 41.4% in female versus 14.8%, 42.0% and 43.2% in male, respectively ($p<0.01$). There was also a difference in frequency of vitamin D deficiency/insufficiency in the second half of the year, corresponding to winter and spring months in south hemisphere (48.2% first half vs 66.7% in second half; $p<0.01$). In our data, multiple linear regression showed a correlation of 25OHD levels with lipids profile values ($p<0.01$) as well as for apolipoprotein A-1 levels ($p<0.01$). Results were not able to show correlation with apolipoprotein B and homocysteine. **Conclusions:** Our data support the literature where other tropical regions also have a high level of patients with deficiency / insufficiency of 25OHD, which was more frequent in winter and spring months. This study also supports an association between low 25OHD levels and lipids profile and ApoA-1, markers that contribute to cardiovascular diseases, suggesting that Vitamin D may be important in maintaining cardiovascular health.

A-329

Comparability of whole blood folate results from the microbiologic assay with a high-throughput LC-MS/MS method: a pilot study

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Background: The microbiologic assay (MA) responds nearly equally to biologically active folate forms and is therefore regarded as a gold standard for the measurement of serum and whole blood folate. The advantage of LC-MS/MS is that it provides information on different folate forms and is less prone to interferences such as antibiotics. While we showed earlier good agreement (within ±10%) between the MA and LC-MS/MS for serum folate, we observed larger differences (~25%) for whole blood folate, with the LC-MS/MS method measuring on average lower. We were interested in an up-to-date comparison of whole blood folate data between these two assays using our newest high-throughput LC-MS/MS method.

Methods: We used conventionally prepared hemolysates (whole blood diluted 1:11 with 1% ascorbic acid; $n = 289$) to measure total folate by MA ($tFOL_{MA}$) and five folate forms (5-methyltetrahydrofolate [5-methylTHF], folic acid, THF, 5-formylTHF, and 5, 10-methenylTHF) and one oxidation product of 5-methylTHF (MeFox; pyrizino-s-triazine derivative of 4α-hydroxy-5-methylTHF) by LC-MS/MS. The sample vials were shared and processed by each assay at the same time. For the LC-MS/MS method, we added $^{13}C_5$-labeled folate analogs to serve as internal standards and subjected the hemolysates to 4-h incubation at 37ºC to deconjugate polyglutamates to monoglutamates. For the MA, we processed thehemolysates directly without further incubation. We calculated $tFOL_{LC\text{-}MS/MS}$ as the sum of folate forms measured by LC-MS/MS without including the biologically inactive MeFox form, because the MA does not respond to MeFox. We evaluated the comparability of the two methods by assessing weighted Deming regression and relative Bland-Altman bias to account for an increase in variance with increasing concentration.

Results: We found excellent correlation ($R^2 = 0.95$) and goodcorrespondence between $tFOL_{LC\text{-}MS/MS}$ (mean [SD]: 432 [158] nmol/L) and $tFOL_{MA}$ (459 [178] nmol/L). While both the Deming slope (estimate [95% CI]: 0.89 [0.85 to 0.92]) and intercept (24.6 [10.3 to 38.9] nmol/L) were significant, they almost cancelled each other out, leaving a small but significant negative relative bias (-5.5 [-6.7 to 4.2] %). Most of the $tFOL_{LC\text{-}MS/MS}$ was composed of 5-methylTHF (mean [SD]: 404 [161] nmol/L), with the other folate vitamers contributing on average only 7%. The mean [SD] MeFox concentration was 66.7 [25.3] nmol/L, which corresponded to 17% of the 5-methylTHF concentration. This was a larger proportion of MeFox than what we typically observed in serum samples (~5%) and may be a result of 5-methylTHF oxidation during the 4-h incubation at 37ºC. The strong correlation (Spearman $r = 0.94$) between MeFox and 5-methylTHF in these whole blood samples, compared to a weak correlation (Spearman $r = 0.25$) observed earlier in serum samples, seems to support this hypothesis.

Conclusions: In this pilot study, we found good agreement for whole blood tFOL between the two test methods, with our new high-throughput LC-MS/MS method measuring on average only ~5% lower than the MA. However, based on the higher than expected proportion of MeFox, we need to explore whether we can shorten the incubation period and whether that would better maintain 5-methylTHF and other folate vitamers.

A-330

Determination of B-Carotene in Serum by Ultra Performance Liquid Chromatography

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Clinical interest in evaluation of vitamin A and vitamin E nutriture has increased in recent years, mainly owing to thepossible roles of retinol (vitamin A), β-carotene, and α-tocopherol (vitamin E) in decreasing the risk of cardiovascular disease and cancer.

The aim of this study was to validate by comparison a simple, rapid, sensitive and cheap method for the determination of β-carotene in serum by Ultra Performance Liquid Chromatography with UV-VIS detection. The instrument used was a chromatograph Acquity UPLC Waters® with a sample manager FTN and TUV detector.

The reference method was developed by ChromSystems® and contains commercial reagents kit for the HPLC analysis of β-carotene in serum/plasma. The method was previously validated in the laboratory to confirm that the analytical procedure employed is suitable for the intended use.

In clinical measurement comparison of a new measurement technique with an established one is often needed to see whether they agree sufficiently for the new to replace the old.

The extraction procedure is based on protein precipitation and liquid-liquid extraction, in which 100 µL of serum were mixed with 450 µL of a solution of ethanol/n-butanol (50:50, v/v) (precipitant solution). Then vortex mixed for 30 seconds, followed by centrifugation at 14.000 rpm for 10 minutes. 400 µL of the clear supernatants were transferred to vials. In UPLC equipment are injected 10 µL.

B-carotene was separated by isocratic elution with a mixture of acetonitrile:ethanol (90:10 v/v) as mobile phase. The flow rate was set to 400 µL/min, using an Acquity UPLC BEH C18 1.7µm 2.1x50 mm column kept under thermostatization at 30

°C. The eluate absorbance was monitored at 453 nm to quantify β-carotene. The chromatographic running time is approximately 3.0 minutes.

There was a comparison test on 60 samples, two levels of commercial control and a commercial standard extracted and analyzed with the two methods in order to verify the performance between the method developed by the laboratory method used.

It was made a direct comparative analysis in which a method is confronted with each other. The correlation coefficient was 0.9960. The regression line (Passing-Bablok) shows In house = 0.9157 ChromSystem- 11.567. The recovery was calculated taking as the true value the value found in the reference method. There was obtained an average recovery of 95.8%, with acceptable values for each sample are 80 to 110%. The mean coefficient of variation was 6.2%. Of the 60 samples and 2 commercial control tested by both methods obtained an absolute agreement of 93.6% in clinical classification of patients.

With the data obtained and the statistical analysis, we can conclude that the tested methods are equivalent and produce results with the same agreement, without causing any difference in clinical interpretation.The performance after the migration to the new methodology was significantly better, and observed the reduction of time analyses, cost, reduction troubles with the instruments and also higher rate of approval of controls.

A-331

Hair trace elements concentrations in obese females and their relation to type 2 diabetes in Saudi Arabia

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Backgrounds: Trace elements excess or deficiency could induce metabolic disorders and cellular growth disturbance; their regulatory, immunologic and antioxidant functions resulting from their action as essential components or cofactors throughout metabolism. Obesity is a worldwide disease affecting population of all age groups. In Saudi Arabia, the population is going through a nutrition transition where customary and traditional food are being replaced by fast food high in fat, sugar, salt as well as changes in lifestyle and reduced physical activity leading to increase the overall obesity prevalence above 35% and type 2 diabetes mellitus (DM) which is projected to rise from 890,000 in 2000 to 2,523,000 in 2030.

Objective: This study aimed to explore the correlations of hair trace elements as long-term biomarkers with obesity and type 2 DM in adult female Saudi patients.

Patients and Methods: This study included 169 women grouped as 64 diabetic obese (BMI > 30 kg/m2), 45 non-diabetic obese and 60 healthy non-obese (BMI 18-25). All subjects were randomly selected among the volunteers of matched age and similar socio-economic status. Renal or liver diseases patients, smokers and individuals who were taking trace elements supplements for the past three months, were excluded from the study. Full history, clinical data and anthropometric measurements were taken for all subjects. Hair samples collected from the nape section and hair trace elements Se, Zn, Cu, Mn and Fe concentrations were analysed by ICP-MS (Perkin- Elmer 7300, USA). Serum levels of total cholesterol, triglyceride, high and low density lipoprotein cholesterol (HDL and LDL) were analysed. DM confirmed by both fasting blood glucose (FBS) and glycated haemoglobin (HbA1c) levels. Odd Ratio of hair trace elements concentrations were adjusted for family history.

Results: FBS, HbA1c, cholesterol, LDL and triglycerides levels of the diabetic obese females were significantly higher than non-diabetic obese and healthy women ($P<0.005$). Although serum HDL levels had no statistical difference between diabetic and non-diabetic obese women, they were significantly lower than healthy group ($P<0.05$). Hair Cu concentrations of the diabetic and non-diabetic obese women were significantly higher than healthy group ($P<0.05$). On the other hand, hair Zn, Mn and Fe concentrations were significantly lower than those of the healthy women ($P<0.05$) as well as hair Zn concentrations was negatively correlated with FBS and HbA1c levels in the diabetic obese women ($P<0.05$). Hair Cu/Zn ratio was significantly higher in the diabetic obese than healthy women ($P<0.05$). Hair Se concentrations had no statistical differences among studied groups. However, they were positively correlated with FBS and HbA1c levels in the diabetic obese women.

Conclusion: Impaired trace-element metabolism may have a role in the pathogenesis and progression of both obesity and type-2 DM. Obesity is a clinically manifested metabolic disorder, including mineral imbalances, our findings showed significant association between obesity and Zn and Fe deficiencies. Hair trace elements can be a useful diagnostic tool as long-term biomarkers for metabolic disorders; however, larger prospective studies are warranted to validate their diagnostic value in obesity and type 2 DM.

Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Animal Clinical Chemistry

A-332

The Inhibitory Effect of DENND1B Overexpression on the Release of Cytokine IL-4 in a Murine Ovalbumin-induced Asthma Model

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Background: The role of asthma-associated gene DENND1B has not been defined in the pathogenesis of asthma. This study aimed at evaluating the effects of DENND1B overexpression in vivo on the release of inflammatory cytokines in a murine model of asthma induced by ovalbumin (OVA).

Methods: Female C57/BL6 mice at 5-6 weeks of age were sensitized on days 0 and 7 and 14 by intraperitoneal injection of 20 mg OVA added in 2 mg of aluminum hydroxide. On days 21 and 22 and 23, mice were exposed to aerosolized OVA once a day for 3 days. Lentivirus containing DENND1B coding sequence vector (OVA + DENND1B vector) or empty vector (OVA + Empty vector) was intravenously injected on day 11. The mice were sacrificed and evaluated 24hrs after the last OVA challenge. Total Immunoglobulin E (IgE) in plasma and the levels of interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) in bronchoalveolar lavage fluid (BALF) were measured by ELISA kits. The number of leucocyte in BALF was counted on an automatic blood cell analyzer.

Results: Systemic sensitization with OVA significantly increased the plasma level of IgE, but the levels were not significantly changed in any of OVA-treated groups. The number of leucocyte significantly increased in the BALF of OVA and OVA +Empty vector groups. By contrast, the number of leucocyte in the BALF significantly decreased in OVA+DENND1B vector group. The levels of IL-4, IL-5, IL-6 and TNF-α in BALF were significantly increased in three OVA-treated groups. However, the level of IL-4 in BALF was significantly lower in the OVA+DENND1B vector group than in OVA group mice.

Conclusions: The release of cytokines IL-4 was suppressed by the overexpression of DENND1B in the lung. The overexpression of DENND1B could play partial protective effect against inflammatory damage in the OVA-induced murine model.

Table. The level of IgE in plasma, leucocyte number and cytokines levels in BALF

Analytes	Normal Control	OVA	OVA + Empty vector	OVA + DENND1B vector
IgE in plasma（ng/ml）	244.01±53.44	1161.56±62.04	1048.67±84.74	1159.01±39.80
Leucocyte number in BALF ($\times 10^5$/ml)	1.67±0.54	48.70±15.23	38.52±14.78	24.81±9.23*
IL-4 in BALF (pg/ml)	51.89±18.61	215.22±72.75	160.67±20.71	91.13±42.73*
IL-5 in BALF (pg/ml)	91.59±22.98	184.72 ±23.48	179.56±15.20	143.63±27.57
IL-6 in BALF (pg/ml)	170.5±62.22	385.54±50.86	359.25±72.12	318.08±34.12
TNF-α in BALF (pg/ml)	231.42±88.27	449.50±119.67	473.47±84.51	409.71±99.10

* P<0.05 vs. OVA mice

A-333

Organ Function and Oxidative Stress Indices in Streptozotocin-induced Diabetic Rats Administered Aqueous or Ethanolic Extract of Uvaria Chamae Roots

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Background: *Uvaria chamae* is a medicinal plant that is used in some regions of the world in the treatment of many diseases including diabetes, cough and gastroenteritis. The chemical constituents include C-benzylated monoterpenes, aromatic oils, flavanones, C-benzylated flavanones, and C-benzylated dihydrochalcones. However, the scientific basis for the traditional use of this plant extracts in the management of diabetes is not well understood. In this study, we evaluated organ function and oxidative stress indices in the blood of normal and streptozotocin-induced diabetic rats administered aqueous or ethanolic extract of *Uvaria chamae* roots. **Method**: Thirty six (eighteen adult normal and eighteen streptozotocin-induced diabetic rats) Sprague Dawley rats were assigned by weight into six groups [6 rats per group, average body weight 265.23 ± 7.20 g]. The six groups were composed as follows: Healthy rats receiving de-ionized water (Normal Control); Normal rats receiving aqueous extract (Normal plus Aqueous Extract); Normal rats receiving ethanolic extract (Normal plus Ethanolic Extract); Diabetic rats receiving de-ionized water (Diabetic Control); Diabetic rats receiving aqueous extract (Diabetic plus Aqueous Extract); and Diabetic rats receiving ethanolic extract (Diabetic plus Ethanolic Extract). Diabetes was induced using a single injection of streptozotocin (Sigma-Aldrich, 60 mg/kg body weight in 0.05 M-citrate buffer, pH 4.5) intraperitoneally. Normal and diabetic rats were then administered the aqueous or ethanolic extract (300 mg/kg body weight/day) for 35 days. Animals were then euthanized by decapitation and blood was collected for assays. **Results**: We noted a significant ($p<0.05$) decrease in blood glucose levels in the diabetic groups treated with each extract compared to the diabetic control group. The levels of serum total cholesterol, triglycerides, LDL- cholesterol and VLDL- cholesterol were not significantly reduced by each extract. However, there was a significant ($p<0.05$) increase in HDL- cholesterol levels in the diabetic groups administered each extract compared to the diabetic control group. Serum uric acid, blood urea nitrogen and creatinine levels in the diabetic groups were not significantly altered by the treatments. We also noted non-significant changes in serum amino transferase activities, and total protein, albumin and globulin levels in the treated diabetic groups compared to diabetic control group. The consumption of each extract resulted in elevated serum total antioxidant capacity and superoxide dismutase (SOD) activity compared to the diabetic control group. The administration of each extract to normal rats resulted in elevated serum uric acid levels and decreased SOD activity. **Conclusion**: The observed reduced blood glucose and elevated HDL-cholesterol levels are indicative of the beneficial effects of each extract in the management of diabetes.

The administration of each extract may be protective against oxidative stress that is often associated with the development of diabetic complications as evidenced in the observed improved antioxidant defense system. However, the consumption of each extract in non-diabetic states should be done with caution because of the observed elevated serum uric acid levels and decreased SOD activity in the normal treated groups.

A-334

The role of ethanol and dietary fat in the disruption of intestinal barrier integrity and liver injury in an animal model of alcoholic liver disease

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Introduction/Aim: Alcoholic liver disease (ALD) ranks among major causes of morbidity and mortality in the United States and worldwide. ALD includes a spectrum of pathologies from simple steatosis to steatohepatitis characterized by inflammation with the potential progression to fibrosis and cirrhosis over time. Although, animal models of ALD do not recapitulate all components of human ALD, they are important tools in understanding the molecular mechanisms underlying alcohol-induced liver steatosis, inflammation and injury. Dietary fat and alcohol both play important roles in the pathogenesis of ALD. Diets enriched in saturated fatty acids protect against ALD, whereas linoleic acid (LA), a major unsaturated fatty acid in the American diet, is known to exacerbate alcohol-induced liver injury. However, the underlying molecular mechanism(s) are not completely understood. It is well documented that ethanol-induced endotoxemia due to the disruption of intestinal barrier integrity plays an important role in the ALD development. The aim of the present study was to examine the effects of different types of dietary fat on intestinal barrier integrity and consequent liver injury in an animal model of ALD.

Materials and Methods: In this study we employed a Lieber-DeCarli ad libitum EtOH feeding model, a widely accepted animal model of ALD. C57BL/6N mice were fed either an unsaturated fat (USF, LA enriched) or a saturated fat (SF, medium triglycerides enriched [MCT]) control or EtOH-containing diets for 8 weeks. Control mice were pair-fed on an isocaloric basis. Initially, all mice were given the control liquid maltose dextrin diets (SF or USF, no EtOH) for one week. Ethanol was gradually increased every 3-4 days from 11.2% to 35% of total calories. Liver injury and steatosis; intestinal morphology and inflammation; intestinal permeability and blood endotoxin levels were evaluated.

Results: After 8 weeks of EtOH feeding significant liver injury and steatosis were observed in USF+EtOH compared to pair-fed group. These effects of EtOH were blunted by SF diet containing MCT. Serum ALT levels, as a marker of liver injury, was significantly higher ($p<0.05$) in USF+EtOH group (44.91+2.81 IU/L) compared to SF+EtOH fed animals (27.27+1.92 IU/L). Hepatic triglyceride content was also higher in USF+EtOH compared to SF+EtOH fed animals (100.2+8.1 vs 68.76+7.96

mg/g liver, p<0.05). The effects of ethanol on intestinal integrity associated with different types of fat were evaluated by measuring the intestinal permeability to FD-4. Significantly increased gut permeability was observed in the ileum segments of the mice fed USF+EtOH, this event was accompanied by a 4.5-fold (p<0.05) increase of blood LPS levels compared to control animals. A moderate, but not significant, increase of LPS levels was found in SFD+EtOH compared to pair-fed group. Significantly elevated intestinal TNF-α and MCP1 mRNA levels as a markers of inflammation were detected only in USF+EtOH group.

Conclusion: The current data demonstrated that EtOH and unsaturated, but not saturated, fat promote liver injury associated with increased gut permeability. Intestinal inflammation caused by USF+EtOH likely play a critical role in the gut leakiness, elevated blood endotoxemia and consequent liver injury in an animal model of ALD.

A-335

Effects of hypoxia time on the extracellular matrix accumulation and development of renal fibrosis in the adriamycin-treated rats

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Background: Tubulointerstitial fibrosis (TIF) is characterized by the accumulation of interstitial fibroblasts and the deposition of extracellular matrix (ECM), which lead to end-stage renal failure. Its presence correlates with impaired excretory function and the degree of fibrosis is a strong pathologic marker of progression. The histopathology of TIF indicates the deposition of ECM in association with inflammatory cells, tubular cell loss, and fibroblast accumulation. Recent experimental evidence suggests that hypoxia and prolonged activation of hypoxia-inducible factor (HIF) are associated with tubulointerstitial injury, which leads to fibrosis and further tissue damage. In this study, the effects of hypoxia time on the ECM accumulation and the development of fibrosis are investigated in experimental nephropathy model.

Methods: Adriamycine-injected nineteen male Wistar Hannover rats were assigned into three groups as sham operation, 15 min ischemia-reperfusion (IR), and 45 min IR (n: 6, 7, and 6, respectively) in the left kidney. Tissue sections were stained with Masson's Trichrome stain, and immunohistochemical (IHC) staining was performed for fibronectin, integrin, laminin, and TGF-β1, and the levels of these parameters were also measured by ELISA in serum samples. Additionally, connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), α-SMA, and HIF-1α tissue expressions were examined through IHC method.

Results: When compared to the sham group (Masson's Tricrom), there were seen periglomerular fibrosis, segmenthal sclerosis and thin interstitial fibrosis in the 15 min IR group. Also, when compared to 15 min IR group; a marked fibrosis in glomerules and interstitium in the 45 min IR group was seen. Dense staining fibronectin, integrin, laminin, TGF-β1, and α-SMA were seen in the evaluation of the IHC staining. Moreover, the serum levels of fibronectin, integrin, laminin, TGF-β1 and VEGF were found to be higher both in the 15 min IR and in the 45 min group than the sham group.

Conclusion: The current study shows that hypoxia and increased renal HIF-1α expression are associated with tubulointerstitial injury, which lead to further tissue damage and fibrosis. The levels of fibronectin, integrin, laminin and TGF-β1 can be used as biomarkers in the diagnosis of renal fibrosis. Hence, understanding the changes in ECM-associated proteins and biomarkers in the progressive renal injury will facilitate in the design of novel strategies to treat chronic kidney disease, such as hypoxia-induced TIF and glomerulosclerosis.

A-336

LOW AND HIGH MOLECULAR WEIGHT HYALURONAN IMPACT AIRWAY HYPERRESPONSIVENESS IN A MURINE MODEL OF ASTHMA DURING INTRAVENOUS METHACHOLINE CHALLENGE

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BACKGROUND Asthma is a chronic lung disease characterized by inflammation, airway remodeling, and airway hyperresponsiveness (AHR). Hyaluronan (HA), a non-sulfated glycosaminoglycan, is present in the pulmonary secretions of asthmatic patients, as well as in murine models of asthma. In normal lung tissue, HA exists primarily in high molecular weight (HMW) form. Abnormal extracellular matrix (ECM) turnover is a hallmark of tissue injury and inflammation in asthma, with accumulation of low molecular weight (LMW) HA fragments. However, the precise role of HA size in asthma is still largely unknown. To investigate the impact of HA size on a murine model of asthma, mice with OVA antigen-induced asthma were treated with either LMW or HMW HA and development of inflammation and airway hyperresponsiveness was determined.

METHODS BALB/c wild-type mice (n= 6-9/group) were treated intratracheally (IT) with 100-µg doses each of LMW HA (~35 kDa) or HMW HA (~2MDa) in 50-ul saline several hours prior to OVA aerosol challenge (~45 min) for 6 days. Control mice were given 50-ul saline only. Mice underwent aerosol or tail vein intravenous (IV) of methacholine (Mch) challenge to obtain AHR parameters based on the Forced Oscillation Technique (FOT). Inflammation was characterized by inflammatory cells in bronchoalveolar lavage (BAL) and lung histology.

RESULTS BAL cell counts and differentials demonstrated no significant impact of either LMW or HMW HA treatment on asthmatic mice compared to asthmatic control mice. Lung histology confirmed BAL results. Aerosol Mch challenge demonstrated no impact of HMW HA on AHR in asthmatic mice, while LMW HA treated mice had minimally elevated AHR. By contrast, AHR was increased dramatically in LMW and HMW HA treated asthmatic mice during IV Mch challenge compared to asthmatic controls.

CONCLUSIONS These data suggest that neither LMW or HMW HA, delivered IT, impacts the development of inflammation in a murine model of asthma.

Additionally, our results indicate that the impact of HA size on AHR is dependent on Mch delivery method. HMW HA treatment did not alter AHR response during aerosol challenge compared to asthmatic controls, while an aggravated AHR response was observed during IV MCh challenge. LMW HA treated mice demonstrated minimally elevated AHR during aerosol challenge compared to asthmatic controls, and elevated AHR during IV Mch challenge. Our results indicate a likely role for HA in AHR in a mouse model of asthma, but the importance of HA size is still unclear. One possible theory is that HA may interfere with methacholine in the airways, affecting aerosol challenge results. More studies are needed to clarify the underlying mechanisms of HA size in asthma.

A-337

Role of RNase L in Kidney

N. Alghamdi, Q. Zheng, D. Liu, A. Zhou. *cleveland state university, Cleveland, OH*

Objective: Investigate the role of RNaseL in kidney function and investigate the mechanism by which RNaseL regulates the EGF excretion in urine.

Clinical Relevance: Renal diseases have been continuing to be a prevalent problem. Current data indicate that 1% of patients admitted to the hospital are diagnosed initially with acute kidney injury (AKI), while about 2-5% of hospitalized patients develop AKI secondarily. It has been reported that epidermal growth factor (EGF)/EGFR activation contributes to the development and progression of renal diseases such as obstructive nephropathy, diabetic nephropathy, hypertensive nephropathy, and glomerulonephritis through mechanisms involved in induction of tubular atrophy, overproduction of inflammatory factors, and/or promotion of glomerular and vascular injury.

Methodology: In this study, we used an animal model of wild type and RNaseL knockout mice to show that 2-5A dependent RNaseL (RNaseL), one of the key enzymes playing an important role in the molecular mechanisms of interferon functions against microbal infection and cell proliferation, mediated EGF/EGFR activation. Interestingly, we found that the dissected kidney from aged (18months) RNaseL deficient mice was significantly smaller than that in wild type mice under the same condition. Histological staining revealed that there were remarkably a higher number of vacuoles in the kidney of RNaseL deficient mice than that in wild type mice although the biological significance of the observation is largely unknown. Using Westernblot analysis and proteomic analyses of urine protein excretion discovered that lack of RNase L exclusively blocked EGF excretion. Further investigation of the molecular mechanism showed that RNaseL regulated the shedding of EGF precursor through inhibiting some specific proteases responsible for the event.

C**onclusions**: Lack of RNaseL may affect the kidney function and development, and altered protein production. Our findings provide new insight into the pathogenesis of renal diseases and RNaseL may be considered as a target molecule for therapeutic treatment of the diseases.

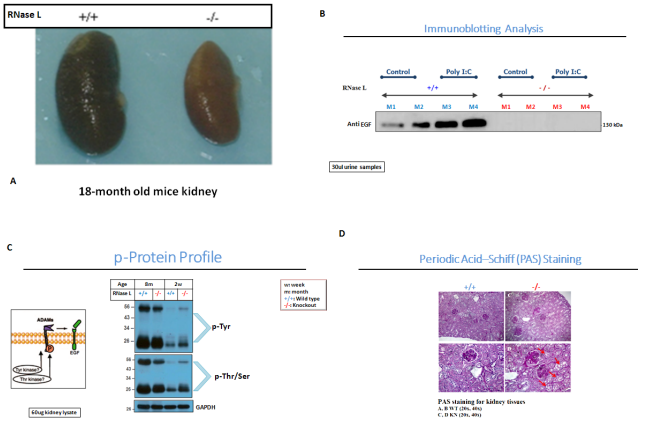

Fig 1: A: Aged mice kidneys of Wild type and knockout RNase L show differences in size and color. B. urine samples obtain from Wild type mice (M1-M4 blue) and RNase knockout mice (M1-M4 red), subjected to SDSPAGE analysis then immunobloted with anti-EGF antibody (ABCAM). C. protein extracted from kidney of wild type and RNase L knockout mice by NP-40 lysis buffer subjected to SDSPAGE immunobloted with anti phosphotyrosin and phosposerin and phosphthrionin antibodies from (Santa Cruz). D. PAS staining was performed on formaldehyde fixed kidney tissues. A, B: wild type (20x, 40x); C, D: Knockout type (20x, 40x)

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A comparison of the Jaffee creatinine and Enzymatic creatinine in pre-clinical species

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This study was designed to compare the non specific colorimetric (Jaffe reaction) and the enzymatic methods of creatinine measurements in monkey, dog, rat, and mouse serum and urine samples. Siemens Healthcare Diagnostics manufacturers both the Jaffe creatinine (CREA_2), and Enzymatic creatinine (ECRE_2) which are optimized for the Siemens Advia® 1800 automated clinical chemistry system. These assays utilize liquid-ready –to- use reagents and require the same Siemens chemistry calibrator. The Jaffe reaction of creatinine and picric acid is the oldest clinical methodology still in use for the measurement of creatinine. There are many analytical problems associated with the use of the Jaffe's reaction, in particular those relating to positive and negative interference by non-creatinine chromogens. Common interfering substances of the Jaffe's based methods include glucose and bilirubin. Glucose and bilirubin both inhibit the reaction between creatinine and alkaline picrate which is the principle method of the Siemens CREA_2 method. Enzymatic creatinine assay is the most accurate routine method available at present. The enzymatic method exhibits several advantages over Jaffe based methods. The enzymatic method has greater specificity. Glucose does not interfere with the enzymatic method however bilirubin may have a negative interference depending on the concentration.The correlation of the CREA_2 and the ECRE_2 methods showed no difference in the results for monkey, dog and rat in serum and urine samples exhibiting an overall average R^2 value of 0.978. When comparing mice urine samples the two methods correlated well with an R^2 of 0.994 however, mice serum results showed no correlation exhibiting an, R^2 value of 0.2995. Since non-creatinine chromogens are a known interference in the Jaffe method, it is the hypothesis that chromogens, specific in mouse serum, are interfering with this assay, and that the enzymatic method is the method of choice when analyzing mouse serum.

A-339

Changes in the specific extracellular matrix protein levels are related with the renal fibrosis in rats with adriamycin-induced nephropathy

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Background: Adriamycin nephropathy is characterized by the reduction in glomerular filtration rate, proteinuria, glomerulosclerosis and tubulointerstitial fibrosis (TIF). Proposed stimuli for TIF include proteinuric glomerulopathy, inflammatory mediators, cytokines and growth factors, and tubulointerstitial ischemia secondary to glomerular capillary injury. In this study, we aimed to investigate the levels and expression of specific ECM proteins (fibronectin, integrin and laminin) and transforming growth factor-beta1 (TGF-β1). In addition to, levels of the other growth factors; connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), and as well as expression of α-smooth muscle actin (α-SMA), and α-Actinin4 in the adriamycin (ADR) induced renal fibrosis.

Methods: Fifteen Wistar Hannover rats were divided into control (n:6), and ADR injected groups (n:9). Kidney tissue sections were stained with the periodic schiff-methenamine (PAS-M) stain. Fibronectin, integrin, laminin, and TGF-β1 levels were measured by ELISA method in the serum samples, and their tissue expressions were also explored immunohistochemical method as well as α-SMA and α-Actinin4, and were examined under a microscope.

Results: Although normal morphology of the kidney in the control group, histopathologic changes were vizualized in the renal tissue glomerular and tubulointerstitial areas of rats with ADR-inducid nephropaty. The glomerular changes consisted of mild mesangial expansion, glomerular tuft adhesion to the Bowman's space, and glomerulosclerosis. There were dense staining fibronectin, integrin, laminin, TGF-β1, and α-SMA, except for α-actinin4, in the evaluation of the immunohistochemical staining. Moreover, fibronectin, integrin, laminin, TGF-β1 and VEGF serum levels were higher in the ADR group than in the control group.

Conclusion: These results indicate that the levels of TGF-β1, fibronectin, integrin and laminin can be used in the diagnosis of renal fibrosis which is characterised glomerulosclerosis and/or TIF. Hence, understanding the changes in ECM-associated proteins and other biomarkers in the development of fibrosis will facilitate in the design of novel strategies to treat chronic kidney disease and renal failure.

A-340

Investigation of cocaethylene cardiotoxicity in Sprague-Dawley rats

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Background: Cocaine is one of the most abused psychostimulant and leading cause of non-prescription drug related deaths in the United States. It is frequently abused along with alcohol to achieve heightened euphoria. The metabolite of cocaine and alcohol, cocaethylene (CE), has a longer half-life than cocaine; thus the cocaine-induced euphoria is prolonged when alcohol is also present. CE has been associated with an overall increased health risks, especially cardiovascular problems, than either drug alone; however, its mechanism of cardio toxicity is not fully understood. In addition, cocaine abusers tend to have poor diet since it is known to act as an appetite suppressant. Our objective is first to investigate the cardio toxic effects of CE in Sprague-Dawley rats and secondly to determine whether nutrition modulates these effects.

Method: Male Sprague-Dawley rats were divided into three diet groups, normal diet (13.2% of calories from fat, 24.6% protein, 62.3% carbohydrate), low protein diet (10% of calories from fat, 5.2% protein, and 84.7% carbohydrates), and high fat diet (62% of calories from fat, 18% protein, and 20% carbohydrates). Within each diet group (6 animals per group), animals were further divided into three CE dosage groups (0 mg/kg, 15 mg/kg, and 30 mg/kg). All animals received intra-peritoneal injections of either saline or CE daily for 28 days. Last CE dose was administration 24 hours prior to sacrifice. Rat heart tissues were analyzed macroscopically, microscopically, and histologically. Additionally, oxidative toxicity was also investigated. Heart tissue and plasma were analyzed for CE and its metabolite, benzoylecgonine (BE), via LC-MS/MS. CE was provided by National Institutes on Drug Abuse and the study was approved by the Institutional Animal Care and Use Committee (IACUC) of our institute.

Result: Hearts of animals treated with CE showed histological evidence of myocarditis and inflammation without gross macroscopic changes. Furthermore, CE treatment also resulted in oxidative damages to heart tissues. Analyses of the heart tissues for the presence CE and BE via LC-MS/MS, indicated absence of these analytes in the tissues. However, when plasma was analyzed, BE detected in low protein and high fat diet animals were dose dependent. In contrast, animals on normal diet did not show dose dependent increase of BE in plasma. At the highest CE dose (30 mg/kg), animals on low protein and high fat diet showed increased level of BE in plasma compared to the normal diet animals.

Conclusion: Although CE induced oxidative and inflammatory responses in cardiac cells, neither CE nor BE were found to be deposited in those cells. The rate of CE or BE clearance may be influenced by diet. Thus, it can be inferred that chronic co-abuse of cocaine and alcohol via the formation of cocaethylene may result in oxidative and inflammatory injury to the heart which can be influenced by the nutritional status of abusers.

Tuesday, July 28, 2015

Poster Session: 9:30 AM - 5:00 PM

Cancer/Tumor Markers

A-342

Development of magnetic bead-based multiplex immunoassays to evaluate serum biomarkers for the early detection of pancreatic cancer

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Background: Pancreatic cancer is the 4th leading cause of cancer death in the United States. The majority of patients present with unresectable disease leading a median survival of 6 months and an overall 5-year survival of < 5%. The early detection of this disease is critical because surgery at an early stage is the most promising therapy that could greatly improve the prognosis of patients. The current existing serum markers such as CA19-9 lack the necessary sensitivity and specificity. Multiplex immunoassay simultaneously measuring multiple analytes in the same sample using minimum volume allows us to evaluate serum biomarker panels that can potentially complement CA19-9 in early detection of pancreatic cancer.

Objective: To develop magnetic bead-based multiplex immunoassays to evaluate serum biomarkers for the early detection of pancreatic cancer.

Method: Curated results from PUBMED database search using a combination of terms "pancreatic cancer, pancreatic neoplasm, PANIN, pancreatic adenocarcinoma, sensitivity, and fold change" were analyzed. Candidate biomarkers were selected using a weighted scoring system based on 1) fold changes and number of publications, or 2) sensitivity/specificity and study sample sizes. Magnetic bead-based multiplex immunoassays were developed for the selected candidate serum biomarkers using a Bio-Plex 200 suspension array system (Bio-Rad). Briefly, monoplex assays of individual candidates were first developed, cross-reactivity checked, and multiplex assays validated and optimized. All of these proteins plus HE4 (Roche) and CA19-9 (Tosoh) were analyzed in sera of patients diagnosed with pancreatic ductal adenocarcinoma (PDAC: IB/IIA/IIB, n=10; IV, n=10), benign pancreatic conditions including intraductal papillary mucinous neoplasm (IPMN, n=10) and chronic pancreatitis (n=10), and healthy controls (n=19). The performances of these candidate markers were evaluated individually or in combination on their capacity to complement CA19-9 in early detection of pancreatic cancer.

Results: The biomarkers evaluated included 1) a 5-plex assay of OPN, CEACAM-1, MIC-1, MIA, and SPON1; 2) a 2-plex assay of POSTN and HSP27; and 3) a monoplex assay of LGALS3BP. These assays were all in-house developed with negligible cross-reactivity, recovery of 75-119%, and intra-assay or inter-assay precision of 0.3-9.6% or 0-18%, respectively. LOD or LLOQ was 0.179 ng/mL or 0.181 ng/mL (OPN), 0.101 ng/mL or 0.213 ng/mL (CEACAM-1), 0.001 ng/mL or 0.046 ng/mL (MIC-1), 0.009 ng/mL or 0.016 ng/mL (MIA), 0.041 ng/mL or 0.191 ng/mL (SPON1), 0.094 ng/mL or 0.767 ng/mL (POSTN), 0.005 ng/mL or 0.062 ng/mL (HSP27), and 0.035 ng/mL or 0.289 ng/mL (LGALS3BP). Individually, the best biomarkers (AUC in ROC analysis, 95% CI) to separate PDAC from benign pancreatic conditions were CA19-9 (0.9425, [0.85-1.00]), CEACAM (0.845, [0.71-0.98]), MIC (0.79, [0.65-0.93]), and SPON1 (0.68, [0.51-0.85]). However, stepwise backward logistic regression selected a three marker panel of CA19-9, HSP27, and MIA (p-values: <3E-9, <0.03, <0.01, respectively) with an AUC=0.99 [0.97-1.00]. Probably due to the small sample size, the improvement over CA19-9 alone is not statistically significantly.

Conclusion: The multiplex immunoassay workflow provides sufficient analytical performance to evaluate serum biomarker panels that complement CA19-9 in early detection of pancreatic cancer. The biomarker panels identified in this study warrant further validation with a larger number of patient samples.

A-343

Validity of serum Eotaxin-1 in diagnosis of prostate cancer

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Backgrounds: Prostate cancer (PCa) is the second most common male cancer worldwide and ranked the sixth male cancers in most Arab world. Eotaxin-1/CCL11 is a member of chemokines, which are a superfamily of small proteins that bind to G protein-coupled receptors on target cells. Chemokines and chemokine receptors are shown to play an important role in regulation of tumor growth, migration, and invasion of different types of cancers. Although, the best and most sensitive screening test available for PCa is prostate specific antigen (PSA) there is a large overlap between PCa and benign prostatic hyperplasia (BPH) especially in patients with moderately elevated PSA levels, which reinforced the critical need to develop, validate and determine the utility of other diagnostic biomarkers for PCa. Eotaxin-1 offer a hope to overcome these drawbacks by virtue of its cancer specific expression. Objective: This study aimed to explore the diagnostic and prognostic value of serum Eotaxin-1 as non-invasive biomarker for PCa as well as to validate its combination with PSA to improve the overall value of sensitivity, specificity and diagnostic accuracy of PCa patients. Patients and Methods: This study included 62 newly diagnosed PCa patients (preoperative), 84 BPH patients and 70 healthy male subjects of matched age. Full history and clinical data were recorded for all subjects. PCa patients were undergo digital rectal examination (DRE), trans-rectal ultrasonography (TRUS) guided biopsy, computed tomography (CT) scan of the pelvis and histopathological examination, accordingly TNM stages were confirmed and PCa patients grouped as early PCa (T I/II) in 69.4% (n=43) and advanced (T III/IV) in 30.6% (n=19). Blood samples were withdrawn from all patients after at least one-week gap following DRE and prior to any prostate biopsy. Serum levels of Eotaxin-1, PSA and free/total PSA were measured as well as possible association between parameters were assessed. The validity (sensitivity and specificity) were evaluated by ROC curve analysis. Results: Serum PSA levels were significantly higher in PCa than BPH and control groups ($p<0.05$) and attained sensitivity of 85% at 83% specificity with a diagnostic accuracy of 84%. f/tPSA ratio had a sensitivity, specificity and accuracy of 82%, 84% and 86% respectively. Serum eotaxin-1 levels differentiated significantly among PCa, BPH and control groups (p0.05), its ratio with PSA differentiated significantly between advanced and early PCa stages ($p<0.05$) and provide a sensitivity, specificity and diagnostic accuracy of 90%, 84% and 87% respectively for diagnosis of PCa. Combination of serum eotaxin-1 and f/tPSA ration seems to improve the overall value of sensitivity, specificity and diagnostic accuracy (92, 85 and 89% respectively). Conclusion: Serum eotaxin-1 may provide a useful diagnostic tool to help distinguish between BPH and PCa. Combination of this chemokine with standard marker PSA can improve the overall value of sensitivity, specificity and diagnostic accuracy of patients with PCa eventually sparing unnecessary prostate biopsies. However, larger prospective studies are warranted to validate the diagnostic value of serum eotaxin-1 level in PCa.

A-344

CCCTC-binding factor inhibits breast cancer cell proliferation and migration via inactivation of nuclear factor-kappaB pathway

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Background: CCCTC-binding factor (CTCF) is an important epigenetic regulator and an evolutionarily conserved and ubiquitously expressed zinc finger protein. CTCF regulates a wide range of genes associated with tumor development, in particular genes involved in growth, proliferation, differentiation, and apoptosis. In this study, we aimed to observe the expression of CTCF in breast cancer cell lines, tumor tissue and serum of breast cancer patients, and investigate the effect of CTCF on proliferation and migration of breast cancer cells. Methods: Western blot was used to detect CTCF expression in human breast cancer cell lines MCF7, SKBR3, MDA-MB-231 and normal breast cells MCF-10A. Real-time quantitative PCR and immunohistochemistry were applied to detect the mRNA and protein levels of CTCF in invasive ductal carcinoma (n=23), peritumoral tissue (n = 10) and fibroadenoma (n = 10). Enzyme-linked immunosorbent assay (ELISA) was used to detect the concentration of CTCF protein in serum. In addition, CTCF was overexpressed and knock-down in MCF7 cells by virus packaging and infection, and then cell viability and proliferation was detected using MTT assay. Furthermore, cell migration and invasion were measured in vitro by scratch/wound healing and transwell migration assays. Last, Affymetrix U133 Plus 2.0 microarray was performed to screen the

possible target genes and pathways that CTCF regulated. Results: CTCF expression in MCF-10A was the highest, and decreased gradually in MCF7, SKBR3 and MDA-MB-231. CTCF expression in breast carcinoma tissue were significantly lower than that in peritumoral tissue and benign lesions (P <0.01). Moreover, CTCF expression in poorly differentiated breast cancer was significantly lower than that in well differentiated breast carcinoma. CTCF protein concentration in serum of breast cancer patients were also significantly lower than that of healthy control (P <0.01). In addition, CTCF could inhibit the proliferation and migration of MCF7 cells. By transcriptomic analysis and further experimental confirmation, we identified the HIPK2 as a target gene of CTCF and found that CTCF could inhibit the activation of nuclear factor-kappaB pathway. Conclusions: In summary, our findings demonstrate that CTCF expression were declined significantly in invasive breast cancer, compared with the non-invasive breast cancer and normal controls, suggesting CTCF may be a potential marker for breast cancer. CTCF could inhibit the proliferation and migration of breast cancer cells via inactivation of nuclear factor-kappaB pathway.

A-345

Better mutation discrimination for a multiplexed KRAS assay using PASS MNAzyme qPCR.

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Background: Many clinical diagnostic assays are now detecting somatic mutations such as gene insertions, deletions and point mutations. Due to the complicated design of these assays they are often in a singleplex format and/or lack sensitivty and specificity. We have developed a novel method to improve sensitivity and specificity of detecting mutations in a multiplex format referred to as PASS MNAzyme qPCR. In this study we apply this method to detect 7 KRAS codon 12 and 13 mutations in characterised colon cancer and melanoma FFPE samples and we compare mutant cross reactivity to the Qiagen Therascreen assay.

Material and Methods: PASS primers consist of target specific regions separated by a unique insert sequence (INS) which is not complementary to the target region. A long 5' target-specific region anchors the primer and a short 3' region, targeting the variant base/s, directs specific binding and extension. After amplification, the INS sequence is incorporated into the amplicon and acts as a 'barcode' to identify the amplicon and/or genetic variation when coupled with MNAzyme® qPCR. MNAzymes (Multi-component Nucleic Acid enzymes) composed of DNA oligonucleotide "partzymes" can be used to mediate a unique real-time detection technology. Active MNAzymes form when partzymes bind to target amplicons, and catalyse cleavage of fluorescently labelled probes thus generating a real-time signal. Superior multiplexing capacity is enabled by coupling PASS with MNAzyme® qPCR. Each multiplexed target is given a distinct INS and this 'barcode' is specifically detected by the MNAzyme. Previously (1), a multiplexed KRAS PASS MNAzyme qPCR assay, which detects 7 mutations in 3 wells, was evaluated using colon cancer and melanoma FFPE samples and compared to analysis from Sequenom MassARRAY and Illumina MiSeq. In this study, the cross-reactivity between individual mutations at each codon was evaluated and compared to Qiagen Therascreen assay.

Results: Previous results demonstrated the performance of the assay against characterised colon cancer and melanoma FFPE samples demonstrating >96% concordance with Sequenom MassARRAY and Illumina MiSeq. PASS MNAzyme® qPCR demonstrated sensitivities of <1% of mutant alleles (down to 0.01% in well optimised assays) and had excellent specificity with minimal cross-reactivity for all individual targets.

In the current study, this multiplex format showed delta Cq values >8 between mutants and >12 between mutant and WT, in comparison to the singleplex Qiagen therascreen product sheets which states delta Cq values of >3 between mutants.

Conclusion: Since PASS MNAzyme® qPCR affords greater multiplex capacity, along with high specificity and sensitivity, it provides a superior tool for ascertaining mutations from tumour tissues. Its ability to detect low levels of mutant alleles makes it particularly well suited for use with liquid biopsies.

(1)Vandenbroucke et al. Cancer Res October 1, 2014 74; 1502

A-346

Determination of Reference Intervals for Catecholamine and Metanephrine Excretion Using Archived Patient Data

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Objective: Fractionated urinary metanephrines and catecholamines are measured for diagnosing and monitoring treatment of pheochromocytoma tumors and used infrequently to investigate dysautonomias. We sought to establish reference intervals for excretion (ug/day) in pediatric and adult populations using archived patient values.

Methods: A data set of ~122,000 archived urine metanephrine and catecholamine results obtained by LC-MS/MS assay were filtered based on criteria including 24-hour collection (20-28 h), creatinine concentration (>25 mg/dL), and total urine volume (400-3000 mL). Duplicate patient results were removed and Chauvenet's criterion was used to identify and exclude outliers. Age and gender partitions were identified visually by plotting excretion per day against patient age, then confirmed statistically by Student's T-test (gender) or one-way ANOVA analysis (age) and by evaluating the clinical significance of each partition, based on research literature. Reference intervals (central 95%) for dopamine, epinephrine, norepinephrine, metanephrine, and normetanephrine were determined by linear regression analysis of the percent cumulative frequency (Hoffmann method, Am J Clin Pathol. 2010; 133: 180-186) with a 5% error allowance. Graphs depicting excretion per day reference intervals as a function of age range for each analyte (in essence, a two-dimensional Forest plot) were used as a visual comparison between these derived reference intervals and published 24-hour excretion reference intervals established by conventional methods.

Results: Excretion per day (ug/day) reference intervals are summarized in the table.

Catecholamines	Age	RI	**Metanephrines**	Age	Gender	RI
	(y)	(ug/d)		(y)		(ug/d)
Dopamine	4-6	95 - 221	**Metanephrine**	7-17	F	42 - 135
	7-12	76 - 371		≥18		39 - 143
	13-17	137 - 393		7-12	M	45 - 179
	18-69	77 - 324		13-17		61 - 202
	≥70	56 - 272		≥18		62 - 207
Epinephrine	4-17	1 - 9	**Normetanephrine**	7-12	F	52 - 247
	18-69	1 - 7		13-17		73 - 266
	≥70	1 - 5		≥18		109 - 393
Norepinephrine	4-12	6 - 45		7-12	M	70 - 273
	13-17	15 - 57		13-17		92 - 312
	18-69	16 - 71		18-29		95 - 379
	≥70	11 - 60		≥30		125 - 510

Discussion: Catecholamine and metanephrine excretion is age-related while partitioning by gender is necessary only for metanephrines. Excretion per day (ug/day) provides greater clinical sensitivity while reducing interindividual variation when compared to random urine collections that are corrected to creatinine concentrations. Reference intervals derived from linear regression analysis align well with intervals determined by conventional methods. Minor discrepancies appear to be related to the timing of urine preservation and analytical methods employed in the studies.

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Prognostic biomarker of the combination of TROY and LGR5 in patients with colorectal cancer

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Background:

Wnt signaling plays an important role not only in the regeneration of colon mucosa but also in the development of colorectal cancer (CRC). Recent studies have shown an association of two Wnt target molecules with CRC in vitro. One is LGR5 (leucine-rich-repeat-containing G-protein-coupled receptor 5), and the other is a Type I transmembrane receptor member of tumor necrosis receptor superfamily 19 called TROY. LGR5 marks stem cells in multiple adult tissues and cancers including CRC, and TROY is produced in fast-cycling stem cells of the small intestine. Although LGR5 and TROY may be involved in the development and progression of CRC in vivo, in clinical samples, and in vitro, such relationships have not been investigated. To clarify these matters, we performed this study using CRC cell lines and surgical specimens.

Methods:

We used 7 CRC cell lines, 106 primary CRC tissues, and 36 non-tumor tissues. The expression levels of LGR5 and TROY were measured by quantitative real time-PCR.

Results:

Expressions of LGR5 and/or TROY mRNA were detected in 5 of the 7 CRC cell lines, of which 4 cell lines revealed overexpression of both genes. In the clinical samples, the expression levels of LGR5 correlated statistically with TROY ($r = 0.485$, $p < 0.001$, two-tailed Spearman's test). Expression levels of LGR5 and TROY were significantly higher in the CRC specimens of stages I to IV than in the non-tumor tissues ($p < 0.0001$). In addition, the duration of disease-free survival was shorter in the patients with high levels of both LGR5 and TROY than in those without these molecules ($p = 0.0376$ by Kaplan-Meier analysis).

Conclusion:

This study suggests that co-overexpression of LGR5 and TROY may play an important role in CRC progression, and these two genes may be possible biomarkers for the prediction of CRC prognosis.

A-348

Role of detoxifing enzyme Nicotinamide Dinucleotide (phosphate) H :Quinone Oxidoreductase 1C609Tgene polymorphism in bronchogenic carcinoma

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Polycyclic aromatic hydrocarbons are abundant in tobacco smoke and constitute a factor in lung cancer. A single base substitution (C→T) polymorphism at 609 in the NQO1 gene reduces quinone reductase activity. The aim of the current work was to study the role of NQO1 (C609T) polymorphism among a sample of Egyptian male patients presenting with different stages of bronchogenic carcinoma.The study was conducted as a case-control study of 200 subjects; 100 male patients presenting to the Chest diseases unit in the Alexandria University Hospital with bronchogenic carcinoma in different stages . Diagnosis of primary lung cancer was confirmed through a review of each patient's pathology report . 100 age-matched male adults were recruited. We investigated NQO1genotype in relation to lung cancer risk. Genotyping was successfully carried out by PCR-RFLP and PCR-CTPP. In lung cancer patients, the most prevalent histological subtype of lung cancer was Adenocarcinoma with a frequency of 37%, followed by 31% squamous cell carcinoma, 19% SCLC (small cell lung cancer) and 13% with other histological subtypes . As regards the tumour staging, 5 out of 100 patients were stage I, 27 patients were stageII, 32 patients were stage III and 36 patients were stage IV.All subjects were analyzed for the NQO1 609C>T(rs1800566) SNP genotype. The distributions of the NQO1 genotypes among lung cancer patients and controls were in agreement with Hardy-Weinberg equilibrium (p= 0.267, 0.425, respectively). The frequency of NQO1 CC, CT, TT genotypes was 62, 31 and 7%, respectively in patients and 73, 26 and 1% in controls. The relative frequencies of the wild (C) allele and the variant (T) allele of NQO1 609C>T (rs1800566) SNP were 77.5% and 22.5%, among the patients respectively, and were 86% and 14% among the controls, respectively.

Patients with one or two copies of the T variant allele had 2.2 fold increased lung cancer risk than those with CC genotype. As regards tumor histological type, patients with one or two copies of the T variant allele had 4.3- fold increased SCLC risk than those with CC genotype, while, they had 3.8- fold increased risk for lung cancer with other histological type, 2.1-fold increased adenocarcinoma risk, and 1.2-fold increased squamous cell carcinoma risk more than those with CC genotype. When adjustment was made for smoking index pack-years, among light smokers (<21 p-y), the patients with two copies of the T variant allele had 1.9-fold increased lung cancer risk compared to those with CC genotype, while, the heavy smoker (>21 p-y) patients with one or two copies of the T variant allele had 3.6-fold increased lung cancer risk compared to those with CC genotype. There was no discrepancy between the CTPP-PCR and RFLP-PCR results in our study subject samples. From the current study we conclude that NQO1 609C>T (rs1800566) increase lung cancer risk among Egyptian male patients presenting with different stages of in bronchogenic carcinoma. CTPP-PCR offers a simple and effective genotyping method for single nucleotide polymorphisms(SNPs) , allow rapid accurate and inexpensive analysis for NQO1 609C>T polymorphism.

A-349

Cripto 1: A novel tumor marker for Oral Squamous Cell Carcinoma (OSCC)

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Background:

Oral Squamous Cell Carcinoma (OSCC) is one of the commonest cancers, particularly in developing country like India. A high rate of mortality and morbidity reported due to OSCC is majorly attributed to late diagnosis of the disease mainly due to non-availability of a screening tool or tumor marker.

Cripto1 (CR1), a member of the EGF-CFC protein family differentially expresses during early embryogenesis. Expression of CR1 mRNA and/or immune-reactive protein, a key phenomenon in tumor dedifferentiation cancers, is associated with increased number of cancer stem cells, thus makes CR1 a potential target for a prospective tumor marker.

In this we elucidated the potential role of Human CR1 as a tumor marker in the cases of OSCC.

Methods:

Fifty consecutive biopsy proven OSCC cases and fifty age/sex-matched controls were recruited for the study. Serum CR1 level of controls as well as serum CR1 levels (soluble component of CR1) of the cases before and after standard therapy according to the stage of the disease were estimated by ELISA (R&D SystemsTM). Expression of CR1 were also checked at transcriptional mRNA level by Real time RT PCR and at protein level by IHC (Immuno-Histo Chemistry) in the cancer tissue. The data were analyzed by appropriate statistical tests for significance using GraphPad Prism v6.00.

Results:

There is significant ($p=0.003$) raise in the serum CR1 level in OSCC patients (mean 497pg/ml) with respect to controls (207pg/ml), which is significantly reduced ($p=0.046$) after completion of therapy. The difference was more significant in patients with well-differentiated tumors and in early stage disease. There is 4.32-fold increase in the mRNA expression of CR1 in cancer tissue with respect to the cancer free tissue and 68% of the cases showed 3+ cytoplasmic positivity for CR1 in tissue level in IHC. With a cut-off value of 200pg/ml the sensitivity and specificity of CR1 is calculated to be 77.4% and 86.7% respectively for diagnosing OSCC.

Conclusion:

Human Serum CR1 is a potential tumor marker for Oral Squamous Cell Carcinoma. This study also suggests that CR1 may be useful in early diagnosis of OSCC and merits larger, prospective studies.

A-350

Serum human epididymis protein 4 (HE4) is a better tumor marker in the early lung cancer diagnosing

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Abstract

Background:HE4 had been shown to be a novel tumor marker in lung cancer. However, there were few reports about the comparison of serum HE4 with conventional tumor markers. This study aimed to explore the diagnostic value of serum HE4 as a tumor marker in early pulmonary cancer and compared it with CEA,NSE,Cyfra21-1 and proGRP .

Methods: We collected blood from 172 individuals, 112 with lung cancer who had indications for surgery and the postoperative pathology showed Ior II stage according to TNM classification, 60 health examination people as healthy controls in Shandong Tumor Hospital from May 2014. The levels of traditional biomarkers and HE4 were measured through electrochemiluminescence assays (Roche E601 MODULAR Immunoassay Analyzer).

Results: Serum HE4 could accurately distinguish between lung cancer and healthy controls ($p<0.0001$). Using the cut-off value of 66.8 pmol/l, HE4 had a sensitivity of 43.80% and specificity of 95.00% with a receiver operating curve (ROC) of 0.822 in early lung cancer. In terms of histological types, serum HE4 had better diagnostic value than CEA in lung adenocarcinoma (ROC-AUC: 0.859 vs 0.663; sensitivity: 43.50% vs 26.10%; specificity was both 95.00%). HE4 showed a higher sensitivity than Cyfra21-1 in patients with pulmonary squamous cancer (ROC-AUC: 0.87 vs 0.834; sensitivity: 57.10% vs 37.10%;specificity was both 95.00%); However, ProGRP was the more sensitive biomarker than HE4 in diagnosing SCLC.

Furthermore, combination HE4 with traditional biomarkers further improved the sensitivity in diagnosing lung cancer.

Conclusion: Serum HE4 could serve as a biomarker to diagnose early lung cancer and the diagnostic value of it was better than traditional tumor markers.

A-351

CA 19.9 and CA 125 for diagnosis of mucinous ovarian cancer

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Background: Mucinous ovarian cancer (MOC) is an epithelial ovarian cancer that contains cysts and glands lined by mucin-rich cells and constitute 5-20% of ovarian carcinomas. The aim of this study was to determine the accuracy of carcinoembryonic antigen (CEA), cancer antigen 15.3 (CA 15.3), cancer antigen 19.9 (CA 19.9) and cancer antigen 125 (CA 125) for diagnosis of MOC in patients with mucinous ovarian tumors.

Methods: Samples were collected preoperatively from patients with mucinous ovarian tumor. We measured the serum concentrations of the tumor markers by electrochemiluminescence immunoassay (ECLIA) in MODULAR E-170 (ROCHE DIAGNOSTIC®). The reference ranges are: CEA (0-3.4 ng/mL), CA 15.3 (0-30 U/mL), CA 19.9 (0-37 U/mL) and CA 125 (0-35 U/mL). After surgery, histology and stage were determined according to FIGO-classification. Patients were classified into two groups according to the diagnosis of ovarian biopsy: NOT MOC (mucinous ovarian cystadenomas and mucinous ovarian borderline tumor) and MOC. All variables were included in a multivariate regression analysis to identify variables independently associated with MOC.

Results: We studied 94 patients with ages between 15 and 80 years old (median = 43). Eighty-two patients were NOT MOC (68 mucinous ovarian cystadenomas and 14 mucinous borderline ovarian tumor) and 12 were MOC. All MOC patients were in FIGO I or II stages. No statistically significant differences were found between MOC and NOT MOC patients according to CEA and CA 15.3 ($p>0.05$). All MOC patients had abnormal serum CA 19.9 and/or CA 125 levels. Using CA 19.9 and CA 125, we performed a linear regression formula CA 19.9+125 = 0.00102 x CA 19.9 + 0.00057 x CA 125. AUCs values were 0.862 ($p=0.0002$), 0.829 ($p=0.0021$) and 0.911 ($p=0.0001$) for CA 19.9, CA 125 and CA 19.9+125 respectively. CA 19.9+125 exhibited 95.1 % specificity and 66.7% sensitivity, increased by 16.7% sensitivity compared with using only CA 19.9 or CA 125.

Conclusions: Preoperative CA 19.9 and CA 125 levels showed high diagnosis efficacy to predict whether a mucinous ovarian tumour is benign or malignant. Using both markers simultaneously increases the sensitivity for diagnosis of MOC.

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Stability and inter-laboratory performance for the determination of the 4Kscore for prostate cancer

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Background: Panels of prostate-specific biomarkers have been reported to improve diagnostic accuracy of blood tests for prostate cancer. The 4Kscore Test, based on a panel of four biomarkers (total prostate specific antigen(tPSA), free PSA(fPSA), intact PSA(iPSA), and human kallikrein 2(hK2)) has been clinically demonstrated to provide a personalized, accurate risk of aggressive prostate cancer (Gleason score ≥7). Clinical validation in multiple European retrospective cohorts and more recently in a prospective study of 1,012 American patients scheduled for prostate biopsy (Parekh et al. 2014) showed excellent discrimination between men harboring clinically relevant cancer and those with indolent tumors or no cancer (AUC 0.82). In clinical practice, the test improves on the current limitations of detection of prostate cancer. The reliable determination of the four biomarkers in routine laboratory practice is an essential requirement to achieve and reproduce the attractive performance characteristics of the test. This work focuses on thorough characterization of the biomarkers' stability profiles and confirms key assay performance characteristics across three laboratories, necessary for the widespread clinical usage of the test.**Methods:**Marker stability in whole blood, plasma, and serum was characterized along with possible recovery bias in frozen specimens using matched clinical samples (single blood collection per patient). iPSA and hK2 custom assays were performed on an AutoDELFIA utilizing time-resolved fluorescence while tPSA and fPSA assays were performed on the Roche Cobas instrument or the AutoDELFIA. Real-time analyte stability studies compared recoveries measured under various handling conditions to that in EDTA plasma frozen at harvest or recovery at Day 0. Recoveries of all analytes were assayed for up to nine days following collection, with up to three days' storage as blood. Analyte recoveries in approximately 400 freshly assayed plasma were evaluated versus frozen plasma. These recoveries were also evaluated in 400 frozen sera versus matched plasma. The analytical performance of the iPSA and hK2 assays, including analytical sensitivity, precision, and accuracy, was evaluated in EDTA plasma across three independent laboratories using CLSI protocols.**Results:**95% of tPSA, fPSA, iPSA, and hK2 recoveries remained within 5% of control values in EDTA plasma refrigerated seven days; recoveries were also stable in specimens stored as EDTA whole blood 60 hours refrigerated and 24 hours at room temperature. Conversely, iPSA and hK2 recoveries decreased rapidly in stored serum. tPSA, fPSA, and hK2 recoveries in frozen plasma were equivalent to those in frozen serum (slope=0.95-1.05,r2>0.98) while iPSA recoveries in the two matrices are linearly related (slope=1.11,intercept =0.017,r2=0.98). Recoveries of all four analytes in fresh plasma were equivalent to those in frozen plasma (slope=0.95-1.05,r2>0.99). A complete characterization of analytical performance on three instruments confirmed robust reproducibility and accuracy at clinically relevant ranges of iPSA and hK2 in EDTA plasma, with the limit of quantitation for the iPSA assay at 13.9 pg/mL and the hK2 assay at 22.0 pg/mL.**Conclusion:**All four biomarkers used to calculate the risk of aggressive prostate cancer are stable in both EDTA plasma, and in EDTA-anticoagulated whole blood. Inter-laboratory performances demonstrate excellent overall analytical performance for iPSA and hK2 custom assay.

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Value of dFLC data in monitoring response to therapy in a patient with Primary Amyloidosis

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Background: Recent evidence have shown that serum free light chains (sFLC) and, particularly, the difference between involved and uninvolved sFLC (dFLC) are a well-established method for identifying and monitoring the response to therapy in patients with Primary Amyloidosis (AL).

Methods: A 46 years old woman was diagnosed with AL presenting renal and cardiac damage. In the initial study, the patient presented a creatinine of 5.9 mg/dL, total proteins=5,8 g/dL, normal serum protein electrophoresis (SPE), negative serum immunofixation (IFE), Beta-2-microglobulin=17.8 mg/L, albumin=3.19 g/dL, pro-BNP=6233 ng/L, Bence Jones Proteinuria (BJP) positive for free kappa (16.8 mg/dL), 7% of plasma cells in bone marrow, renal biopsy positive for kappa amyloid material and the echocardiography study showed septal hypertrophy. The patient began treatment with Melphalan and Prednisone (MP). The sFLC were measured by turbidimetry (Freelite, The Binding Site).

Results: The sFLC were expressed as dFLC (free kappa / free lambda / ratio). At diagnosis (day 0), dFLC was 71.6 mg/L (94.8 mg/L / 23.2 mg/L / 4.08) and PBJ was 16.8 mg/dL. After two cycles of MP (day +61), the patient achieved a status of partial response (PR) with dFLC value of 25.9 mg/L (52.8 mg/L / 2.03 mg/L / 3.12) and the reduction in dFLC was of 64%. PBJ was 18 mg/dL. After fourth, fifth and sixth cycles of MP, the dFLC increases with value of 28.5 mg/L (41.7 mg/L / 13.5 mg/L / 3.08) at day +120; 39.6 mg/L (53.8 mg/L / 14.2 mg/L / 3.78) at day +150 and 43 mg/L (58 mg/L / 15 mg/L / 3.86) at day +181. PBJ values were 10.0, 13.9 and 12.5 mg/dL, respectively. The increase of dFLC was the only parameter that showed us the existence of biological progression of the disease and the hematologist decided to change the treatment to Bortezomib/Cyclophosphamide/Dexamethasone (VCD). The patient presented good tolerance at this new treatment with dFLC of 28 mg/L (38.2 mg/L / 10.3 mg/L / 3.7) at day +221 (after first cycle of VCD) and 8.32 mg/L (18.1 mg/L / 9.78 mg/L / 1.85) at day +255 (after second cycle of VCD). The BJP were 8.26 and 14.21 mg/dL, respectively. Serum SPE and IFE were negative during the two lines of treatment of the patient.

Conclusion: The quantification of sFLC was the only assay that allows us the monitoring of the response to the treatment in this patient. dFLC was an effective tool that helps the hematologist to evaluate the response to the treatment and the presence of biological progression. PBJ was positive but ineffective to evaluate the response to the treatments. Periodic monitoring of dFLC allows us to predict whether the patient responds correctly to chemotherapy or, conversely, has a relapse and need to change treatment.

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Verification and Validation of LUMIPULSE G CA125II Assay under Development

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BACKGROUND: Serum CA125 values are useful for monitoring the course of disease in patients with invasive epithelial ovarian cancer (OC) (NIH Consensus Conference, JAMA, 1995). The current study was to evaluate the analytical and clinical performance of the LUMIPULSE ***G*** CA125II assay (LUMIPULSE CA125II assay) under development. The LUMIPULSE CA125II assay has not been cleared by the FDA.

METHODS: LUMIPULSE CA125II assay is a second-generation CA125 immunoassay using monoclonal antibody (MAb) OC125 as the solid phase antibody and the MAb M11 as the labeled antibody. Serum or plasma is incubated with the MAb OC125-linked magnetic particles followed by washing and rinsing steps. Alkaline phosphatase-labeled MAb M11 is then incubated with the CA125-bound particles followed by washing and rinsing steps. Substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy)phenyl-1, 2-dioxetane disodium salt) is then added to generate luminescence signals reflecting the amount of CA125 in the samples.

RESULTS: In the monitoring study of serial serum samples collected in SST tubes from 59 patients with OC, a total of 289 pairs of observations were undertaken. A positive change of CA125 values was defined as an increase if the change was at least 20% greater than the previous value. 67% of 52 samples with a positive change correlated with the disease progression of OC while 76% of the 237 samples with no significant change correlated with no progression of OC. The total concordance was 74%. A reference range study showed CA125 ≤ 35.0 U/mL in 99.2% of 240 healthy female subjects, 80% of 40 females with pregnancy, 90.4% of 260 females with benign gynecological disease, 92.5% of 40 females with a non-gynecological benign disease, 80.0% of 40 females with congestive heart failure, and 85.0% of 40 females with hypertension, respectively. Analytically, the assay's LoQ (Limit of Quantitation) was ≤ 2.0 U/mL, and linearity ranged 2.5 - 1000 U/mL. The imprecision studies showed a total CV ≤ 6.4% in a 20-day CLSI EP5-A2 study (8 sera, n = 80), inter-site study with 3 sites and inter-lot study using 3 lots of reagents. Spike recovery ranged from 96% - 115%. The average levels of CA125 in the individual interferent-spiked samples (9 endogenous substances including human anti-mouse antibodies and rheumatoid factor, and 16 therapeutic drugs) were within -5% to 3% difference of that in the unspiked samples. No high dose hook effect was observed for samples with up to 200,000 U/mL of CA125. Method comparison to ADVIA Centaur CA125II generated a Passing Bablok correlation as [LUMIPULSE CA125II] = 0.753 + 0.991 [ADVIA Centaur CA125II] within the range of 3.4 - 17755.2 U/mL (n = 120, r = 0.9998). Specimens collected in SST, K2-EDTA, sodium heparin, and lithium heparin tubes correlated with that in serum tubes with r ≥ 0.9 and a 95% confidence interval of slope ranging 0.9 - 1.1.

CONCLUSION: The LUMIPULSE ***G*** CA125II assay under development demonstrates to be an accurate, precise, sensitive and robust assay for measuring CA125 in human serum and plasma.

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Serum B-Cell Maturation Antigen Is a Novel Prognostic Indicator for Multiple Myeloma Patients and Correlates with Clinical Status and Survival

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Introduction: B-cell maturation antigen (BCMA) is a tumor necrosis factor receptor family member that is expressed on normal and malignant B-cells. Our group has recently shown that BCMA is present in the serum of multiple myeloma (MM) patients (pts) and that its levels may correlate with their clinical status and overall survival (OS). We now have analyzed the relationship between serum BCMA levels and monoclonal (M)-protein levels as well as the relationship of BCMA to response status, progression-free survival (PFS) and OS in a large cohort of MM pts.

Methods: Serum was obtained on 227 MM pts following informed consent. Enzyme-linked immunosorbent assay (ELISA) was used to determine serum BCMA levels (R&D Systems). The Mann Whitney test was used to assess the differences between clinical status groups. Kaplan Meier analysis and multivariate Cox regression models were also used. Kaplan Meier survival of MM pts was determined from the time of initial serum BCMA measurement to death or the date of last follow-up. PFS of MM pts was evaluated from the time of serum BCMA measurement to date of first disease progression. Cox proportional hazards regression was utilized to determine the predictive influence of serum BCMA and various other factors including age, creatinine, hemoglobin, ISS stage, and bone disease on OS and PFS. P-values less than 0.05 were considered statistically significant. Changes in serum BCMA levels were correlated with serum M protein levels for 44 MM pts during their course of disease. Similarly, serum BCMA levels were correlated with bone marrow and PET scan findings for NSD pts.

Results: Serum BCMA levels correlated with the patient's clinical status at the time of its determination (P PR had significantly lower serum BCMA levels (median, 52.49 ng/mL) than those with stable and progressive disease (median, 124.8 ng/mL; $P < 0.0001$). Changes in serum BCMA levels highly correlated with changes in serum M-protein levels among 44 consecutive MM pts that had multiple determinations made during their course of disease. Notably, pts with NSD showed a direct correlation between changes in serum BCMA levels and their clinical status as reflected by PET scan and bone marrow findings during their course of disease. PFS was markedly longer among pts with serum BCMA levels below than among those with levels above the median ($P = 0.006$). We divided pts into quartiles based on their serum BCMA levels. Pts in quartiles 1-3 had a longer PFS compared to pts in quartile 4 ($P < 0.0001$). Among all 227 pts, the 36-month OS of pts whose serum BCMA levels were above the median (≥ 102 ng/mL) was significantly lower than among those whose levels were below the median ($P = 0.02$). In the multivariate regression analyses, serum BCMA levels significantly correlated with only OS ($P = 0.0003$). In contrast, age, bone disease status, serum creatinine, hemoglobin, and ISS staging did not correlate with OS. Lastly, serum BCMA levels were independent of ISS staging and serum creatinine levels.

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The Diagnostic Value Of Serum Alphafetoprotein For Hepatocellular Carcinoma Among Patients With Chronic Liver Disease

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Background:

The aim of this study was to determine the value of using Alpha Fetoprotein for early detection of Hepatocellular Carcinoma among patients with chronic liver disease in Aminu Kano Teaching Hospital.

Methods:

In this study,50 patients with a clinical and radiologic diagnosis of Chronic Liver disease attending the gastroenterology clinic of Aminu Kano Teaching Hospital and 50 healthy volunteers were recruited by systematic samplingover 3 months. The patients were grouped according to their definitive diagnosis into : Hepatocellular carcinoma, Hepatitis B and C co-infection, Hepatitis B infection, Hepatitis C infection, Liver cirrhosis and unclassified/Miscellaneous. Alpha fetoprotein was assayed using an Electrochemiluminescence Immunoassay.

Results:

Hepatocellular carcinoma accounted for 22% of the study group, while Hepatitis B and C co-infection 16%, Hepatitis B infection 12%, Hepatitis C infection 6%, Liver cirrhosis 34% and Miscellaneous group 10%. The sensitivity, specificity and positive predictive value of Alpha Fetoprotein for Hepatocellular Carcinoma at a cut-off value of >200IU/L were 73%, 100% and 100% respectively.

Conclusion:

Screening for HCC using Alpha Fetoprotein in patients with Chronic Liver is useful for early detection.

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Multimarker quantitative PCR-based screening platform for circulating tumor-initiating cells of hepatocellular carcinoma: a large-scale, multicenter study

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Background

Given the prevalence of hepatocellular carcinoma (HCC) worldwide, more timely and accurate diagnosis is essential. Our aim was to facilitate diagnosis of hepatitis B virus (HBV)-related HCC by identifying those circulating tumor cells (CTC) with tumor-initiating cell (TIC) phenotypes.

Methods

Differing subsets of circulating tumor-initiating cells (CTICs) were investigated in two independent cohorts, including 890 healthy and test subjects (with chronic hepatitis B infection [CHB], cirrhosis, and HBV-related HCC) recruited between 2012 and 2013. Initially, 100 subjects were screened for 9 TIC markers of HCC. A working model was then constructed (via logistic regression) from preliminary data (training cohort, n=401) and validated independently (validation cohort, n=389), with area under the receiver operating characteristic curve (AUC-ROC) reflecting diagnostic accuracy.

Results

Four CTIC markers (epithelial cell adhesion molecule, CD133, CD90, and cytokeratin 19) of HCC were established, with a cutpoint of 0·57 by ROC curve after regression (training: AUC=0·88, 95% CI 0·84-0·91; sensitivity, 72·5%; specificity, 95·1%; validation: AUC=0·91, 95% CI 0·88-0·94; sensitivity, 82·1%; specificity, 93·8%). Results were similar for early-stage HCC (training: AUC=0·87, 95% CI 0·83-0.90; sensitivity, 71·8%; specificity, 94·6%; validation: AUC=0·92, 95% CI 0·88-0·95; sensitivity, 85·1%; specificity, 93·3%). Compared with all controls, the panel performed equally well in detecting α-fetoprotein-negative HCC (including early-stage tumors), also distinguishing HCC from CHB and cirrhosis. Panel performance was uniformly satisfactory, regardless of disease status.

Conclusion

A multimarker CTIC screening panel showed considerable clinical benefit in diagnosing HCC, even in patients with AFP-negative or early-stage disease, enabling differentiation of HCC from non-malignant chronic liver diseases.

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Validation of Fluorescence in Situ Hybridization assay for detection of TP53 gene deletion

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Background: Fluorescence in Situ Hybridization (FISH) provides an important adjunct to conventional cytogenetic in the evaluation of chromosomal abnormalities associated with hematologic and other neoplasias. FISH analysis offers a sensitive, specific, and reliable strategy for identifying acquired genetic abnormalities such as loss of a chromosomal region associated with hematologic disorders. Hemizygous deletion of TP53 gene has been identified in the Chronic Lymphocytic Leukemia, Acute Myeloid Leukemia, Multiple Myeloma and others.The presence of TP53 deletion is associated with adverse prognosis. Although the performance of most FISH probes has been evaluated by the manufacturer prior to marketing, it also must be validated prior to implementation of assay for clinical use. Clinical laboratories must independently adopt protocols in order to verify the performance of the assay.

Objective: To validate FISH assay for detection of TP53 gene deletion following recommendations from the American College of Medical Genetics (ACMG).

Methods: We used the P53 deletion probe manufactured by Cytocell®. During the familiarization phase of probe validation the analysts should become familiar with the concepts underlying the probe labeling and testing strategy. Metaphases cells obtained from 5 karyotypically normal male blood samples were used to localize the probe and determine its sensivity and specificity. To establish a reference range (normal cutoff) were estimate the false positive rate from 10 uncultured bone marrow samples and 10 uncultured peripheral blood samples that would be unlikely to harbor P53 deletion. Two analysts score 500 interphase cells (250 per analyst). All P53 probe signal patterns were recorded. The normal cutoff value for the P53 FISH assay was calculated using the beta inverse function (BETAINV) available in Microsoft Excel.

Results:The TP53 deletion probe presents two differentially labeled probes: a probe covering the TP53 gene labeled in red and a control probe labeled in green. A typical result of using this probe should present two green signals and two red signals (2G2R). In the analysis of 10 bone marrow samples were identified six atypical signals patterns: 2G1R, 1G1R, 3G2R, 2G3R, 3G3R, 4G4R. Analyzing 10 peripheral blood samples we observed four atypical signal patterns: 2G1R, 1G1R, 3G2R, 2G3R. The cutoffs obtained with BETAINV function was validated for counting 200 cells. The signal patterns and respective normal cutoffs for bone marrow samples are 2G1R (5,67%), 1G1R (2,34%), 3G2R(3,78%), 2G3R(2,34%), 3G3R(2,34%), 4G4R(2,34%) and for peripheral blood samples are 2G1R(3,78%),1G1R(2,34%), 3G2R(2,34%), 2G3R(2,34%).The clinical validation of FISH showed presence of the TP3 deletion, in agreement with conventional cytogenetic. The probe demonstrated 100% specificity and sensitivity higher than the recommended by ACMG.

Conclusion:The assay FISH for detection of the TP53 gene deletion was considered approved since it showed excellent reproducibility and high quality in different hybridizations, specificity and sensitivity higher than those guaranteed by the manufacturer of the probe.

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A familial adenomatous polyposis case with a novel (c.4091delG; p.Ser1364Metfs*51) mutation

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Familial adenomatous polyposis (FAP) is an autosomal dominant inherited colon cancer syndromecaused by mutations in theadenomatous polyposis coli (APC) gene (OMIM#611731). APC is a tumor suppressor gene that encodes a 2,843 amino acid protein involved in several cellular processes, including the control of β-catenin turnover in the Wnt pathway.Inactivation of the APC gene plays a significant role in FAP and more than1315 variants are described for APC in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), with most of the FAP related mutations being nonsense or small insertions/deletions that lead to a truncated protein. This report describes a 32-year-old male with a familial adenomatous polyposis (father, brother and aunts affected) and a case of an aunt diagnosed with Gardner syndrome, a variant of FAP. PCR and Sanger sequencing of all APC exons and 50bp flanking intronic regions identified a novel case of pathogenic APC mutation in exon 15: a G deletion at cDNA position 4091 (c.4091delG; p.Ser1364Metfs*51 - NM_000038.5). This mutation results in a frame shift and stop signal 51 codons downstream, causing a premature truncation of the APC protein at amino acid position 1415. ClinVar, Ensembl,LOVD, 1000 Genomes Project, ESP6500 and dbSNP141 databases searches revealed this mutation has never been described before. Online prediction program Mutation Taster (http://doro.charite.de/MutationTaster/index.html) classified this mutation as disease causing with a probability value of 1. Genetic investigation of this mutation in at risk family members should be performed to allow presymptomatic diagnosis and prophylactic interventions when necessary, as well as to increase the knowledge about genotype-phenotype correlations of this novel mutation.

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Genetic testing for BRCA Jewish founder mutations in Ashkenazi population from São Paulo, Brazil

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BACKGROUND: There are evidences that germline mutations in *BRCA1* and *BRCA2* genes are associated with the Hereditary Breast and Ovarian Cancer Syndrome, increasing the risk of cancer manifestation in up to 80%. More than 2,000 pathogenic mutations have been described in *BRCA1* and *BRCA2*. Among them, three variants show higher frequency in the Ashkenazi population: c.68_69delAG (BIC 185delAG) and c.5266dupC (BIC 5382insC) in *BRCA1* and c.5946delT (BIC 6174delT) in *BRCA2*. A study conducted by Myriad, a world reference in detecting *BRCA1* and *BRCA2* mutations, showed that approximately 24% of 2539 individuals with Ashkenazi ancestry and personal or family history of breast and ovarian cancer presented a mutation in either of these genes. Among these individuals, approximately 97% had one of the three founder mutations above mentioned. In Brazil, São Paulo city harbors the second densest Ashkenazi population in Latin America (about 65,000 individuals) and a previous survey has shown that all 15 Ashkenazi individuals presenting pathogenic variants in *BRCA1* or *2* carried one of the three founder mutations, corroborating the Myriad study. Based on these evidences, the survey of the three *BRCA* Ashkenazi founder mutations by Sanger sequencing could be an appropriated initial screening for this population, improving turnaround time and avoiding unnecessary costs associated with the *BRCA1* and *BRCA2* full sequencing. Our aim in this study was the validation of a test to accurately detect the three Ashkenazi founder mutations by Sanger sequencing.

METHOD: We selected 34 samples previously fully sequenced for *BRCA1* and *BRCA2*. Among these samples, 17 (eleven positive and six negative), 10 (five positive and five negative) and six (two positive and four negative) were tested for the presence of c.68_69delAG (185delAG), c.5266dupC (5382insC) and c.5946delT (6174delT), respectively. For reproducibility purposes, 13 samples were tested in triplicate (six positive and seven negative) and eight were tested in duplicate (four positive and four negative). The reproducibility tests were performed on different routines by different analysts.

RESULTS: ***All samples corroborated previous results, presenting an accuracy of 100%.*** All replicates reproduced the previous results.

CONCLUSION: We validated the test using Sanger sequencing methodology and demonstrated that this technique is a reliable and useful tool for the detection of the Jewish founder mutations in *BRCA1* and *BRCA2* genes. Based on the relatively high frequency of the founder mutations we reinforce the importance of this test for initial screening of *BRCA* mutations in the Ashkenazi population.

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Gene panel NGS testing for hereditary breast cancer in Brazilian patients: Hermes Pardini Institute case report

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Breast cancer is the most common cancer in women worldwide and alsothe main cause of death from cancer among women.Around 5-10% of these tumors present a strong hereditary component due to mutations in highly penetrant genes. BRCA1 and BRCA2 are the two most frequently mutated genes related to Hereditary Breast and Ovarian Cancer Syndrome (HBOC) and account for up to 45% hereditary breast cancers. The remaining 55% of severely affected families that do not carry mutations in BRCA genes represent a diagnostic challenge from the genetic and clinical perspective, and are less benefited from screening and prevention measurements. Recent advances in sequencing technologies have enabled the discovery of several novel genes related to breast cancer increased risk, such as BRIP1 and PALB2. Additionally, the development of the Next Generation Sequencing (NGS) technologies allowed the clinical use of targeted gene panels that screen multiple susceptibility genes in parallel, increasing the mutation detection rates and reducing results turnaround time. Hermes Pardini Institute, one of the most important diagnostic laboratories in Brazil, developed a NGS gene panel - named as Breast Cancer Panel 2 (BCP2) - for screening 15 breast cancer related genes in BRCA mutation negative patients.This panel includes 15 genes: ATM, BARD1, BRIP1, CHEK2, CDH1, MRE11A, NBN, PTEN, PALB2, RAD50, RAD51C, RAD51D, STK11, TP53 and XRCC2. Since January 2014, 12 female patients were tested with BCP2 panel. From these, no significantly clinical mutation was detected in four patients and 8 patients presented variants of unknown clinical significance (VUS) in one or more genes. We considered as VUS undescribed missense mutations or described variants with minor allele frequency (MAF) <0.02. ATM mutations were identified in four patients (p.F858L and p.P1054R in the same patient, p.F858L, p.P604S, p.K1454N); two NBN mutations (p.F263S and p.P593A) were identified inone patient; MER11A (p.T303I), BARD1 (p.K130T), CDH1 (p.A617T); BRIP1 (p.V193I) and CHEK2 (p.D438Y) mutations were identified in one patient each. One PALB2 (p.G998E) mutation was identified in three patients. Although reported dbSNPMAF for this variant is 0.008, novel studies reported a frequency of 2% in the studied population, reducing the probability of this being a pathogenic mutation. Our study demonstrates the utility of using NGS panel testing to investigate mutations in breast cancer susceptibility genes, establishing it as a cost effective and sensible test for clinical diagnosis. The main challenge for the scientific and clinical communities now resides in increasing the available information on phenotypes and genotypes of these novel susceptibility genes, allowing a correct classification of these genes VUS, as already occurs for BRCA1 and BRCA2 genes.

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Pleural fluid homocysteine levels for diagnosis of malignant pleural effusion

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Background: Malignant pleural effusions (MPE) are a common clinical problem in patients with cancer. A cytologic test is the standard basis for diagnosis of MPE, but positivity rate is only 60%. Pleural biopsy can greatly improve diagnostic sensitivity (90%), however it has a high cost and is associated with injury and other complications and thus is limited in its clinical application. Efforts have been made to find markers that would improve the positivity rate in MPE, including tumor markers. Homocysteine (HCYS) levels in serum are used as tumor marker in colorectal and breast cancer. We have not found any paper published of HCYS levels in pleural fluid. The aim of this study was to measure the accuracy of HCYS in pleural fluid for diagnosis of MPE.

Methods: We studied pleural fluids obtained by thoracocentesis in patients with pleural effusion. HCYS in pleural fluid were measured by nephelometry in BNII (SIEMENS®). Patients were classified into two groups according to the aetiology of pleural effusion: benign pleural effusions (BPE) and MPE. Pleural effusion was categorized as MPE if malignant cells were demonstrated in pleural fluid or pleural biopsy. Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC) using the software MEDCALC®.

Results: We studied 60 patients with ages between 1 and 89 years old (median = 61.2), 23 women and 37 men. Half of the patients were MPE, 3 mesotheliomas, 13 lung cancers, 4 breast cancers and 10 other tumors. The BPE were: 11 transudates, 9 parapneumonics, 2 tuberculosis and 8 other benign aetiologies. The median HCYS value in pleural fluid was significantly higher in MPE 13.95 µmol/L (CI 95%: 12.31-18.37) vs. 8.22 µmol/L (CI 95%: 7.08-12.74) in BPE patients. The AUC of HCYS in pleural fluid for diagnosis of MPE was 0.806 (CI 95%: 0.683-0.896) (p<0.0001). The patients with HCYS in pleural fluid 16.40 µmol/L were MPE.

Conclusions: HCYS levels in pleural fluid showed high diagnosis efficacy to predict whether a pleural effusion is benign or malignant.

A-363

Measurement of Human Epididymis Protein 4 (HE4) may be Closely Associated with Breast Cancer Progression

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Background: Human epididymis protein 4 (HE4) is described as a useful new biomarker in ovarian cancer however, it can be elevated in a variety of benign and malignant diseases and therefore it is neither tumor nor organ specific. For example approximately 10% of ovarian cancers are familial and relate to mutations of BRCA1, BRCA2, and mismatch repair genes. In this study, we have investigated the occurrence of this protein in female with breast cancer to monitor any diagnostic utility with the progression of the disease after therapy in combination with CA153.

Methods: HE4 was measured in the sera of 20 healthy individuals, and 30 patients with breast cancer under treatment. HE4, CA153 and CA125 were measured by immunoassay. The statistical analysis were also performed and p-value was calculated. All clinical and histological data were reviewed.

Results: The sensitivity and specificity of HE4 in patients with breast cancer were found to be 47% and 93% respectively .The median, mean, and standard deviation (SD) for HE4 were found to be 35.6, 38.0 and 9.5 in healthy women respectively. For patients with breast cancer but normal CA153 and CA125, the median, mean, and SD of HE4 were found to be 51.9, 54.1 and 18.2 respectively and 89.6, 117.0 and 78.8 respectively for those with abnormal CA153. There was a significant difference in the mean of HE4 among patients with breast cancer of those with normal CA153 versus those with abnormal CA153 (p< 0.01). Similarly both groups with either normal or abnormal CA153 values have shown a significant difference in the mean of HE4 against the healthy and breast cancer patients (p<0.0001). Among 30 cases of breast cancer, 28 were negatively free from any ovarian or pelvic cancer. One case confirmed with ovarian cancer and the other with endometrium cancer.

Conclusion: HE4 may have a role in monitoring breast cancer patients, however, due to some limitations and low sample size, further studies may be needed to obtain a solid conclusion for the utility of this marker in breast cancer patients.

A-364

Prognostic value of onconeural antibodies in patients with paraneoplastic neurological syndromes

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Background: Onconeural antibodies (OA) are strongly associated with cancer and paraneoplastic neurological syndromes (PNS). PNS can be defined as remote effects of cancer and are seen <1% of patients with cancer. Most of these antibodies are well-characterized (antibodies against Hu, Yo, Ri, CRMP5, amphiphysin, Ma-2 and Tr) and are in common use for the diagnosis of definite PNS. The aim of our study is to determine the percentage of OA detected in our Laboratory of Autoimmunity during last two years (2013-2014) and the possible association with PNS and tumor pathology.

Methods:OA were studied on 421 patients with neurological symptoms during a period of two years. OA were identified in serum sample by indirect immunofluorescence (IIF, Euroimmun AG) and recombinant immunoblot assay (Ravo Diagnostika) that

detects Hu, Yo, Ri, CV-2, Ma-1, Ma-2 and amphiphysin autoantibodies. One result is considered positive when it is confirmed by the two techniques.

Results:OA were positive in 7 patients only (2%). The OA detected were: anti-Hu in 5 samples (72%), anti-amphiphysin in one sample (14%) and anti-Ma-2 in one sample (14%). Three positive results of anti-Hu corresponded to the same patient with multiple sclerosis and suspected of tumor pathology in which the OA were measured periodically during the two years of the study without finding associated neoplastic pathology. The PNS and tumor associated to the other four positive results are shown in the table.

Conclusion: In our study the percentage of OA detected is very low (2%). Except one patient with multiple sclerosis, positive anti-Hu antibodies and absence of tumor pathology, the rest of the OA were associated with tumors and poor prognostic outcome.

Paraneoplastic neurological syndromes, onconeoural antibodies and tumors associared					
Onconeural antiobodies	Gender	Age (years)	Paraneoplastic neurological syndromes	Tumor associated	Survival (months)
anti-Hu	Male	67	Paraneoplastic encephalitis	Lung adenocarcinoma	19 months (still alive)
anti-Hu	Female	50	Paraneoplastic encephalitis	Small cell lung cancer	7 months (exitus)
anti-Ma-2	Female	65	Acute cognitive impairment	Breast cancer	2 months (exitus)
anti-amphiphysin	Male	79	Limbic encephalitis	Squamous cell lung carcinoma	2 months (exitus)

A-365

A quick protocol for the identification of Multiple Myeloma in patients attending Emergency Services with severe bone pain

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Background: Lytic bone metastases are due to a variety of primary tumors that include Multiple Myeloma (MM) and solid tumors like lung, breast, thyroid or prostate cancer. Its effects result in pain refractory to conventional analgesic treatments and osteolysis leading to spinal cord compression and pathological fractures. In 70% of all cases, the pain is the most common symptom of the bone metastases. Sometimes, patients with age over 50 years and intense bone pain are treated with analgesics without assessing the possibility of a MM at Emergency Services. After several visits to the emergency service because of the progressive increase pain and evidence of bone damage as pathological fractures the patient is admitted to study a possible MM. MM is one of the cancers with greater delay in diagnosis. Early study of the presence of pathological bone lesions is crucial for a correct diagnosis and increase the survival time of patients. The combination of quantification of serum free light chains (FLC) and serum protein electrophoresis (SPE) enables sensitive quantification of monoclonal component in the study of MM. This protocol (SPE+FLC) can help us to detect a MM in patients with incidental clinical findings without diagnosis at Emergency Service of the Hospital.

Methods: During a period of 12 months, we studied 44 patients with age>50 years old, intense bone pain and recurrent visits to Emergency Service where imaging methods (X-Rays, CT scan and MRI) showed osteolytic lesions, vertebrae collapse and pathological fractures that may be associated a MM or metastasis from a primary tumor of unknown origin (TU). The protocol (SPE+FLC) was applied to every patient to study a possible MM and the determination of tumor markers to discard a TU with bone metastasis.

Results: The diagnosis was: MM in 16 patients (36%), TU with bone metastasis in 14 patients (32%) and 14 patients without tumoral pathology (32%). In MM patients, the median age was 68 years (range 58-75) and the median time from symptoms to diagnosis was 5 months (range 2-7) with a median number of visits to Emergency Service of 3. The diagnosis was intact immunoglobulin MM in 13 patients and Bence-Jones MM in 3 patients. According to ISS system for MM; there were 2 patients in stage 1 (12%), 4 patients in stage 2 (25%) and 10 patients in stage 3 (63%). During the study there were 3 MM related deaths. The protocol "SPE+FLC" had a sensitivity of 100%, specificity of 97%, PPV of 94% and PNV of 100%.

Conclusion: In patients with age>50 years, intense bone pain with pathological bone lesions, the application of the protocol "SPE+FLC" allow us to detect a possible MM in order to apply an early treatment and increase the survival time of the patient.

A-366

QClamp a rapid and highly sensitive assay for detection of tumor FGFR3 mutations

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Background: Biological fluid-based noninvasive biomarker assays for monitoring and diagnosing disease are clinically powerful. A major technical hurdle for developing these assays is the requirement of high analytical sensitivity so that biomarkers present at very low levels can be consistently detected. In the case of biological fluid-based cancer diagnostic assays, sensitivities similar to those of tissue-based assays are difficult to achieve with DNA markers due to the high abundance of normal DNA background present in the sample.Somatic mutations in the FGFR3 gene have been associated with several cancers, most notably bladder cancer. Activating mutations in FGFR3 occur in approximately 50% of all bladder cancers and at higher frequencies in tumors of low-grade and low stage (approximately 60%-70%).There are nine common FGFR3 mutations associated with bladder cancer that are located in three exons, i.e, exons 7, 10, and 15, with the exon 7 (S249C) mutation being the most prevalent (about 62%).

Methods:Here we describe a new urine-based assay that uses a xeno-nucleic acid (XNA) clamping technology to detect low frequency somatic mutations of fibroblast growth factor receptor 3 (FGFR3) DNA that are indicative of bladder cancer. Detection of FGFR3 mutations in urine will provide clinicians with a noninvasive means of diagnosing early-stage bladder cancer.

Results:The XNA enrichment real-time qPCR assay (QClamp) detects activating oncogenic point mutations in Exon 7 (R248C and S249C) and Exon 10 (G370C and Y373C) of FGFR3 tumor derived DNA when present at an allelic frequency as low as 0.1% in a background of wild-type DNA.

Conclusion:We have developed a highly sensitive real-time PCR assay for detection of activating mutations in the FGFR3 gene that can be used to monitor the cancer recurrences and the development of tumor resistance to targeted therapies. The technology is rapid, noninvasive and can be used to detect mutations in tumor DNA found in the urine of bladder cancer patients, presenting a patient-friendly diagnostic alternative to standard-of-care cystoscopy.

A-367

Utilization of specific intact human chorionic gonadotropin analysis for pregnancy screening significantly reduces false positive results in cancer patients with the potential to improve patient care and institutional efficiency

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Background: It is standard protocol at our cancer center that women of childbearing age undergo pregnancy screening prior to any major medical procedure. False positive (FP) results are an issue as they cause delays to procedures, undue stress on the patient, scheduling conflicts, and delays for other patients. These FP results occur because a number of epithelial neoplasia can produce beta-human chorionic gonadotropin (bHCG). This subunit of human chorionic gonadotropin (HCG) is detected by the majority of HCG quantitative pregnancy assays available in the United States as they all target the beta subunit without differentiating between intact HCG (iHCG) and bHCG.

Objective: Since it is reported that iHCG predominates in early pregnancy, we believe that a reagent specific for iHCG would be useful in cancer patients. We will show that such an assay can potentially eliminate FP pregnancy tests due to tumor-derived bHCG.

Methods: From 10/21/14 – 01/20/15, patient serum samples tested on the Tosoh AIA 2000 by immunoenzymometric assay were identified having total HCG (tHCG) values ≥5 mIU/mL. This method uses enzyme labeled monoclonal antibody specific to the beta subunit to quantify the total amount of HCG. These samples were retested using a quantitative assay (Tosoh) specific for iHCG on the Tosoh AIA 2000 analyzer. Patient specimens with tHCG >14 mIU/mL, our institutional cutoff, were considered compatible with pregnancy. This unique use of 14 mIU/mL as the upper limit of normal was validated in our patient population because the cutoff of 5 mIU/mL resulted in an unacceptable amount of FP results. Results were compared with clinical history as the gold standard.

Results: Samples from 57 female cancer patients of childbearing age were attained among 2241 specimens analyzed for tHCG during the time period. Of them, 32 had tHCG >14 mIU/mL and 25 between 5-14 mIU/mL. Of the 32 with >14 mIU/mL tHCG,

eleven had iHCG below 14 mIU/mL attributable to tumor-derived bHCG yielding FP results. There were an additional two non-pregnant patients with elevated iHCG possibly due to their cancer. Together this makes up 13 FP cases due to HCG secreting tumors, of which 11 (85%) would be eliminated via iHCG analysis. The remaining 19 cases were due to pregnancy and exogenous HCG from oocyte harvesting procedures. Of the patients with results between 5-14 mIU/mL, two showed low amounts of iHCG with comparatively higher tHCG (iHCG of 0.7 and <0.5, and tHCG of 13.5 and 7.6 mIU/ml, respectively) consistent with tumor origin. The remaining 23 of these patients had similar values for both iHCG and tHCG suggesting that iHCG is more commonly elevated above 5 mIU/mL in cancer patients.

Conclusion: Pregnancy screening within the cancer population should utilize iHCG specific analysis. Based on our preliminary data, this method definitively rules out pregnancy and markedly reduces the FP rate in this setting at a cutoff at14 mIU/mL. In turn, interruptions to patient care are decreased, patients experience less undue stress, and efficiency in scheduling and delivering of medical procedures and treatments would be improved. Additional studies are recommended to further support these conclusions.

A-368

A comparison study on the test results of serum tumor markers conducted by four different automatic immunoassays

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Background & Objective:

The method of serum tumor markers tested by automatic immunoassay has significant values in the areas of early screening, treatment assessment, prognosis and recurrence monitoring. The purpose of this paper is to pinpoint the consistencies and conduct imprecision analysis on tumor markers tested by different automatic immunoassay.

Methods:

Seven tumor markers AFP, CEA, CA 125, CA 19-9, Ferritin, total PSA (tPSA) and freee PSA (fPSA) were conducted by four automatic immunoassays, namely, Architect i2000 (Abbott Diagnostics, Abbott Park, IL), AxSYM (Abbott Diagnostics), Advia Centaur (Siemens Diagnostics, Tarrytown, NY) and E170 (Roche Diagnostics, Indianapolis, IN). The consistencies on test results of AFP, CEA, CA 125, CA 19-9, Ferritin, tPSA and fPSA, as well as imprecision on the above-mentioned seven tumor markers of four immunoassays were analyzed.

Results:

The imprecison of AFP, CEA, CA 125, CA 19-9, Ferritin, tPSA and fPSA was in the range of 2.8% and 6.5% by the four immunoassays. The r coefficient on the test results of AFP, CEA, CA 125, CA 19-9, Ferritin, tPSA and fPSA by the four immunoassays were 0.996, 0.987, 0.993, 0.804, 0.994, 0.993 and 0.988 (AxSYM and Architect i2000); 0.995, 0.965, 0.990, 0.975, 0.991, 0.993 and 0.953 (Advia Centaur and Architect i2000); 0.993, 0.985, 0.990, 0.815, 0.989, 0.990 and 0.972 (E170 and Architect i2000) respectively; the slope of them were 0.962, 0.846, 0.849, 0.399, 0.852, 0.976 and 1.086 (AxSYM and Architect i2000); 0.922, 0.609, 0.846, 0.625, 0.874, 0.931 and 0.826 (Advia Centaur and Architect i2000); 1.014, 1.227, 0.999, 0.620, 1.102, 0.945 and 0.884 (E170 and Architect i2000) respectively.

Conclusions:

The test results of AFP, CA 125, Ferritin and tPSA by four immunoassays is highly identical while the results of CEA, CA 19-9 and fPSA differs in some extent, especially in the case of CA 19-9 which means the standardisation of the tumor marker testing should be further improved.

A-369

Validation of the Abbott Architect alpha-fetoprotein and total beta-human chorionic gonadotropin assays in cerebrospinal fluid for the management of central nervous system germ cell tumors

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Background: Central nervous system (CNS) germ cell tumors are a heterogeneous group of malignant brain tumors formed from primitive germ cells that primarily afflict children and young adults. The measurement of alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG) in cerebrospinal fluid (CSF) is of clinical utility for diagnosis, assessment of treatment efficacy and monitoring for recurrence. However, commercial assays for AFP and hCG are validated for use in serum or plasma but not for CSF. Therefore, the objective of this evaluation is to validate the analytical performance of the Abbott Architect alpha-fetoprotein (AFP) and total beta-human chorionic gonadotropin (beta-hCG) assays in cerebrospinal fluid (CSF) for their use in the management of CNS germ cell tumors. **Methods:** The evaluation was performed using a pool of nineteen AFP and beta-hCG-negative CSF pediatric samples to which known concentrations of AFP or beta-hCG from serum samples were added. Potential matrix effects were assessed in recovery experiments comparing serum versus CSF. Functional sensitivity was determined by dilutions to obtain the lowest concentration within 20% of the expected value having a coefficient of variation (CV) less than 20%. Linearity was evaluated with dilutions spanning the lower end of the measuring range. For imprecision, pooled samples at three different concentrations were measured in duplicate over 10 days. To determine values in the absence of disease, beta-hCG and AFP were measured in 30 pediatric CSF samples from individuals free from pregnancy, trophoblastic disease or malignancy. **Results:** Recovery values ranged from 81% to 90% for beta-hCG and 87-101% for AFP in CSF compared to serum, suggesting no significant matrix effects. Functional sensitivity was determined to be 1.59 IU/L for total beta-hCG and 0.26 ug/L for AFP, below conventionally used diagnostic cut-off values for CNS germ cell tumors of 5 IU/L and 10 ug/L. Linearity was confirmed from 2.8 to 27.8 IU/L for beta-hCG and 2.4 to 45.2 ug/L for AFP, both spanning the lower ends of their measuring ranges where the cut-off values lie. Between-day imprecision was low, with CV values from 4.1-7.7% for beta-hCG and 1.8-2.6% for AFP. Measurements in control samples were all below the measuring range of 1.2 IU/L for beta-hCG and ranged from 0-0.05 ug/L for AFP, confirming that both these markers are normally absent in CSF. **Conclusions:** The Abbott Architect assays for total beta-hCG and AFP can be used to detect beta-hCG and AFP in CSF at the low end of the analytical measuring range without significant matrix effects and with good precision, linearity and functional sensitivity. Measurements of control samples indicate that, normally, beta-hCG and AFP are essentially absent in CSF. Thus, these assays provide a suitable means to assist in the detection of malignancy for the management of CNS germ cell tumors.

A-370

Serum tumor markers in lung cancer patients

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Background: Lung cancer (LC) is the most common malignancy in the world and a leading cause of cancer deaths. The aim of this study was to determine the accuracy of serum tumor markers: carcinoembryonic antigen (CEA), cancer antigen 15.3 (CA 15.3), cancer antigen 19.9 (CA 19.9), cancer antigen 125 (CA 125), neuron-specific enolase (NSE) and cytokeratin 19 fragment (CYFRA 21-1) for diagnosis of LC.

Methods: We studied patients with clinical suspicion of LC (tos, hemoptysis, chest pain, pulmonary node, pleural effusion). We measured serum tumor markers (CEA, CA 15.3, CA 19.9, CA 125, NSE and CYFRA 21-1) by electrochemiluminescence immunoassay (ECLIA) in MODULAR E-170 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the diagnosis of bronchial cytology or lung biopsy: NOT LC and LC. The accuracy of serum tumor markers for diagnosis of LC were determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC). The optimal cuts off value of tumor markers for diagnosis of LC were considered with a high specificity (>90%), to avoid detection of numerous false positives, because of the low prevalence of cancer. The statistical software used was MEDCALC®.

Results: We studied 191 patients with ages between 33 and 86 years old (median = 65 years old). Ninety patients were LC (9 small cell lung cancer, 78 non small cell lung cancer and 3 cancer metastasis), and 101 were NOT LC. No statistically significant differences were found between LC and NOT LC patients according to CA 19.9 ($p>0.05$). AUC values were 0.732 ($p<0.0001$), 0.665 ($p<0.0001$), 0.666 ($p<0.0001$), 0.645 ($p=0.0005$) and 0.768 ($p<0.0001$) for CEA, CA 15.3, CA 125, NSE and CYFRA 21-1 respectively. The optimal cuts off value were 8.4 ng/mL (37.7% sensitivity and 90.7% specificity), 30 U/ml (34.1% sensitivity and 90.7% specificity), 55.3 U/ml (34.9% sensitivity and 91.8% specificity), 18.1 ng/mL (25.9% sensitivity and 90.5% specificity) and 2.9 ng/mL (51.3% sensitivity and 90.5% specificity) for CEA, CA 15.3, CA 125, NSE and CYFRA 21-1 respectively.

Conclusion: CYFRA 21-1 is the serum tumor marker that shows a higher accuracy for diagnosis of LC, exhibited higher sensitivity than CEA, CEA 15.3, CA 19.9, CA 125 and NSE.

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Tube type selection to support next generation sequencing of single cells

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Introduction: The ability to analyze a single cell using next generation sequencing (NGS) is of great value when analyzing rare cells like circulating tumor cells (CTCs) and capabilities are rapidly evolving. Sequencing a single cell is technically challenging due to many factors including cellular stability, therefore specialized tubes have been developed and optimized to retain cellular stability. Because the NGS process is labor intensive and expensive, the use of quality control checks to monitor both quality and quantity are necessary throughout the workflow. To select the most appropriate blood collection tube type when single cells will be analyzed by NGS, we chose to use one such quality control method (QuantiMIZE, Qiagen) due to its quantitative capabilities. Here, we report DNA quality from 12 single cells that were incubated in one of two blood collection tubes, K2EDTA or Streck Cell-Free DNA BCT.

Objective: The purpose of this study was to determine the most appropriate blood collection tube type to use when single cells are collected from patient samples for NGS.

Methods: We used a QC check (QuantiMIZE) that is a component of our NGS workflow to determine DNA quality of single cells incubated in 2 different tube types. Breast cancer cells (SKBR-3) were incubated for 2 hours in either K3EDTA or Streck Cell-free DNA blood collection tubes and were then sorted by DEPArray technology or hand sorted into single cell samples. A single cell whole genomic amplification (RepliG, Qiagen) was performed on each cell followed by a QC check for quality and quantity. The QC check uses 2 pools of 20 primers and probes that are either 100 or 200 base pairs long to analyse each sample as well as provided controls. Each sample and control is interrogated by 6 reactions: 3 (triplicate) reactions using both pools. Each sample is compared to the controls to determine ΔCts and a ratio of ΔCts between the 200 and 100 group is calculated. Ratios <0.04 (values are often negative) are indicative of high quality DNA. If Ct values are not reported (as is often the case for single cells), then the ratio is indeterminate and the quality is reported as poor.

Results/Validation: Out of the 6 single cells incubated in K2EDTA tubes, 4 cells (66%) were found to be of high quality with QC ratios of -.045, -.0129, -.084 and -.0154. The 2 cells determined to be poor quality had indeterminate values. Out of the 6 single cells incubated in Streck DNA tubes, only 2 (33%) were found to be of high quality with reported QC ratios of -.023 and -.051. Of the 4 cells determined to have low quality scores, the QC ratios were reported as 0.056 or indeterminate.

Conclusion: Although Streck tubes were designed to stabilize cells, this pilot found that single cells incubated in K2EDTA tubes resulted in overall higher quality DNA than cells incubated in Streck tubes. Further optimization of patient sample processing may be needed to fully maximize the stability of cells when collected in Streck tubes.

A-372

The Role of Circulating Angiogenesis-Associated Factors in Early-Stage Non-Small Cell Lung Cancer: Implications for Mechanistic Insights and Improved Methods for Prognostication

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Background:

Non-small Cell Lung Cancer (NSCLC) is the most common cause of mortality from cancer disease worldwide. The overall 5-year survival rates for NSCLC are less than 15%, given only a small portion of the cases being diagnosed at stage I where surgical resection being the most curative option. However, even in stage I, survival rates may still be as low as 50% due to occult diseases, mainly in nodal metastasis, leading to a high risk of recurrence. Angiogenesis being one of the hallmarks of cancer is believed to play a main role in cancer metastasis. Tie -2, an angiopoietin receptor, one of the angiogenesis related molecules expressed by vascular endothelium, yet to be elucidated in the relation to tumor progression. The objective of this study is to develop a panel of serum biomarkers based on circulating angiogenesis factors and to measure the levels of Tie-2 to improve prognostication of disease recurrence in stage I patients.

Method:

Serum levels of commercially available distinct angiogenesis biomarkers (EMD Millipore®, Billerica, MA) were evaluated against a cohort of 279 cases of NSCLC using quantitative multiplex bead based immunoassays (Luminex FlexMAP 3D) with concentrations calculated based on a five-parametric fit algorithm. Cohort was comprised of 188 cases of stage I ($T_{1-2}N_0M_0$) disease, compared to a cohort of 91 cases of lymph node positive ($T_{1-4}N_{1-3,\ x}M_0$) disease. In addition, a novel immunobead based assay for Tie-2 was validated in its performance both analytically and clinically against a subset of cases from previous cohorts of stage I and node positive serum cases. Statistical significance was determined using Mann-Whitney Rank Sum test (two-tailed) and Log-Rank to identify associations of biomarkers with nodal status or recurrence. Finally, the Random Forest algorithm was used to identify the optimal combination of biomarkers for prognosticating disease recurrence.

Results:

Significant associations were identified between nodal status and clinical outcome parameters including progression free and overall survival (r=0.331 and 0.336, respectively with significance of p<0.001 for both). TNF-RI, HB-EGF, pg130 and EGFR were found to be an optimal combination of biomarkers capable to predict the recurrence in stage I cases with good performance parameters (specificity = 89.5%; sensitivity = 24.3%; and a negative predictive value =75.3 %). Tie-2 was found to have a significant correlation in tumor differentiation (r= 0 .296, p=0.018). Further, significant increases in Tie-2 levels between well and poor differentiation (p=0.048) and between poor and moderate differentiation (p=0.018) were also identified.

Conclusion:

Precise identification of nodal status is crucial to develop an accurate treatment plan for patients with stage I NSCLC. In this study we evaluated a series of angiogenesis biomarkers and developed a panel that significantly can be utilized to predict recurrence in stage I. Combined with improved early detection methods, this panel can be a platform for developing a clinical laboratory diagnostic tool that could help further improve long term survival in this dreaded disease.

A-373

miR-25 targets the modulator of apoptosis 1 gene in lung cancer

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Background: To investigate the potential mechanisms of miR-25 in non-small cell lung cancer development and progression

Methods:we first detected miR-25 expression in clinical specimens and lung cancer cell lines by quantitative real-time polymerase chain reaction (qRT-PCR).

Results:The expression of miR-25 was elevated in the plasma of non-small cell lung cancer (NSCLC) patients and NSCLC cell lines. Transfection of A549 and 95-D cells with a miR-25 inhibitor resulted in reduced cell proliferation and enhanced apoptosis. Moreover, the modulator of apoptosis 1 (*MOAP1*) gene was identified as a novel target of miR-25. The ability of miR-25 to promote cell proliferation and block apoptosis is attributable to its effect on MOAP1 inhibition. In addition, miR-25 antagomirs significantly inhibited lung cancer growth via upregulation of MOAP1 in a mouse xenograft model.

Conclusion: these data demonstrate that miR-25 is an important marker for lung cancer, and inhibition of miR-25 suppresses proliferation and induces apoptosis in NSCLC cells by promoting MOAP1 expression.

A-374

Use of Prostate-Specific Antigen Test in National Samples of Privately-Insured, Medicare and Medicaid Patients, 2007-2012

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Background: Prostate-specific antigen (PSA) is currently the most commonly used cancer screening test. The U.S. Preventive Services Task Force (USPSTF) recommended against PSA-based screening for prostate cancer in men older than 74 and younger than 50 years of age in 2008, and against PSA screening of all men in 2012. However, other organizations, such as American Cancer Society and American Urological Association recommend that PSA screening be provided to men aged 50-74 years only after provision of information on the screening's known harms and potential benefits. The objective of this study was to evaluate the use of PSA screening in different age groups from 2007 through 2012 by using claims data from convenience samples of privately insured, Medicare and Medicaid populations, hence providing baseline information to inform interventions needed to implement evidence-based recommendations. Methods: Outpatient claims data were collected using Truven Health Analytics' MarketScan databases for 3 enrollee population samples: commercial claims and encounters (CCAE), Medicaid, and Medicare supplemental

for the years 2007 through 2012. These populations constituted an average of ~30% of privately insured US population (range, 14.0-26.0 million), as well as ~10% of Medicaid (range, 2.2-3.5 million) and Medicare (range, 1.0-2.2 million) enrollees. In order to consider PSA testing for screening only, all encounters with any one or more of 62 prostate or urinary conditions implying use of PSA for purposes other than screening were deleted. Annual claims rates per 10,000 enrollees were evaluated for up to 14 age cohorts ranging from <20 to ≥90 years. Annual claims rates for all Medicare Part B enrollees obtained from Centers for Medicare & Medicaid Services were also determined for each year. Results: Annual claims for PSA-based screening tests per 10,000 enrollees for the 3 enrollee samples in 2007-2012 ranged from 0.3-2.3 in 0-19-year age cohort to 484-3,715 in 55-59-year age group. There were 12-36 screening tests performed per 10,000 enrollees in 20-29-year age group, increasing to 428-1,726 in men 45-49 years of age. There were 188-2,158 screening tests per 10,000 enrollees in 75-79-year age group, decreasing to 69-878 in the ≥ 90-year age cohort. While there were no significant temporal trends for privately ensured ($P = 0.13$) populations, both Medicaid and Medicare enrollees showed significant downward trends ($P = 0.02$ and $P = 0.04$, respectively) while Medicare supplemental enrollees showed a significant upward trend ($P < 0.0001$). Conclusion: Prostate cancer screening with PSA continues to be done in men younger than 50 years of age and those older than 74 years old, contrary to all recommendations. In view of the current USPSTF recommendations, these results can serve as the baseline for future studies beyond 2012, and can be further extended to also evaluate insights into geographic variability in PSA screening rates. Furthermore, these findings call for the design and evaluation of intervention studies to dissuade the use of PSA screening, particularly in men younger than 50 and older than 74 years of age.

A-375

Evaluation of Chromogranin A assay on the automated Kryptor Compact Plus analyzer and comparison with current manual Elisa method

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Background: To evaluate Chromogranin A assay on the BRAHMS KRYPTOR COMPACT PLUS analyser and compare it with the current manual ELISA assay.

Methods: We evaluated the Chromogranin A assay based on its performance in the imprecision, sensitivity,linearity, carryover and correlation studies. For imprecision studies (total & within run) Brahms CGA quality control materials were used. For correlation studies between the two methods patient's serum specimens were used. The 'Analyse It' software was used for computing the evaluation statistics

Results: Onthe BRAHMS KRYPTOR COMPACT PLUS, we obtained a within-run imprecision of <1.2% CV and a total imprecision of <2.3% CV. Linearity studies showed good recovery (100 %-110 %). The lower limit of detection for the assay was10.7µg/L. Comparison between the two methods showed 91.5 % concordance for test results.

Conclusion: The performance of the automated Chromogranin A assay on the BRAHMS KRYPTOR COMPACT PLUS is comparable and acceptable to that of the current manual ELISA assay.Automated assay is better than manual assay in terms of precision and accuracy and faster turnaround time.

A-376

Results from National Colorectal Cancer Screening Program by fecal occult blood test in South Korea: analysis using the Korean National Health Insurance Corporation databases of recent 8 years (2006-2013)

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Background: There have been controversies over the clinical utility of fecal occult blood test (FOBT) as a screening tool for colorectal cancer (CRC) in the general population. Many countries, including United Kingdom, France, Italy, Croatia, and Finland, have published the results of national colorectal cancer screening by various screening strategies such as FOBT, colonoscopy and sigmoidoscopy. Since Korea is one of Asian countries with the most rapidly increasing CRC prevalence and mortality, the Korean government implemented national CRC screening in 2006, which obligated all Korean individuals who are the national health insurance subscribers and aged over 50 years to take FOBT annually. Herein, we report the results of the Korea national CRC screening by FOBT in 2006-2013, which is the largest data ever, and evaluate implementation of the program as well as diagnostic significance of FOBT for CRC.

Methods: With database from the national cancer screening program, the results of participants of CRC screening from 2006 to 2013 were analyzed. After eliminating erroneous results, the results of a total of 20,609,909 individuals who participated in the CRC screening program were included in the study. For the participants with a positive result in FOBT, colonoscopy or double-contrast barium enema test was conducted as a further additional screening test. When colonoscopy found abnormal findings, biopsy was performed. Results of FOBT, colonoscopy or double-contrast barium enema, and biopsy were analyzed by medical statistics specialists.

Results: Considering the uptake rate, the proportion of actual examinees divided by eligible target population ranged from 27.1 % to 36.8 % in 2008-2013. Continuous increasing pattern of uptake rate was observed from 2008 to 2011. Consistently, female participated more thoroughly than male in all years. Positivity rate of FOBT ranged from 5.0 % to 9.1 % in 2006-2013, which did not show significant differences by gender, age, year, or type of FOBT (quantitative or qualitative). Even though sensitivity and specificity were unavailable to be calculated due to lack of the control group, positive predictive values (PPVs) of FOBT for cancer, adenoma, and polyp were 6.3 %, 60.9 %, and 32.3 %, respectively. Male gender and older age were associated with higher PPVs of FOBT for cancer throughout study years. Complications of colonoscopy as further evaluation for CRC after positive FOBT result included bleeding, bowel perforation, and infection, all of which occurred in less than 0.5 %. Follow-up colonoscopy uptake accounted for 57.5 % of all FOBT tests performed in CRC national screening program during 8 years. **Conclusion:** These results suggest a need for intervention strategies that include organizational changes and educational activities to improve awareness of CRC screening usefulness and increase participation rates. Even though false positive rates of FOBT were still high as criticized by previous researchers, implementation of FOBT as a screening modality for Korean national CRC screening could be assessed as a success based on key performance indicators. More studies are needed to establish the value of FOBT with feasibility of scaling-up organized CRC screening by satisfactory process measures.

A-377

Comparison of pre and post radiotherapy serum butyrylcholinesterase levels in oral cancer

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Background: Oral squamous cell carcinoma (OSCC) is one of the most common malignancies recognized nowadays, and represents a public health problem. The clinical and histological features alone cannot always accurately predict whether potentially malignant disorders of the oral mucosa remain stable, regress or progress to malignancy. Identification of molecular markers which can predict disease progression is necessary for better management of these disorders. Studies have shown correlation of butyrylcholinesterase with tumorigenesis, cell proliferation and cell differentiation. So, we sought to estimate and compare serum butyrylcholinesterase levels among healthy controls and biopsy proven oral cancer patients before and after radiotherapy.

Methods: Institutional Ethics Committee gave us the permission conduct this study. After obtaining consent from biopsy proven oral cancer patients (n= 39) 2 ml of blood was taken twice once before onset of any definitive treatment and again one day after completion of radiotherapy. Simultaneously, same amount of blood was taken form age and sex matched healthy controls (n = 20). After clot formation samples were centrifuged and serum was collected for estimation of butyrylcholinesterase.

Results : Median values of pretreatment serum butyrylcholinesterase levels were significantly elevated ($p \leq 0.0001$) in oral cancer patients [6956 IU/l] as compared to that of controls [1725.5 IU/L]. There was a significant increase in median values of pretreatment serum BChE levels with advancement of oral cancer. The median values of post treatment BChE levels of cancer patients in different stages were significantly decreased as compared to their respective pretreatment levels. Conclusion: Thus, there could be a role for butyrylcholinesterase in the management of oral cancer.

A-378

Tumor markers and diabetic patients

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Background: Carcinoembryonic antigen (CEA), cancer antigen (CA) 15.3 and CA 125 are glycoproteins, and CA 19.9 is high molecular weight glycolipid. They all have been widely used as tumor biomarkers. Our aim was to investigate values of these tumor markers in diabetic patients and compare them with non-diabetic patients.

Methods: Two groups of subjects were included in the study: patients without history of diabetic (NDP) and diabetic patients (DP). Patients with cancer or other pathology that increase the serum tumor markers levels were excluded. All tumor markers were determined by electrochemiluminescence immunoassay (ECLIA) in MODULAR E-170 (ROCHE DIAGNOSTIC®). Statistical analysis was performed using the software SPSS®.

Results: A total of 1718 patients enrolled in the study, 562 (32.7%) were NDP and 1156 (67.3%) were DP. The medians of serum tumor markers levels in NDP and DP were: CEA: 1.93 ng/ml vs. 2.53 ng/ml; CA 15.3: 9.29 U/mL vs. 12.65 U/mL; CA 19.9: 15.87 U/mL vs.18.73 U/mL and CA 125: 12.37 U/mL vs. 12.47 U/mL. No statistically significant differences were found between NDP and DP according to the CA 125 levels ($p>0.05$). Serum CEA, CA15.3 and CA19.9 levels were significant higher in DP (all $p<0.0001$).

Conclusions: Serum CEA, CA15.3 and Ca19.9 levels are increased in diabetic regarding non-diabetic patients.

A-379

FREND-LC Fluorescence Immunoassay for Quantitation of SAA and Haptoglobin in Lung Cancer

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Background: The FREND™ System is a portable FREND™ cartridge reader which is based on immunoassay technology capable of quantifying single or multiple analytes by measuring laser-induced fluorescence in a single-use disposable reagent cartridge. Acute-phase proteins (APPs) have been reported in many literatures that altered levels of various APPs are correlated with different types of cancers. Our study focused on the measurement of two highly abundant serum proteins – haptoglobin(HP) and serum amyloid A(SAA) - from APP class, which were previously reported to be altered in serum from patients with lung cancer. We developed a fluorescence immunoassay (FREND™-LC*) to measure the levels of SAA and HP on the FREND™ system.

Objective: The objective of this study was to evaluate the analytical and clinical performances of the FREND™-LC assay.

Methods: The imprecision, linearity, method comparison and detection limit of FREND-SAA/FREND-HP were evaluated according to CLSI guidelines EP05-A2, EP 06-A, EP 09-A2IR and EP 17-A2. The FREND-SAA and FREND-HP assays are standardized against NIBSC 92/680 and ERM®-DA470k/IFCC respectively. For the method comparison, aliquots of serum samples over the measuring ranges were measured with FREND-SAA/FREND-HP assays on NanoEnTek FREND™ system. The comparative assays were N-latex SAA kit and N Haptoglobin assay on Siemens BNII system. For the clinical performance evaluation, the retrospective cohort study was designed, where enrolled 120 lung cancer patients and 120 apparently healthy subjects under the oversight of Kyungbook National University Hospital Institutional Review Board. Plasma specimens from the enrolled subjects were procured to NanoEnTek Research Laboratory and run on the FREND™-LC assays, and Receiver-Operating Characteristic (ROC) curves analysis was conducted. Reference Interval for each analyte was investigated using 120 serum samples from apparently healthy subjects according to CLSI guideline C28-A.

Results: The imprecision for SAA produced coefficient of variation (CV) of <10% (range 5.8-9.3%) at concentrations of 29.71, 60.85 and 119.4 mg/L. The imprecision for HP also produced CV of <10% (range 5.7- 8.0%) at concentrations of 78.67, 158.47 and 310.14 mg/dL. The AMR of the assays were 20 ~200 mg/L(SAA) and 30~400 mg/dL(HP) with Passing-Bablock regression fits of $y = 0.912x+7.040$ ($r^2=0.987$) (SAA) and $y = 1.013x -3.205$ ($r^2=0.995$) (HP), respectively. LoDs were determined to be 3.3 mg/L(SAA) and 11.3 mg/dL(HP). In the method comparison studies with SIEMENS BNII N-Latex SAA and N Haptoglobin assays, the spearman correlation coefficients were 0.9789 (SAA, N=40) and 0.9247 (HP, N=43), and the slopes /intercepts were 0.9908/-0.0204 (SAA) and 0.9395/10.3(HP) by Passing-Bablock regression fit. The area under the curve (AUC) was 0.745 when SAA and HP assay values were combined, where the sensitivity and specificity were 70.6% and 75.0%. Reference Intervals were established 0.9~9.8 mg/L (SAA) and 1.2~184.6 mg/dL(HP), respectively.

Conclusion: Data indicates that the newly developed FREND™-LC assay exhibits reliable analytical performance and can be useful as an easy-to-use lung cancer screening kit.

This work was supported by the R&D program of MKE/KEIT (10040393, Development and commercialization of molecular diagnostic technologies for lung cancer through clinical validation)

*Assay currently under development and not for clinical use

A-380

Dermcidin isoform-2 induced abolition of the effect of insulin in breast cancer being the causal pathophysiology behind the development of CAD through the nitric oxide inhibition.

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Background: Insulin plays an important role as antithrombotic humeral factor for the prevention of CAD. We have also found that dermcidin isoform 2 (DCN-2), small 11Kda stress protein, was responsible for the hypertension and diabetes and predisposing them to atherosclerosis. It has been also found that levels of estrogen and progesterone were decreased in breast cancer patients. Lower amount of estrogen cannot produce enough nitric oxide (NO) to take part in physiological function. It has been also claimed that dermcidin is the potent inhibitor of all forms of nitric oxide synthases. In fact, insulin resistance has a greater effect on breast cancer patients.

Methods: Blood was collected from the breast cancer patients and equal numbers of age and sex matched normal volunteers. The insulin resistance was measured by HOMA score analysis [HOMA is found from glucose (mmol/L) and insulin level (micro Unit/L)]. Triglycerol, HDL, HbA1c% and CRP were determined for the validity of the experiment. DCN2 level was measured by enzyme linked immunosorbant assay by using dermcidin antibody and amount of NO was assayed by methomoglobin method (Zia et al)through spectral changes.

Results: Some new interesting data were found from the experiment. Insulin resistance (IR) was confirmed by HOMA score analysis which was found to elevate in these patients than healthy normal volunteers. Triglycerol, HDL, HbA1c% and CRP levels were found to increase in this case. DCN2 protein which was elevated in acute myocardial infarction was also found to increase in the breast cancer patients and the decreased level of nitric oxide was also obtained from the experiment.

Conclusion: From the above experiment it can be concluded that insulin resistance plays the key role in pathophysiology of breast cancer development. Here we point out the role of DCN-2 protein in resistance of insulin and also examined the correlation of Triglycerol, HDL, CRP with insulin and as well as DCN-2 protein. The above experiment also demonstrates that nitric oxide is the important factor for the activity of insulin and its resistance. Therefore, insulin might have the mechanism in the development of breast cancer.

A-381

TMCO1 is a novel marker in cancer metastasis

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Objective: Discovered that transmembrane and coiled-coil domains 1 (TMCO1) could be as a novel prognostic marker in cancer therapy.

Clinical Relevance: Transmembrane and coiled-coil domains 1 (TMCO1) is highly conserved in amino acid sequence among species and ubiquitously expressed in all human tissues. Homozygous frameshift mutation in TMCO1 causes distinctive craniofacial dysmorphism, skeletal anomalies, and mental retardation. However, its physiological functions, particularly in cancer biology, are largely unknown.

Method and Results: In this study, we have found that knock down of TMCO1 in HeLa cells, a human cervical cancer cell line, and U2OS cells, an osteosarcoma cell line, remarkably inhibited their migratory capability; TMCO1 was highly expressed in the cells of the invasive front of high grade lung cancer and metastatic cancer cells in the clinical specimens, and lung cancer cells at the metastatic bone site in our animal model; Immunohistostaining revealed that TMCO1 was co-localized with

microtubules and was able to be co-sedimentated with microtubules in the presence of paclitaxel and GTP; and deficiency of TMCO1 in cells dramatically increased acetylation of tubulin. In this study, other investigation demonstrated that TMCO1 impacted microtubule dynamics, which is closely correlated with cancer metastasis, TBA drug response and therapeutic prognosis.

Conclusion: In summary, our findings provide new mechanistic insights into cancer metastasis and demonstrate that TMCO1 can be as a novel prognostic marker in cancer therapy.

A-382

Expression of Endoglin (CD105) in Experimental Colorectal Cancer

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Background:

Endoglin (CD105) is a receptor for the TGF-β1 molecule with crucial role in tumor angiogenesis. It has been shown to be a more useful marker to identify proliferating endothelium involved in tumor angiogenesis especially in cancer patients at risk of developing metastases. The aim of our study was to investigate the efficacy of COX-2 inhibitors on tumor development incidence and endoglin expression in Sprague Dawley rats in which an experimental model of colorectal cancer (CRC) was created.

Methods:

Rats were divided into 4 groups. Control group received 1 mM EDTA saline (sc, weekly) for 12 weeks and DMSO (po, daily) throughout experiment (25 weeks). DMH group received 25 mg/kg DMH in 1 mM EDTA-saline (sc, weekly) for 12 weeks and DMSO (po, daily) for 25 weeks. The groups received 8 mg/kg diclofenac and 6 mg/kg celecoxib in DMSO (po, daily) simultaneously with DMH throughout experiment were identified as treatment groups. The rats were sacrificed by decapitation at the end of experiment. Quantitative assessment of endoglin protein expression was performed on each colorectal tissue specimen by Western blot analysis.

Results:

In histopathological evaluations, no pathological change was observed in control rats, while adenocarcinoma (62.5%), dysplasia (31.25%) and inflammation (6.25%) were detected in DMH group. In treatment groups, a marked decrease was observed in adenocarcinoma rate. Expression of endoglin protein was significantly elevated in DMH group compared with the controls ($p< 0.001$). When compared to DMH group, a decrease was detected in endoglin expression in celecoxib-treated groups but no statistically significance. However, no differences in endoglin expression were observed between diclofenac-treated group and controls.

Conclusion:

In conclusion, it was confirmed by histopathological and western blotting results that COX-2 inhibitors, particularly celecoxib, decrease rate of disease and slow down progression of existing disease in CRCs. These data show that endoglin expression may have an important role in tumor angiogenesis and predict of tumor invasion.

A-383

Comparison of K2EDTA Tubes and a Specialized Tube to Stabilize RNA for Rare Single Cell Gene Expression Analysis

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Background:

Messenger RNA (mRNA) within a cell reveals a picture of real time activity and is highly sensitive to changes in the cellular environment. Therefore, to gain a true assessment of a native cellular state, collection methods must maintain the state of the cell at time of collection. This is especially important in RNA expression profiling of circulating tumor cells (CTCs), which may yield useful information in biomarker discovery for disease diagnosis and prognosis. Therefore, specialized blood collection tubes containing preservatives have been developed (i.e. Streck Cell-Free RNA BCT™ (BCT)). While these tubes have been validated for cell-free RNA they have also been shown to work with intracellular RNA and have been used to enumerate CTCs. But as diagnostic applications of rare, single cells in blood, (i.e. CTCs) continues to increase, BCT tubes must be shown to be compatible with gene expression assays demonstrating comparable or better results than traditional blood collection tubes (i.e. K2EDTA).

Methods:

Millions of SKBR3 cells, a breast cancer cell line, were spiked into either K2EDTA or BCT tubes. At time points 1 and 24 hours, cells were isolated as either ten or single cell aliquots (n=3). Additionally, cells at time point 0 were also isolated and aliquoted as described above but without being spiked into a tube for baseline comparison. Cell aliquots were then lysed, reversed transcribed and preamplified using the single cell-to-Ct kit (Life Technologies). Following cell lysis, xenoRNA a synthetic RNA transcript was spiked into each lysate to serve as an internal control. Finally, real-time PCR (qPCR) was performed and Ct values of two housekeeping genes (i.e. Actin and GAPDH) accessed to determine quality of the RNA.

Results:

Successful amplification was obtained in each ten and single SKBR3 cell aliquots at all time points in both tubes as determined by the presence of a Ct value for both housekeeping genes. For actin, the mean Ct value at time point 1 hour for a single cell was 33.35±0.69 in K2EDTA and 33.96±1.45 in BCT. At time point 24 hours, it was 33.55±4.12 in K2EDTA and 35.42±0.70 in BCT, suggesting bad RNA quality. The same Ct trends were seen in the GAPDP expression and for a single cell versus ten cells. Additionally, for single cells at time point 0, cells not spiked into either tube showed no statistical difference compared to cells in either K2EDTA ($p>0.23$) or BCT ($p>0.25$) tubes at 1 hour or K2EDTA tubes at 24 hours ($p>0.71$). However, there was a statistical difference seen for 10 cells in BCT tubes at 24 hours compared to 10 cells in K2EDTA tubes ($p<0.01$), suggesting K2EDTA superior to BCT at higher cell concentrations. Finally, to ensure validity of our methods, we demonstrated precision between run was within ±2SDI of the mean Ct value for xenoRNA expression (28.58±1.69).

Conclusion:

We have shown that BCT tubes are promising as an alternative to standard K2EDTA tubes for use in single cell gene expression assays. However, further refinement of RNA extraction and amplification methods after cell removal from BCT tubes is still needed.

A-384

Waldenstrom's Macroglobulinemia Express B-Cell Maturation Antigen and Serum Levels Correlate with Disease Status and Conventional M-Protein and IgM Levels

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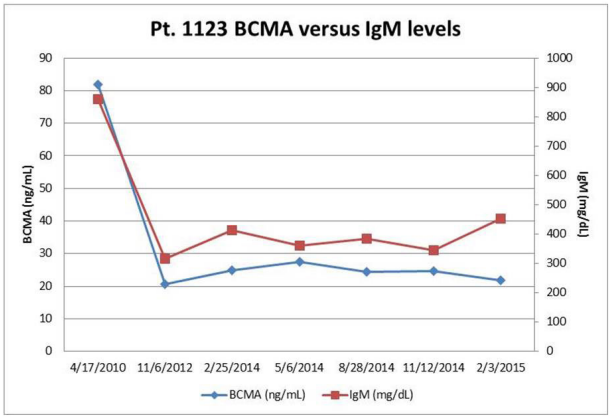
Waldenström's macroglobulinemia (WM) is an incurable B-cell lymphoplasmacytic lymphoma. B-cell maturation antigen (BCMA) is expressed on malignant B cells and we have previously shown that serum BCMA levels are elevated in multiple myeloma (MM) patients and correlate with disease status in multiple myeloma (MM) patients. Our objective was to determine whether BCMA is present in WM; and, furthermore, whether its serum levels also correlate with disease status and track with conventional tumor markers for patients with WM. Data was obtained on 20 WM patients who received treatment in a single clinic specializing in monoclonal gammopathies that was established 10 years ago. Mann-Whitney analysis was used to measure statistical significance ($p<0.05$ considered significant). Untreated WM patients with partial response (n=7) contained significantly lower levels of BCMA than samples from patients with stable disease (n=4) or progressive disease (n=7; $p=0.003$ and $p=0.0003$, respectively). Untreated WM patients (n=9) also had significantly higher BCMA levels than healthy individuals (n=14; $p<0.0001$). Additionally, the BCMA levels of seven WM patients were correlated with the serum M-protein and IgM levels of these patients during the course of their therapy. Changes in BCMA levels corresponded with changes in serum M-protein as well as changes in IgM levels. These results indicate that BCMA is present on the malignant cells from WM patients and serum levels of this protein can be used as a potential marker for tracking the course of their disease.



A-385

Identification of the Long Interspersed Nuclear Element-1 (L1) Product in Human Plasma as an Epigenetic Biomarker for Environmentally-Induced Diseases

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Background:

Environmental stressors such as particulate air pollutions, chemical carcinogens and inducers of oxidative stress can cause hypomethylation of the Long Interspersed Nuclear Element-1 (L1) promoter. Hypomethylation of L1 promoter region results in L1 activation and increased expression of open reading frame 1 (ORF1) and 2 (ORF2) proteins involved in retrotransposition. Previously published research has used genomic analysis for assessing L1 methylation and activation. Here, for the first time, we report the use of a select portion of the ORF1 protein as a target for polyclonal antibody production and measurement of L1 protein abundance in plasma. Using this antibody, a new ELISA assay was developed which has now paved the way for development of a simple blood test for assessment of L1 activation. Therefore, the objective of this study was to detect ORF1 in human plasma and to assess differences in its plasma concentrations based on gender and smoking status

Methods:

A competitive ELISA assay was developed for measuring ORF1 protein utilizing a custom-made antibody against a selected amino acid sequence of the protein. The concentration of ORF1 protein in test samples was analyzed using a standard curve generated with calibrators of known concentration. To detect and assess the concentrations of ORF1 protein in human samples, we used 61 plasma samples of known gender and smoking status from an ongoing epidemiological study.

Results:

The measuring range of the new ORF1 protein assay in plasma was determined to be linear from 2.0 to 30 ng/ml. The lower limit of detection was 2.0 ng/ml. The overall imprecision of the assay resulted in CVs of 20%, 4% and 13% at concentrations of 3.0, 6.0 and 20 ng/mL, respectively. The subjects tested for detection of ORF1 were 61 individuals with an average age of 63(SD=10.7). We found that ORF1 protein is present in the plasma of males and females, with males exhibiting a mean of 6.03 ng/ml (SD=6.7) which was significantly higher than that of females with a mean of 2.09 ng/ml (SD=1.9) (P = 0.011). A large number of female samples was below limits of detection. The mean ORF1 concentrations measured in current, former and never smokers were 7.1, 5.1 and 3.3 ng/ml respectively. The differences in ORF1 concentration were significant in former smokers vs. non-smokers (P = 0.015), as well as between current smokers vs. nonsmokers (P=0.032). Our data show for the first time that the L1 activation product is present in human plasma. Since L1 activation has been linked to various types of cancers, the new assay has great potential as a novel biomarker for assessing cancer risk due to environmental insults. We also have shown that there are gender and smoking status differences in the expression of this biomarker.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Cardiac Markers

B-001

Heart-type Fatty Acid Binding Protein as a Marker of Ischaemia in Patients Undergoing Percutaneous Coronary Intervention

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Objective: To evaluate the potential of heart-fatty acid binding protein (HFABP) as a marker of myocardial ischaemia.

Methods: Percutaneous coronary intervention (PCI) was used as a model for myocardial ischaemia. Fifty patients undergoing planned or elective PCI were prospectively enrolled. Blood samples were taken pre and post balloon inflation and 3 hours after the procedure. Samples were allowed to clot, the serum separated and stored at -20° C until analysis as a batch. Heart-fatty acid binding protein was measured using the Advia 2400 analyser (Siemens healthcare diagnostics). The assay range is 0.747 to 120 ng/mL with a 6.85% CV at 5.47 ng/mL and a 99th percentile of 6.32 ng/mL. Cardiac troponin I was measured on the Advia Centaur with an analytical range of 17 to 50000 ng/L. The CV is stated as 10% at 30 ng/L with a 99th percentile of 50 ng/L.

Results: There was no statistical difference in HFABP pre and post balloon inflation, but HFABP was significantly higher at 3 hours ($p<0.05$). HFABP was significantly higher in patients who were troponin positive (defined as troponin >50 ng/L or showing a dynamic change) versus those who were troponin negative. There was no significant difference in HFABP in men versus women or those that have had previous PCI procedures. There was no correlation between HFABP and age of patient, number of balloon inflations or total inflation time.

Conclusion: HFABP is not a marker of ischaemia. However, HFABP results were consistent with paired troponin results indicating that it is a marker of cardiac necrosis. This study indicates the ongoing need for cardiac markers for ischaemia and non-MI events.

B-002

High-sensitivity cardiac troponin I in a large community-based population at risk for cardiovascular disease

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Background: With the development of high-sensitivity cardiac troponin assays and the ability to measure cardiac troponin in essentially all healthy individuals, comes the possibility to use this assay for risk stratification for future cardiovascular disease (CVD).

Objective: To determine plasma levels of hs-cTnI and identify the impact of co-morbidities on cTnI concentrations in a large at CVD risk population.

Methods: We previously reported the development of a high-sensitivity Single Molecule Counting assay to quantify plasma cTnI. This assay was further developed into a laboratory developed test and offered in a CLIA licensed, CAP accredited laboratory with a LLoQ of 0.4 pg/mL and the (95%tile upper limit of normal (ULN) of 4.6 ng/L. Blood samples were measured for hs-cTnI, LDL, HDL and HbA1c in 23,832 (56% female) community-based patients at risk for CVD. Parametric and non-parametric analyses were performed in de-identified data using SAS v9.3.

Results: The distributions of cTnI in the study population are shown in Table. cTnI was quantifiable in 88 % patients. Seven percent of the population was above the ULN cutoff. cTnI was significantly higher in patients > 50 yrs, males, as well as those with CVD risk factors of pre-diabetes, diabetes and HDL dyslipidemia, with those patients having HbA1c >5.9% having the highest concentrations.

Conclusions: This is the largest community cohort study assessing hs-cTnI in patients at risk for CVD, as determined by their primary care physician. Differences exist between gender and age which may represent subclinical disease; as evident by patients with HbA1c >5.9% having the highest hs-cTnI concentrations. Based on these results, it may be possible to establish hs-cTnI cutoff concentrations for subgroups that may more accurately define at risk patients than use of a single 99th percentile for a healthy population. Health outcome studies are required for validation of these levels.

hs-troponin results				
	Median, ng/L	95th%, ng/L	99th%, ng/L	%>99th%
Entire cohort	0.9	6.0	21.5	7.0
Females	0.8	4.6	15.5	4.9
Males	1.2	7.6	27.0	9.4+
	0.6	3.4	10.9	3.1
>=50 y	1.1	7.0	24.4	8.5+
HbA1c <=5.9%	0.9	5.1	17.6	5.8
HbA1c >5.9%	1.3	9.1	34.6	11.3+
LDL <= 129 mg/dL	1.0	6.4	22.3	7.6
LDL > 129 mg/dL	0.9	5.8	20.1	6.7
HDL at target*	0.9	5.8	20.1	6.8
HDL less than target*	1.0	7.5	25.4	8.6+
HDL target	>=45 mg/dL females	>-=35 mg/dL males	+P<0.0001	

B-003

Changes in the utilisation of evidence-based recommendations for the biochemical diagnosis of acute myocardial infarction.

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Objective. To assess the progress towards the utilisation of evidence-based practice for the diagnosis of acute myocardial infarction in Europe.

Methods. Three consecutive audits in 2006 (pilot study), 2010 and 2013 were performed using a web-based questionnaire distributed to European biochemical societies for circulation to their membership. Questions covered cardiac biomarkers measured, decision thresholds and their derivations, sampling strategies, repeat sample interval and use of decision-making protocols. Results were collated and linked into using a central database, the data coded and then analysed using comparative and descriptive nonparametric statistics.

Results. Returns were obtained from 8 (pilot),39 and 35 respectively by year, countries throughout Europe (220, 303, 442 responses). Central or University hospitals provided respectively (by year) 55%, 58% and 50% of responses with 39%, 35% and 39% from local (community) hospitals (Chi^2 2.83 ns).

The measurement of cardiac troponin has now become the preferred cardiac biomarker in 99.5% of hospitals and the first line marker in 97.7% (95.3% in 2006 and 2010) with 37.7% of those hospitals offering troponin alone as cardiac biomarker (28.6% 2006, 31.4% 2010) Chi^2 6.05 p =0.48). The proportion of non-recommended markers offered has fallen significantly (Chi^2 104.2, p,0.0001). Aspartate transaminase was offered by 52.7% as part of the cardiac profile in 2006, 34% in 2010 but is now offered by only 1.1%. Lactate dehydrogenase or hydroxybutyrate dehydrogenase is offered by 15.6% of laboratories, (25.5% in 2006, 35.6% in 2010). In acute units in 2013, creatine kinase (CK) or CK MB isoenzyme continue to be offered as supplementary markers either as part of a cardiac marker panel or on request in 72% of hospitals. However, 25.6% continue to offer CK-MB activity. The usual reason cited was clinician familiarity or need for a short term marker.

There has been a statistically significant shift from the use of assay imprecision at the 10% coefficient of variation to the use of the 99th percentile as the decision limit with an increase in use of the 99th percentile from (35.4% 2006, 37.9% in 2010, 52.1% in 2013, p <0.0001). There has been an increased use of the manufacturers data sheet as a source of data (51.9% in 2006, 50.2% in 2010, 62.2% in 2013) Chi^2 89.4, p<0.0001.

11.1% used values taken from the literature but local validation of the 99th percentile was only reported in 0.9% of those measuring troponin (2013 data).

Conclusion. Although there has been a significant improvement in use guideline-based practice there is still a lack of use of the 99th percentile and lack of independent assessment of the analytical performance claims.

B-004

Reference Range Study Using High Sensitivity cTnI Assay in Development for the Sgx ClarityTM Single Molecule Counting Platform

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Background:

The Research Use Only (RUO) cardiac Troponin I (cTnI) assay developed by Singulex for the ERENNA® instrument provided the first evidence that cTnI is measurable in almost all healthy subjects. With that knowledge, it has become clear that cTnI is not only a valuable marker for diagnosing acute myocardial infarction, but it may also be useful as a prognostic indicator for underlying heart disease. Singulex is now developing the Sgx Clarity System, a fully-automated in vitro diagnostics platform that, similar to the ERENNA system, utilizes Single Molecule Counting technology. The new system will be a clinical diagnostics instrument utilizing a second generation scanning-based detection system which has equal sensitivity to the ERENNA with an improved throughput and an extended dynamic range. The cTnI assay in development for the Sgx Clarity System is based on the 2 + 2 antibody concept introduced by HyTest, with two capture and two detection antibodies. One antibody of each pair recognizes an epitope in the stable central portion of the cTnI molecule, while the second recognizes an epitope in the N- or C-terminal region, thus conferring overall robustness to known cTnI-specific interferences.

Objectives:

To estimate the 99th percentile of cTnI values in apparently healthy subjects on the Sgx Clarity system using a set of reference range samples and to assess preliminary performance characteristics of the assay.

Methods:

For the reference range study 120 male and 120 female EDTA plasma samples from self-declared healthy donors were collected from five states across the US. The 99th percentile was calculated using a nonparametric method using StatisProTM software and gender specific results were compared. Additional performance characteristics were assessed according to protocols based on CLSI guidelines where available.

Results:

The 99th percentiles were 13.38 pg/mL and 4.90 pg/mL for male and female donors, respectively. The 99th percentile for the overall population was 11.11 pg/mL. Based on this analysis, the recommendation for gender specific reference ranges should be considered. A precision profile was generated from the duplicate samples less than 1 pg/mL in the reference range study, and the within-run 10% and 20% functional sensitivities were calculated at 0.31 and 0.13 pg/mL, respectively. Troponin values were quantifiable above the 20% functional sensitivity in 100% of samples tested. The reportable range goes up to 25 ng/mL with no high dose hook effect up to 1000 ng/mL, thus giving the assay a 5 log linear dynamic range. No significant impact from common endogenous interferences was observed and the assay formulation was shown to be robust against common cTnI-specific interferences such as heparin, proteolytic cleavage, phosphorylation, and cTnI-C complex. Furthermore, minimal cross reactivity was observed when tested with 1000 ng/mL of sTnI (0.019%), cTnT (0.03%) and TnC (0.00005%).

Conclusion:

The cTnI assay in development for the Sgx Clarity System demonstrates sensitivity and precision that is equivalent to the ERENNA RUO assay, sufficient to quantify cTnI in 100% of apparently healthy donor samples. This sensitivity allows for the determination of gender specific differences in the 99th percentile of Normal cTnI values.

B-005

Association of Blood Homocysteine levels with Subclinical Coronary Atherosclerosis in Asymptomatic subjects

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Background: Atherosclerotic plaques progression has been known to be correlated to elevated circulating homocysteine (Hcy) due to increased thrombogenicity, oxidative stress and endothelial dysfunction. But it remains to be unclear whether the level of Hcy is related with the coronary atherosclerosis in subclinical state. Therefore, we performed this study to investigate the relationship between blood Hcy levels and subclinical atherosclerosis in asymptomatic self-referred subjects.

Methods: We retrospectively enrolled 2,968 self-referred asymptomatic subjects (1,374 men, 1594 women) who had undergone both coronary CT angiography (CCTA) and coronary artery calcium scoring. The relationships between atherosclerosis, Hcy, and other clinical factors were assessed. The subjects were divided into 4 quartile groups (<7.7, 7.7-9.0, 9.1-10.9, >10.9 μmol/L). We investigated the association of each Hcy quartile with coronary artery calcium score (CACS), coronary plaque, coronary stenosis.

Results: High level of Hcy was related with age (P <0.001), male gender (P <0.001), body mass index (BMI) (P <0.001), waist circumference (P <0.001), Blood pressure (P <0.001), high density lipoprotein (HDL) (P < 0.001), Triglyceride (P = 0.003), Blood glucose (P < 0.001), HbA1c (P = 0.01), hsCRP (P = 0.006), the number of plaques (P <0.001), extent of coronary stenosis (P <0.001), CACS (P <0.001). The factors associated with CACS were age, Hcy, HbA1c and hsCRP. Logistic regression analysis adjusted for gender and confounding factors showed that the third- and fourth-quartile Hcy level groups had higher odds ratios [odd ratio (OR) 3.980 (1.723-9.195), P = 0.001, 7.355 (3.291-16.439), P <0.001, respectively] for high CACS (CACS > 400) than did the first quartile group. Coronary plaque was more frequently found in higher Hcy quartile groups (21.3%, 28.8%, 34.4% and 34.3%, P <0.001). Significant coronary artery stenosis (stenosis>50%) was also more frequently found in higher Hcy quartile groups (1.8%, 5.4%, 5.0%, 6.6%, P <0.001).

Conclusion: High levels of blood Hcy were related with an increased risk of the presence and the extent of subclinical atherosclerosis in asymptomatic subjects.

B-006

Characterization of plasma Endothelin-1 in a large community-based patient population at risk for cardiovascular disease

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Background: Endothelin (ET-1, (aa1-21)) is a potent physiological vasoconstrictor that plays a pivotal role in vascular dysfunction. Elevated, plasma ET-1 correlates with both the occurrence and severity of atherosclerosis and heart failure; however, no reports characterize ET-1 in a population, judged at-risk for cardiovascular disease (CVD) using traditional biomarkers (lipids, hsCRP, HbA1c).

Objective: To determine plasma levels of ET-1 and identify the effect of co-morbidities on its levels in a large at CVD risk study population.

Methods: We previously reported the development a Single Molecule Counting immunoassay to quantify plasma ET-1. This assay was further developed into a laboratory developed test and offered in a CLIA licensed, CAP accredited laboratory with a LLoQ (20%CV) of 0.2 pg/mL and a 95%tile (upper limit of normal; ULN) of a healthy reference population of 3.7 pg/mL. Plasma ET-1 as well as other blood biomarkers including LDL, HDL and HbA1c were measured in a non-selected community-based population at risk for CVD (n=8,422 patients). Identifying information was removed and the data were analyzed via parametric and non-parametric analyses (SAS v9.3, p<0.05 considered significant).

Results: The characteristics of ET-1 in the CVD risk population are shown in Table. ET-1 was quantifiable in >99.9 % patients and 5.9% were above the ULN. ET-1 concentrations were slightly higher in patients > 50 yrs, males, as well as those with CV risk factors of pre-diabetes, diabetes and HDL dyslipidemia. Of note ET-1 was paradoxically higher in patients with low LDL.

Conclusions: This is the first report of ET-1 concentrations in a large community-based population at risk for CVD, as determined by their primary care physician. Minor differences exist between subgroups; however, an overall ULN of 3.7 pg/mL appears to be appropriate, and unlike high-sensitivity cardiac troponin I, age and gender specific reference ranges may not be required.

Endothelin results				
	Median, pg/mL	95th%, pg/mL	99th%, pg/mL	%>95th%
Entire cohort	2.4	3.9	5.5	5.9
Females	2.4	3.8	5.4	5.4
Males	2.5	4.0	5.9	6.6++
	2.2	3.4	4.7	2.1
>=50 y	2.5	4.1	5.8	7.5+
HbA1c <5.9%	2.4	3.8	5.4	5.2
HbA1c >=5.9%	2.6	4.4	6.1	8.8+
LDL <= 129 mg/dL	2.4	4.0	5.7	6.6
LDL > 129 mg/dL	2.4	3.7	5.4	4.9+
HDL at target*	2.4	3.8	5.5	5.7
HDL less than target	4.5	4.2	5.8	7.6++
HDL target	>=45 mg/dL females	>=35 mg/dL males	+p<0.001	++P<0.05

B-007

Current use of evidence-based recommendations for the biochemical diagnosis of acute myocardial infarction in routine clinical practice - a comparison of European and North American practice.

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Objective. To assess current use of evidence-based guidelines for the use of cardiac biomarkers for the diagnosis of acute myocardial infarction in Europe and North America.

Methods. In 2013/14 a web-based questionnaire was distributed to North American and European biochemical societies for circulation to their membership. Questions covered cardiac biomarkers measured, analytical methods used, decision thresholds and their derivations, sampling strategies, repeat sample interval, use of rate of change and use of decision-making protocols. Results were collated using a central database and analysed using comparative and descriptive nonparametric statistics.

Results. In Europe (E), returns were obtained from 442 hospitals, 50% Central or University hospitals and 39% from local hospitals from 35 countries. 395/442 (89%) provided an acute service and were analysed further. In North America (NA) there were 91 responses (63.7% Central or University hospitals, 19.8% community hospitals) with 76/91 (83.5%) providing an acute service. Cardiac troponin was the preferred cardiac biomarker in 99.5% (E) and 98.7% (NA) and the first line marker in 97.7% (E) and 97.4% (NA). Of those markers no longer recommended, in Europe aspartate transaminase was still offered in 1.0% with lactate dehydrogenase in 15.2%, with only 0% and 2.6% in North America., respectively. Creatine kinase (CK) or CK MB continue to be offered as supplementary markers either as part of cardiac marker panel or on request in 72% (E) and 39.5% (NA).

There were significant differences in the choice of decision limits and their derivations. Decision limits (E vs NA) were: 20% CV - 16.2% vs 24.2%; 10% CV - 2.0% vs 8.1%; 99th percentile - 52.3% vs 45.2%; other - 29.5% vs 22.6%; p 0.02. The origin of the information was also significantly different, with E vs NA as follows: package insert - 61.9% vs 40%; publications - 17.1% vs 15.0%; local clinical or analytical validation choice - 21.0% vs 45.0%; p 0.0003.

Conclusion. There are significant differences between European and North American use of cardiac biomarkers. This probably relates to different availability of assays between Europe and North America (such as high sensitivity troponin assays) and different laboratory practices on assay introduction (greater local evaluation of assay performance occurred in North America). Better awareness, agreement, adaptation and adherence with evidence-based guidelines is needed. This survey indicates that more effective strategies for disseminating choice of cardiac marker testing and diagnostic cut-off limits is needed.

B-008

An ARCHITECT Assay for Quantitation of Galectin-3 in Human Serum and Plasma

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BACKGROUND: Galectin-3 has been implicated in a variety of biological processes associated with heart failure (HF). An assay for galectin-3 on ARCHITECT *i* series (ARCHITECT Galectin-3) has been cleared in the US for the quantitative determination of galectin-3 in human serum and EDTA plasma and may be used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure (CHF).

METHODS: ARCHITECT Galectin-3 is a chemiluminescent microparticle immunoassay (CMIA) utilizing paramagnetic microparticles coated with mAb M3/38 and an acridinium- labeled mAb 87B5 conjugate. Sample and microparticles are combined and incubated in the first step. After a wash, the conjugate is added to the mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting signals in relative light units (RLUs) is directly proportional to the amount of galectin-3 in the sample and allows quantitative determination of galectin-3 in serum and EDTA plasma.

RESULTS: The calibration range for the assay is 0.0 to 114.0 ng/mL. The LoB, LoD and LoQ were 1.0, 1.1 and 2.8 ng/mL, respectively. Linearity supports a measurement range of 5.5 to 103.1 ng/mL. There was no High Dose Hook effect when samples containing up to 1420.5 ng/mL of galectin-3 were assayed. In the sample tube type study, a matrix comparison of > 40 matched serum and EDTA plasma samples from CHF patients demonstrated a linear correlation (Slope: 0.9 - 1.1; $r \geq 0.9$) within the measurement range for each tube type when compared to the Red Top serum samples. In the interference studies, 11 endogenous substances spiked individually into sera and plasma were tested at the following levels: (≥) 1000 ng/mL HAMA, 800 IU rheumatoid factor, 40 mg/dL conjugated bilirubin, 40 mg/dL unconjugated bilirubin, 5 g/dL human gama globulin, 5 mg/dL creatinine, 5 mg/mL whole blood lysate, 3000 mg/dL triglycerides, 250 mg/dL hemoglobin, 500 mg/dL cholesterol, and 12 g/dL human serum albumin. The average percent difference between test and control samples for all endogenous interferents was ≤ 10%. Percent difference in the presence of 35 potentially interfering drugs was ≤ 4.0%. The potential cross-reactants (galectin-1, galectin-2, galectin-4, galectin-7, galectin-8, galectin-9, galectin-12, collagen type I, and collagen type III) were evaluated at a concentration of 500 ng/mL. The percent cross-reactivity of the potential cross-reactants was ≤ 0.3%. The Precision study of 3 controls and 5 panels demonstrated a total %CV ≤ 5.8%. In a clinical performance study of 405 CHF patients, elevated baseline levels of galectin-3 (> 17.8 ng/mL) in CHF patients were shown to be significantly and independently associated with a higher risk of hospitalization due to worsening HF, ventricular assist device placement, cardiac transplantation or all-cause mortality (first to occur).

CONCLUSION: The ARCHITECT Galectin-3 assay is an accurate, precise, and sensitive assay for the quantitative determination of galectin-3 in human serum and EDTA plasma. The ARCHITECT Galectin-3 assay may be used in conjunction with clinical evaluation as an aid in assessing the prognosis of patients diagnosed with chronic heart failure.

B-009

High MMP-9 Level in Serum Is an Independent Predictor of Non-Calcified Coronary Atherosclerotic Plaques in Unselected Population

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Background:

Lipid enriched non-calcified plaques are considered more instable and rupture prone than calcified lesions. Our previous small case-control study found higher levels of circulating Matrix metalloproteinase-9 (MMP-9) in patients with non-calcified plaques contrast to those with calcified plaques or non-plaques, consistent with that expression of MMP-9 in lesions is associated with plaques vulnerability. In the present study we investigated the value of serum MMP-9 and MMP-2 levels in predicting non-calcified plaques and screening feasibility during routine hospital practice.

Methods:

862 unselected consecutive outpatients with stable chest pain who undegone coronary CT angiography (CCTA) were divided into four groups by CCTA: non-plaque (n=474), calcified (n=159), non-calcified (n=80) and mixed plaques (n=149).

Demographic data and biochemical indicators were collected and serum levels of MMP-9 and MMP-2 were determined by ELISA. All statistical analyses were performed using SPSS version 17.0.

Results:

Serum MMP-9 levels of non-calcified plaque group was significantly higher than non-plaque, calcified plaque and mix plaque group (731.0±271.2 ng/mL vs 535.6±246.1 ng/mL, 589.2±272.6 ng/mL, and 588.2±246.7 ng/mL, $P < 0.001$), implying that MMP-9 may identify non-calcified plaques, while MMP-2 had no statistic difference between non-calcified plaque and other groups. Furthermore, multivariate logistic regression analysis suggested MMP9 was an independent predictor of non-calcified plaques adjusting for covariables including age, sex, HDL-c, Hs-CRP, CREA, GLU, stenosis, branch, MMP-2 ($P < 0.001$). Interestingly, the AUC value of circulating MMP-9 for predicting non-calcified plaques was 0.701 (95% CI, 0.644-0.758; $P < 0.001$) alone, and no significant increases combined with other indicators. In addition, we also found that serum levels of MMP-9 and MMP-2 increased in patients with plaques compared non-plaque (609.2±256.2 ng/mL vs 518.6±219.9 ng/mL, 197.8±56.5 ng/mL vs 181.7±43.5 ng/mL, $P < 0.001$). Similarly, both MMP-9 and MMP-2 were independent predictors for presence of plaques ($P < 0.001$, $P = 0.003$). And the AUC value were 0.717 (95% CI, 0.683-0.752; $P < 0.001$) when combining MMP-9, MMP-2 and age.

Conclusion:

High serum MMP-9 level is an independent predictor of non-calcified coronary atherosclerotic plaques in unselected population. Additionally, both MMP-9 and MMP-2 may identify patients with or without plaques. In conclusion, serum MMP-9 and MMP-2 could be biomarkers of screening plaques during routine hospital practice.

B-010

Circadian Rhythm of Cardiovascular Disease-Related Micro RNAs from HemaSpot ™Dried Blood Samples

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Background:

Disruption of the body's circadian rhythm has been linked to numerous chronic cardiovascular diseases (CVD) such as insomnia, and hypertension. Changes in gene expression of cardiovascular pathways by mirco RNAs (miRNA) have been identified as potential biomarkers of CVD. miRNA are short (~22 nucleotides), single-stranded, non-coding, posttranscriptional regulators expressed in various tissues including circulating blood. MicroRNAs are ideal biomarkers for disease as they are abundant, relatively stable, and easily quantifiable. Dried blood spot (DBS) sampling technology has advanced to provide assessment of numerous analytes and blood-based biomarkers including proteins, DNA and micro RNA (miRNA). In this study we utilize the HemaSpot blood collection device to analyze the expression profiles of miRNAs that have been shown to be differentially regulated in individuals with cardiovascular diseases. By understanding the expression patterns of CVD related miRNA in healthy individuals, we seek to identify changes in expression of these biomarkers on DBS samples from at-risk or CVD patients.

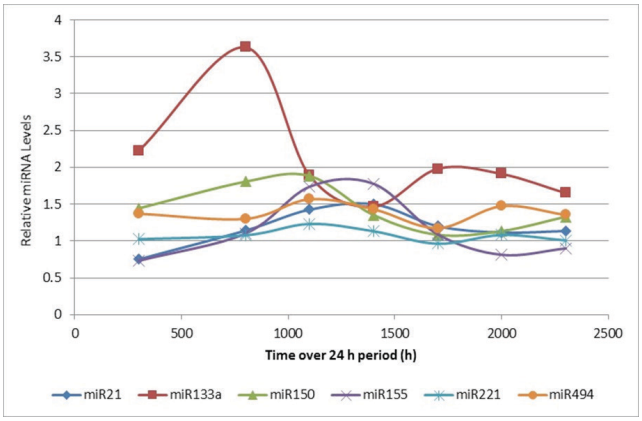
Methods:

2-3 drops of whole blood were collected by finger stick onto the HemaSpot HF device every four hours (8 time points) over a 24 hour period from five healthy individuals. Total RNA was extracted from DBS samples and Taqman ® RT-PCR analysis was performed to determine the expression profile of six miRNAs (miR21, miR133a, miR150, miR155, miR494, and RNU48). The relative quantitation of each CVD-related miRNA was normalized to that of the non-variable small RNA molecule RNU48.

Results:

MiR133a shows a cyclic expression pattern with an average early morning peak approximately 9:30 am all five individuals over a 24-hour period. MiR155 displays peak expression at noon (mid-day) of the 24-hour cycle.

Conclusion: The HemaSpot device proves to be an optimal tool for studying circulating miRNA expression profiles from dried blood spot samples. Cyclic expression of miRNAs may prove to be significant biomarkers for regulation of gene expression of cardiovascular diseases.



B-011

Generation and Characterization of Monoclonal Antibodies against H-FABP for Use in Rapid and Sensitive Immunological Diagnostic Assays Development

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Background:

Heart-type fatty acid-binding protein (H-FABP) is a small cytoplasmic protein (15 kDa) released from cardiac myocytes, detectable as early as 30 minutes following an ischemic episode. Although H-FABP has been studied as a cardiac biomarker since early 1990s, early progress in clinical studies was hampered due to the lack of monoclonal antibodies (MAbs) and highly quantitative and accurate assays. The objective of this study was to prepare a set of MAbs against H-FABP with high specificity and affinity for use in the rapid and sensitive immunological diagnostic assays development.

Methods:

The recombinant H-FABP protein prepared in-house was used as the immunogen. On day 1, BALB/c mice were immunized at dose 20 µg/mouse in 200 µl of Freund's complete adjuvant intraperitonially. The second and third immunizations were done on day 15 and day 29 with the same immunogen in Freund's incomplete adjuvant, respectively. Mice were bled on day 36 and the immune response to H-FABP protein was assessed by in-house developed enzyme-linked immunosorbent assay (ELISA). Three mice with the highest antibody titer were given a final pre-fusion boost intraveneously, and the mice were sacrificed and the spleens were removed four days later. The splenocytes were fused with the mouse myeloma cell line SP2/0 using PEG-1500. The microplate tissue culture wells exhibiting hybridoma growth were screened for the production of specific antibodies against H-FABP. The H-FABP specific antibody-producing cells with higher antibody titer were then subjected to three subsequent rounds of subcloning steps, by limiting dilutions, for obtaining stable hybridomas. All established 63 MAb clones secreting the desired antibodies were purified by using protein G affinity chromatography. The most suitable matched pairs had been tested by colloidal gold-based lateral flow test (LFT) to ensure the compatibility in forming a sandwich with H-FABP. Most importantly, there was no cross-reactivity with other types of FABP proteins, e.g. FABP-1, FABP-2, FABP-4, FABP-5. Furthermore, we obtained four well-matched MAb pairs for further assay development such as fluorescence-based lateral flow assay (FLFIA). The application of these MAbs in the latex-enhanced immunoturbidimetric assay (LEITD) was also evaluated. The comparative studies from clinical samples tested by our FLFIA and LEITD with the Randox H-FABP clinical chemistry assay were performed.

Results:

Sixty-three stable monoclonal hybridoma cell lines and four well-matched antibody pairs were generated and characterized eventually using the above-mentioned methods. In FLFIA, the best antibody pair (H-FABP08:H-FABP31) was selected with the sensitivity of 0.5 ng/ml, linearity range to 64 ng/ml and inter-assay CVs <15%. In LEITD, the MAbs showed a great ability with the sensitivity of 2.5 ng/ml, linearity range to 160 ng/ml and inter-assay CVs <15%. The comparative results from clinical samples revealed that both our FLFIA and LEITD kits correlates well with the Randox's method (R2=0.95 and 0.98, respectively).

Conclusion:

We described in this study that we have successfully generated and characterized 63 highly sensitive and specific MAbs against H-FABP. A superior antibody pair (H-FABP08:H-FABP31) was proved to be the best candidate with promising and potential applications in rapid and sensitive immunological diagnostic assays development, e.g., FLFIA and LEITD kits.

B-012

Retrospective analysis of 160,000 BNPs shows that initially low NT-pro BNP is highly predictive of future negative NT-proBNP

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Background:

Low BNP or low NT-proBNP are strong negative indicators of congestive heart failure. Based on our findings of low biologic variation in patients with normal BNPs, we recommended that emergency department patients have no more than 2 consecutive negative BNP's before a computer-generated message is sent to the attending physician stating that another BNP was superfluous, unless there was a change in the patient's clinical status. This encouraged us to study NTproBNP.

Methods:

We analyzed 6.5 years of Salzburg University BNP data (164490 NT-proBNP from 52103 patients). We identified all patients who had repeatedly low results (two, three, or four times consecutively), under 200, 150, or 100 pg/mL. For three age groups, we determined the probability that the subsequent BNP would be positive (> 450pg/mL and >900 pg/mL for ages <55y and >55y, respectively).

Results:

The Table presents the probabilities of a positive test following a series of negative NT-proBNP's as well as the reduction in BNP testing. The greater the number of consecutively low NT-proBNP or the lower the BNP limit, the lower the probability of a positive test but at a cost of increased testing.

Conclusion:

Repeatedly low initial values signify a high probability of another negative test result. In the situation of prior low BNP test results in a newly re-admitted patient, either the laboratory information system or clinical information system should inform the attending physician: "In the absence of new clinical symptoms, another NT-proBNP would offer no new diagnostic

information." Between 1 and 5.5% of NT-proBNP tests would not be done, depending on replicate/cutoff combination and resulting in savings ranging from 34100 € to 452168 €

Number of consecutive BNPs & upper BNP limit	Age < 55y (N=27,119 BNP's)		Ages 55-75y (N=78,248 BNP's)		Ages >75y (N=59,123 BNP's)		
	% missed	BNP's not done	% missed	BNP's not done	% missed	BNP's not done	Total BNPs not done
2 <200pg/mL	5.0	3026	4.0	5115	8.3	852	8993
2 <150	4.0	2505	3.5	3791	7.3	507	6803
2 <100	3.5	1806	2.4	2273	5.8	224	4303
3 <200	3.3	1750	2.7	2922	5.7	436	5108
3 <150	2.3	1409	2.2	2089	4.5	245	3743
3 <100	1.8	979	1.7	1190	2.9	105	2274
4 <200	2.8	1150	2.1	1871	3.2	251	3272
4 <150	2.1	906	1.5	1300	2.8	143	2349
4 <100	1.9	582	1.4	725	3.5	57	1364

B-013

Automated approaches to wrangling wayward troponins

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Background: Quantification of cardiac troponin I (cTnI) serum concentration is a critical part of evaluating patients for acute myocardial infarction. Errors in this quantification can lead to significant clinical mismanagement. To mitigate this risk, we sought to quantify the frequency of false-positive cTnI results and develop strategies to prevent them.

Methods: We reviewed clinical cTnI results (N=22,976; 1/21/2014 - 11/20/2104) performed using the Beckman Coulter AccuTNI+3 assay across two academic medical centers. cTnI results for individual patients were grouped and then serial tests were compared based on their resulted concentrations and collection times.

Results: Retrospective analyses of the temporal patterns of cTnI concentrations for individual patients revealed decay rates that were faster than physiologically expected and not explained by other clinical factors. Focusing on results > 0.04 ng/mL with follow-up testing within 24 hours (N=6,205; 27%), we developed specific criteria ($t_{1/2}$ < 3.7 hours; %Δ[cTnI] ≤ -25%; 1st [cTnI] > 0.05 ng/mL; 2nd [cTnI] 0.03 ng/mL, (B) rapid concentration increases (t_2 < 4.7 hours; %Δ[cTnI] ≥ 35%), and (C) unexpectedly rapid concentration decreases ($t_{1/2}$ < 5 hours; %Δ[cTnI] ≤ -20%). Retrospective analyses indicate that the application of these rules to our study data would have triggered repeat assessment in 15% of cTnI tests and identified all of our suspected false elevations. Additionally, we expect this intervention to identify false elevations that our retrospective analyses could not flag because there was no timely follow-up testing.

Conclusions: Retrospective analyses of serial cTnI concentrations have identified a set of potentially falsely elevated results (~1 in 200) with important clinical ramifications. We are leveraging the power of our middleware system to prospectively identify these potential errors in real-time and automatically trigger repeat evaluation. Ongoing studies, including cross-institution and cross-instrument comparisons of the temporal patterns of cardiac troponin concentrations, will help to understand the sources of these potential false-elevations and enable the refinement of strategies to prevent patient harm.

B-014

Correlation between platelets volume indices and risk of cardiovascular disease factors: Results from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

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Background: Pro-inflammatory and pro-thrombotic activities of platelets play a key role in the development of atherosclerotic plaque and in the formation of intra arterial thrombus. In this account, a better knowledge of such mechanisms has increased the interest in platelet functions measurements. Platelet function can be indirectly estimated by platelets volume indices (PVI), which are easily obtained by automated hematology analyzers, which provide platelet count and some indices, such as mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR). Several studies have related cardiovascular diseases (CVD) and their risk factors with PVI. Yet, the papers' results are conflicting, probably because of methodological differences as well as lack of measurement standardization. The aim of this study was to investigate the relationship of the PVI with CVD risk factors included on the Framingham Risk Score (FRS), and the score itself.

Methods: We used the baseline data (2008-2010) of 3115 civil servants (aged 35-74 yr) from a university and a research institution located in Minas Gerais state and enrolled in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Venous blood sampling was performed after 12- to 14- hour-fasting, using tubes containing ethylenediaminetetraacetic acid (EDTA). The time between sampling and exam procedure was strictly controlled to be within 2 hours, and blood samples were kept at room temperature until the PVI measurements were performed. The population was distributed according to their exposure to different risk factors (age, sex, systolic blood pressure, total and high-density lipoprotein cholesterol, smoking, diabetes status, and use of antihypertensive medications), and stratified for cardiovascular risk, based on FRS. In the comparison between subgroups, with and without different risk factors, Wilcoxon test was performed. Additionally, multiple linear regression analysis was used to estimate the association of PVI measures with the FRS, after adjusting for variables that are not part of the FRS, but can increase CVD risk was applied for analysis.

Results: MPV (adjusted r^2=0,084; p=0.013), PDW (adjusted r^2=0,086; p<0.001), and P-LCR (adjusted r^2=0,085; p=0.004) was independently associated with the FRS after adjustment for education, skin color, alcohol use, and physical activity. It was observed that a one unit increase in MPV, PDW, or P-LCR increases the FRS by 0.59%, 0.40%, or 0.08%, respectively. In the comparison between subgroups, with and without different CVD risk factors, PVI is associated only with diabetes and systolic hypertension. Diabetics had higher MPV (p=0.003), PDW (p<0.001), and P-LCR (p=0.001) median. The same was observed for systolic hypertension: MPV (p=0.031), PDW (p=0.004) and P-LCR (p=0.020) median was higher in the hypertensive subjects.

Conclusion: In this large cohort of free living Brazilians, ours results showed that increased PVI is independently associated with diabetes, systolic hypertension, and higher CVD risk based on the FRS. It is unclear if the relationship with the FRS is mostly due to diabetes and hypertension, as they are part of the FRS. Prospective follow up of this population may help to clarify if PVI is independently associated to the development of CVD and can add accuracy to the CVD risk stratification.

B-015

Circulating myostatin levels in heart failure with preserved ejection fraction

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To date, heart failure with preserved ejection fraction (HFPEF) represents a growing medical and economic challenge since, in contrast to HF with reduced EF (HFREF), there is no evidenced-based therapeutic approach that lowers mortality. Hence, a better comprehension of the complex pathophysiology of HFPEF is urgently needed. Myostatin, a negative regulator of skeletal muscle growth, has been implicated in the regulation of myocardial hypertrophy and cardiac cachexia; however, no data are available on myostatin levels in HFPEF. This observational study investigated circulating myostatin levels in femoral artery (A) and vein (V) as well as coronary sinus (CS) of 43 patients (n = 17, controls without HF; n = 19, HFPEF; n = 7, HFREF) undergoing ablation for atrial fibrillation.

First, we detected significant differences between myostatin levels measured at A, V, and CS neither in controls, HFPEF, nor HFREF; nor were there significant differences between controls, HFPEF, and HFREF. In a multiple linear regression analysis including clinical and echo parameters, we determined the following: Relative LV mass and CS myostatin correlated inversely (univariate r = -0.43, P = 0.02) in 28 patients (9 controls, 13 HFPEF, 6 HFREF), in which the quality of the procedure-related CT scan allowed for determination of LV mass. In all 43 patients, diastolic dysfunction as measured as E/E' correlated directly with CS myostatin (r = 0.50, P = 0.001).

In conclusion, the circulating levels of myostatin appear not to be altered in HFPEF patients as compared with patients displaying atrial fibrillation without heart failure. Nevertheless, the strong correlations of CS myostatin with E/E' and LV mass point to significant myocardial actions of the peptide: The positive association with diastolic dysfunction may reflect myostatin-induced cardiac fibrosis while the inverse relation to LV mass presumably indicates its anti-hypertrophic effect.

B-016

High Sensitivity Cardiac Troponin I and T Assays for Predicting Death in a Hemodialysis Population

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Objective: This study determined the prognostic value of hs-cTnI and hs-cTnT assays for all-cause death in 2499 hemodialysis patients. **Methods**: Hazard ratios of all-cause death were determined using hs-cTnI (Abbott ARCHITECT *i*1000$_{SR}$) and hs-cTnT (Roche Cobas e411) assay concentrations (EDTA plasma) based on quintile distributions from a hemodialysis patient biorepository enrolled between May 2011 and November 2012. Increased concentrations were based on sex-specific 99th percentiles: hs-cTnI male 34 ng/L, female 16 ng/L; hs-cTnT male 20 ng/L, female 13 ng/L. Unadjusted relative risks of death were compared between dialysis vintage of <1 year (n= 316) vs vintage ≥1 year (n=2176). **Results**: 463 deaths occurred during a 3-year follow-up, n = 49 for vintage <1 y and n = 414 for vintage ≥1 y. For vintage <1 y, three-year probabilities of death were 12.4% [CI 8.0%, 19.1%] (n =236) with normal hs-cTnI and 47.3% [26.2%, 74.0%] (n=80) with increased hs-cTnI (p <0.0001); 0% (n=8) with normal hs-cTnT and 22.2% [14.6%, 32.8%] (n=308) with increased hs-cTnT (p=0.26). For vintage ≥1 y, three-year probabilities of death were 20.2% [16.6%, 24.5%] (n =758) with normal hs-cTnI and 37.1% [30.9%, 44.1%] (n=1418) with increased hs-cTnI (p <0.0001); 5.2% [1.4%, 19.1%] (n =47) with normal hs-cTnT and 26.5% [23.2%, 30.1%] (n=2129) with increased hs-cTnT (p=0.023). Unadjusted hazard ratios by quintiles for both hs-cTnI and hs-cTnT by vintage year, with corresponding concentrations, are shown in Table. Both high sensitivity assays showed a greater risk of death over increasing hs-cTn concentrations. hs-cTnI was significantly more predictive of death compared to hs-cTnT in vintage <1 year (p=0.001) and trended at p=0.07 at ≥1y. **Conclusions**: hs-cTnI and hs-cTnT are both predictors of three year mortality in hemodialysis patients. However, hs-cTnI appears to be a particularly powerful predictor of death in incident (vintage <1 y) hemodialysis patients.

Unadjusted hazard ratios and concentrations by quintile for hs-cTnI and hs-cTnT by vintage year

Quintile	hs-cTnI				hs-cTnT			
	hs-cTnI, ng/L		HR (95%CI)		hs-cTnT, ng/L		HR (95%CI)	
Vintage	<1 y	≥1y	<1 y	≥1y	<1 y	≥1y	<1y	≥1y
Q1	7	8	1.0 (ref)	1.0 (ref)	35	39	1.0 (ref)	1.0 (ref)
Q2	11	14	0.4 (0.1, 2.4)	1.4 (0.9, 2.0)	53	59	1.9 (0.5, 6.4)	1.9 (1.3, 2.9)
Q3	17	22	2.5 (0.7, 8.0)	1.5 (1.0, 2.2)	81	85	2.6 (0.8, 8.2)	2.1 (1.4, 3.2)
Q4	31	38	3.5 (1.1, 10.7)	2.4 (1.6, 3.4)	124	136	2.5 (0.7, 8.1)	3.3 (2.2, 5.0)
Q5	391	10653	5.4 (1.8, 16.0)	3.7 (2.6, 5.2)	1090	3750	3.9 (1.3, 11.9)	4.1 (2.8, 6.1)

B-017

Verification of the Analytical Performance of an Assay to Determine the Enzymatic Activity of Circulating Lipoprotein-Associated Phospholipase A2 (Lp-PLA2)

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Objective: Characterize analytical performance of the PLAC Test for Lp-PLA2 Activity Assay for use as a laboratory test to augment clinical risk assessments of coronary heart disease (CHD).

Background: While lipid levels are key parameters in assessing an individual's risk for CHD, the occurrence of myocardial infarction in patients who have achieved targeted levels of LDL, suggests the need for additional clinical tools to improve patient management. The PLAC Activity test uses a synthetic substrate that shares the cleavage site of the endogenous substrate of Lp-PLA2 to determine the enzyme's activity in plasma or serum.

Methods: Testing for Lp-PLA2 activity was done using an open user-defined channel of the Beckman Coulter AU400. Activity was determined by spectrophotometrically monitoring the rate of 4-nitrophenol formed and calibration was achieved using a 5-point calibration curve (0-400 nmol/min/mL). The analytical performance of the test was assessed to characterize its performance for clinical use.

Results: Males had higher Lp-PLA2 activity (median: 176, 95th percentile: 295 nmol/min/mL) compared to that for females (median: 154, 95th percentile: 264 nmol/min/mL), with expected values established using samples from all-comer donors (154 males and 146 females, age range: 35 to 75 yrs). Analyses of Lp-PLA2 activity in EDTA plasma and serum yielded similar results including a slope of 1.00, a y-intercept of 0.05 and R value of 0.988 (n=131). Sample stability for activity determinations was acceptable for different temperatures and storage durations, including ambient for 24 h, 4°C for 2 wks, -20°C for 18 mos, and -70°C 2 yrs, with a mean recovery of 90-103 %. Five freeze/thaw cycles had minimal influence when samples were stored at -20 or -70°C, with a mean recovery of 95-97 % (95% CI). Analytical sensitivity, limit of quantitation (LOQ) was ≤ 10 nmoL/min/mL. Total lab precision with 4 samples and 2 controls assayed in duplicate twice a day over 20 days with 3 reagent lots yielded a coefficient of variation of <3.8% (113-315 nmol/min/mL). Linearity determined in 3 reagent lots with 3 pairs of plasma samples demonstrated an assay measuring range from 10-382 nmol/min/mL. Analysis of endogenous substances, including albumin 60 g/L, unconjugated bilirubin 20 mg/dL, conjugated bilirubin 12 mg/dL, cholesterol 300 mg/dl, triglycerides 400 mg/dL, hemoglobin 1 mg/mL) demonstrated no interference. Similarly, analysis of drugs at low/high concentrations in µmol/L, including acetaminophen 33/1324, aspirin 720/3600, atorvastatin 2/20, Diphenhydramine 2/20, fenofibrate 42/125, lisonopril 0.25/0.74, niacin 480/4800, tolbutamide 400/2300, warfarin 10/33, Metformin 31/310, clopidogrel bisulfate 10/100, vitamin C 14/342, demonstrated no interference. Recovery of Lp-PLA2 tested with spiked solutions and 3 reagent lots, yielded regression slopes of 0.99-1.10, y-intercept -2.87-4.21, and R2 values of 0.997 - 1.000. On-board stability was validated up to 4 weeks for reagents and open bottle stability of 3 months was validated for calibrators and controls.

Conclusions: Key performance parameters of sample stability and assay precision demonstrate the analytical robustness of the assay, and its suitability and convenience for use on automated chemistry analyzers in diverse clinical laboratory settings.

B-018

Evidence-based diagnostic decision limits for cardiac troponin for the biochemical diagnosis of acute myocardial infarction in routine clinical practice.

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Objective. To assess current use of evidence-based decision limits for cardiac troponin for the diagnosis of acute myocardial infarction (AMI) in Europe.

Methods. And of current practice in 2013-14 was performed using a web-based questionnaire by distribution to European biochemical societies for circulation to their membership. Questions covered cardiac biomarkers measured, analytical methods used, decision thresholds and their derivations. Results were collated using a central database and analysed using comparative and descriptive nonparametric statistics.

Results. Returns were obtained from 442 hospitals, 50% Central or University hospitals and 39% from District (Community) hospitals from 35 countries. 395/442 (89%) provided an acute service 11% were non acute laboratories. In 98.6% troponin measurement was the preferred biomarker for diagnosis of AMI.

The decision limit for diagnosis was based on assay imprecision in 71/441(16.1%) with the 10% CV in 61 (13.8) and the 20% CV in 10 (2.3%). The 99th percentile was used in 196 (44.3%) an optimised decision thresholds from receiver operating characteristic curve analysis in 5 (1.1%) and a local decision in 104 (23.5%). No data was available for 66 (15%). The choice of value for the decision limit was derived from the manufacturers package insert in 244 (55.2%), from peer-reviewed literature, national or international recommendations in 68 (15.5%) and from locally-based consensus review in 80 (18.1) %. No data was available for 42 (9.5%) and 3 laboratories reported they did not use a decision limit.

A detailed analysis of the decision limits used was performed for the Roche diagnostics high sensitivity troponin T (n = 183) and the largest single troponin I group, the Abbott diagnostics standard assay (n = 84). For troponin T the 99th percentile is 14 ng/L, 10% CV of 13. Only 92 (50.3%) of laboratories were using the 99th percentile for cardiac troponin T recommended. The decision limit used varied from 2 ng/L to 700 ng/L with peaks of utilisation at14 ng/L, 30 ng/L, 50 ng/L and 100 ng/L. For the 10% CV a value of 14-100 ng/L was reported and for the 99th percentile 14-400 ng/L. For the Abbott assay (99th percentile 28 ng/L, 10% CV 32 ng/L 7 (8.3%) used 28 and 3 (3.6%) 32. The range used was from 25 ng/L to 500 ng/L with peaks at30 ng/L, 40 ng/L and 300 ng/L. The 10% CV was reported as 28-500 ng/L and the 99th percentile 28-300 ng/L

Conclusion. There is currently a lack of understanding of the decision thresholds and their derivation which should be in routine clinical use for they diagnosis of acute myocardial infarction using cardiac troponin measurement. Recent publications show that this lack of understanding will result in under diagnosis of preventable disease.

B-019

Cardiac biomarkers for early detection and prediction of cardiotoxicity in patients undergoing chemotherapy: Can they help?

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Background

Cardiotoxicity after anticancer-drug therapy is an important issue in survivors. Left ventricular ejection fraction (LVEF) is used to diagnose it. Tools for early detection and prediction of cardiotoxicity are needed. Cardiac biomarkers can detect myocardial injury and may play a role in subclinical detection of drug-related toxicity.

Methods

331 patients undergoing chemotherapy with potential cardiotoxicity with a 24m follow-up were enrolled. Blood samples were drawn at baseline, 21d, 3m, 6m, 12m, 18m and 24m. Transthoracic echocardiograms (LVEF) were performed at all visits. Chemioluminiscence assays were used for biomarkers: hs-cTnT(Roche Elecsys), Galectin-3 and hs-cTnI(Abbott Architect), c-TnI and NT-proBNP (Siemens Vista) and 99th percentile (P99) was used as cut-off (hs-cTnT: female 13 male 20 pg/mL CV=10%, hs-cTnI female 15,6 male 34,2 pg/mL CV=4% and c-TnI 27 pg/mL CV=7.7%). Cardiotoxicity was defined as: LVEF decrease >10% without or >5% with heart failure to a value <55%.

Results

Patient's age: 58,1±14,3 years; 84.3% women. Diagnosis: 71% breast cancer; 27,7% leukemia or lymphoma; 1,3% other tumours. Incidence of cardiotoxicity was 10.3% (decreased LVEF from baseline 63.8% to 61.8% at 24m). Maximun value for all troponins occurred at 3m (table1). Using P99 as cut-off, hs-cTnT identified additional patients when compared to hs-cTnI and c-TnI. Percentage of troponins >P99 were higher in patients with cardiotoxicity. Sensitivity for hs-cTnI at 3m for early detection of cardiotoxicty was 76,3% and specificity 94,5%. Positive predictive value was 87,3%, and negative predictive 89,1% and hs-cTnT S=70% E=42,7% NPV=91,1% PPV=14,7%. No significant relation was observed between NT-proBNP and Galectin-3 and cardiotoxicity.

Table1. Media ±SD for troponins

pg/mL	B	21D	3M	6M	12M	18M	24M
Hs-cTnI	2,9±4.5	13,9±33.4	41,1±24.1	10,5±20.8	4±4.4	15,2±7.4	3,7±4.3
Hs-cTnT	7,4±5.6	11,4±5.3	16,5±9.1	15,4±12.8	9,3±4.9	11±4.6	11,1±6.2
cTnI	16,6±15.4	18±18.9	27,5±41.5	25,5±42.7	16,9±9.4	16,1±5.3	16,7±10.3

Conclusions

Increased hs-cTnT and hs-cTnI occurred in the cardiotoxicity group with a maximum concentration at third month.

Hs-cTnT identifies more patients with cardiac injury than hs-cTnI and c-TnI, and has higher NPV (91,1%%) at 3m for early detection of cardiotoxicity.

NT-proBNP and Galectin-3 doesn't seem to add value in this scenario.

B-020

Are Heart Failure Recommendations And Guidelines Established In Practical Laboratory Medicine In Europe, US And Canada? The CARdiac MArker Guideline Uptake in Europe (CARMAGUE)

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Objective and Relevance: The well-established heart failure (HF) markers B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) are widely used by clinicians. Analytical and clinical recommendations for measurement of these biomarkers have been published. The aim of this survey was to investigate how well these guidelines for measurement of NP have been implemented in laboratory practice in Europe and as a comparison in the United States (US) and Canada. Methodology: Member societies of European Federation of Clinical Chemistry and Laboratory Medicine were invited in January 2013 to participate in a web-based audit-questionnaire. Laboratories in the US and Canada were also invited to participate. The survey was implemented using a web-based survey system consisting of a HTML-AJAX interface and a CGI program storing the results in XML files on a database server. Results from raw data XML files were joined into a tabular format and the numbers of different answers for each question were calculated for further analysis with Microsoft Excel. The questionnaire requested information including type of tests performed, reason for method choice, decision limits for HF and laboratory

accreditation status. The survey closed in August 2014. Validation: Preliminary results show that participating laboratories consisted of 29% and 41% university laboratories in Europe and US/Canada, respectively. In Europe, 305 responders out of 494 measured NP as well as 64 out of 135 US/ Canadian responders. NT-proBNP was most widely used in Europe (67% of NP offering laboratories) and BNP slightly more (55%) in US/Canada. The reason was availability of instrument in both regions, and in Europe additionally stability and clinician preference. The preferred methods were Roche and Abbott in Europe, and in US/Canada Roche, Abbott and Siemens (Advia Centaur). Most laboratories used ng/l or pg/ml. with pmol/l only rarely stated as a unit. More than half of the European responders used the guideline recommended BNP rule-out cut-off of 100 ng/l for acute HF. However, cut-off values for NT-proBNP were diverse and only 5% used the guideline recommended rule-out cut-off of 300 ng/l. Several laboratories (18%) used age dependent cut-off values, which were recommended by the European Society of Cardiology to rule-in HF (<50 years: 450 ng/l, 50-75 years: 900 ng/l, >75 years 1800 ng/l). In Europe, age dependent decision limits for chronic HF were reported only in one third of responders and were mostly stated to be the same as for acute HF. In US/Canada the same cut-off levels for acute and chronic HF were used in the majority of laboratories. There were still laboratories not performing external quality assurance in Europe (Europe: EQA 11%; US/Canada: EQA: 1%). In Europe, one third of laboratories were not accredited for NP, whereas in US/Canada almost all were. Conclusion: NP measurement for HF diagnosis was available in more than half of the responding laboratories. However, guideline recommended cut-off values for acute HF were still not adequately implemented. Further, accreditation is an ongoing process in Europe, and full EQA participation for HF biomarkers remains incomplete in European laboratories.

B-021

In vitro Studies of Human Cardiac Troponin I Degradation.

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Background. Cardiac troponin I (cTnI) is a golden marker of myocardial cell death. It was shown that a wide diversity of cTnI forms, including proteolytic fragments could be found in patients' blood. Partial cTnI proteolysis happens from N- and C-terminal parts of the molecule, whereas central part of cTnI remains relatively stable. Though N- and C-terminal degradation of cTnI was described more than 15 years ago, still it is not clear, how it happens - is it a sequential truncation, or relatively big fragments from both ends are cleaved on the very first steps of cTnI degradation.

Methods. Cardiac proteins were extracted from human cardiac tissue and incubated at 37ºC for 3 hours. In the preliminary experiments it was shown that in such conditions cTnI is partially cleaved by co-extracted endogenous proteases. After incubation extracted proteins were separated by gel-filtration (GF) chromatography. cTnI immunoreactivity in GF fractions was measured by sandwich immunoassays utilizing pairs of antibodies specific either to N- or to C-terminal parts of the molecule. On the next step cTnI fragments from GF fractions were purified by means of affinity and reverse-phase chromatography. Purified fragments were analyzed by mass spectrometry.

Results. Both types of immunoassays (specific to N- and C-terminal parts of cTnI) revealed two peaks of immunoreactivity in GF fraction. In both cases the first peak was detected in fractions corresponding to the proteins with molecular masses about 60-80 kDa (most likely cTnI in ternary complex), whereas second peak of immunoreactivity was found in fractions corresponding to the proteins with significantly lower molecular masses. By MS studies we were able to identify only few peptides (27-36 amino acid residues long) truncated from the N-terminal part of the molecule and multiple peptides (20-40 amino acid residues long) from the C-terminal part. Peptide sequence analysis suggested that the N-terminal part of cTnI has a very limited number of cleavage sites, whereas C-terminus contains much more sites of protease degradation.

Conclusion. Using different biochemical methods and mass spectrometry analysis we were able to purify and identify peptides that are formed *in vitro* after cTnI cleavage by endogenous proteases. Our studies suggest that relatively big and relatively stable peptide(s) is (are) truncated from the N-terminal part of cTnI. C-terminus of cTnI molecule contains multiple possible sites of proteolysis and protease cleavage results in formation of wide diversity of different size peptides.

B-022

Clinical Study to Validate the Use of a New Point of Care BNP Test as an Aid in the Diagnosis of Heart Failure

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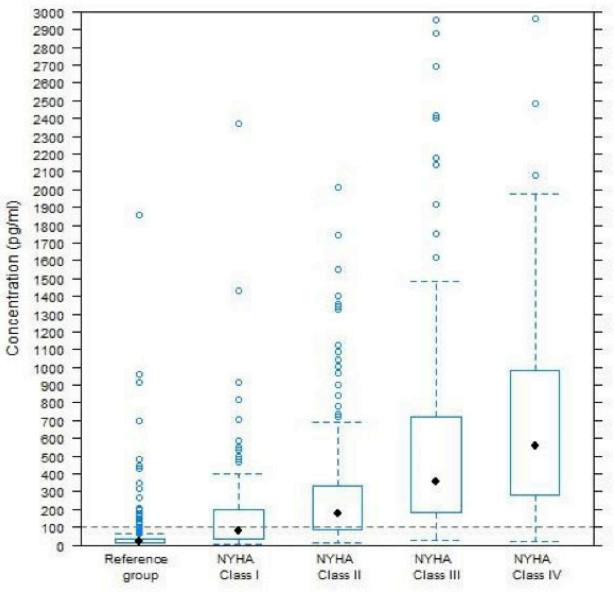
Background: The Trinity Biotech Meritas BNP single-epitope assay for detecting BNP is a Point of Care test used in conjunction with the Meritas Analyzer for quantitative determination of BNP in whole blood or plasma to aid in the diagnosis of heart failure (HF).

Objective: The objective of this study was to validate the clinical performance of the Meritas BNP test (Trinity Biotech) for the quantitative determination of BNP for use as an aid in the diagnosis of heart failure (HF).

Methods: The study was designed as a retrospective study of banked EDTA plasma from 665 eligible adult subjects (281 females, 384 males) with a diagnosis of HF. The diagnosis was based on the NYHA classification I-IV. The normal range was determined using banked EDTA-plasma from 1424 non-HF patients (822 females, 602 males), including individuals with comorbidities such as diabetes, hypertension, chronic obstructive pulmonary disease (COPD) and renal disease.

Results: A box and whiskers plot of the clinical study population, classified according to NYHA, is presented the Figure. A progressive increase in BNP concentrations with increasing NYHA classifications shows a relationship between the severity of the clinical signs and symptoms of HF and the median BNP concentrations of each NYHA class. The diagnostic sensitivity and specificity using a decision threshold of 100 pg/mL (ng/L) for various age groups (< 45, 45-54, 55-64, 65-74, 75+ years) within each gender were as follows: male: sensitivity, range 64 to 79%, specificity, range 81 to 100%; female, sensitivity, range 63 to 85%, specificity, range 75 to 100%; The ROC curve area for HF based on BNP was 0.938 (95%CI 0.927-0.949).

Conclusion: The data indicate that BNP measurements provide objective information for use in the diagnosis of heart failure. The sensitivities and specificities were determined to be acceptable according to the performance claims.



B-023

Diurnal and Longitudinal Variations of Lipoprotein-Associated Phospholipase A2 (Lp-PLA2) Enzyme Activity

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Objective: Characterize the diurnal and longitudinal variations of serum measurements of Lp-PLA2 enzyme activity, a biomarker of vascular inflammation in coronary heart disease (CHD).

Background: Since clinical interpretation of laboratory values requires comparisons of results within and among individuals, understanding both intra- or inter-individual and also temporal biovariability of assays is important. To assess the relative variability of Lp-PLA2 activity in the context of tests used in clinical assessments of CHD risk, we compared the biovariability of Lp-PLA2 activity to lipid profiles and C-reactive protein (CRP).

Methods: To assess diurnal variation, phlebotomy was performed in 15 subjects at baseline (fasting) and at 3 hr intervals during a 24 hr period to obtain serum samples, with measurements of Lp-PLA2, CRP and lipids performed on all specimens. To assess longitudinal variation, phlebotomy was performed on 23 subjects at baseline and at wks 2, 4, 8 and 12 to obtain serum samples which were stored at -70°C. Assays for Lp-PLA2 activity, lipase, total cholesterol, HDL, triglycerides, ApoA1, ApoB, and lipoprotein (Lp) (a) were performed for all samples at all-time points. Lp-PLA2 activity was measured by the PLAC Test for Lp-PLA2 Activity using the Beckman Coulter AU400. Variations in intra-individual and inter-individual results were determined for both the diurnal and longitudinal analyses.

Results: An analysis of diurnal variation showed mean percent intra-individual diurnal variation (95% CI) for Lp-PLA2 activity of 4.1 (3.2-5.0), with corresponding results as follows: lipase, 7.1 (5.1-9.2); total cholesterol, 2.7 (2.2-3.2); triglycerides, 28.8 (22.6-35.0); HDL, 4.0 (2.6-5.3); ApoA1, 1.7 (1.2-2.2); ApoB, 2.6 (1.8-3.4); and Lp(a), 5.8 (2.3-9.2). Inter-individual diurnal variation was 28.4% for Lp-PLA2 activity, compared to a range of 12.9 to 33.4% for lipase, total cholesterol, triglycerides, HDL, ApoA1 and ApoB; the mean inter-individual diurnal variation for Lp(a) was 116.8%. An analysis of longitudinal variation over 12 wks demonstrated a mean percent intra-individual variation of 5.3% for Lp-PLA2 activity, with corresponding published data as follows: LDL cholesterol , 8.3%; total cholesterol, 5.4%; HDL cholesterol, 7.1%; oxidized cholesterol, 21.0%; Lp(a), 20.8%; and CRP, 42.2%. The mean percent inter-individual longitudinal variation for Lp-PLA2 activity was 29.7%, compared to 25.7% for LDL cholesterol, 15.2% for total cholesterol, 19.7% for HDL cholesterol, 50.0% for oxidized LDL, 18.1% for Lp(a) and 76.3% for CRP. The mean index of individuality for Lp-PLA2 activity was less than 0.2.

Conclusions: The diurnal variation of Lp-PLA2 activity was similar to that of lipase, total cholesterol, HDL, triglycerides, Lp(a), ApoA1 or ApoB, and less than that for triglycerides. The longitudinal variation of Lp-PLA2 activity was similar to that for total cholesterol, LDL, HDL, and Lp(a), and less than that for oxidized LDL or CRP. These data suggest that the diurnal and longitudinal variations for Lp-PLA2 activity results are similar to those for lipid biomarkers measured in clinical assessments of CHD risk, suggesting the suitability of measuring Lp-PLA2 activity for patient management.

B-024

Fully automated ultrasensitive digital immunoassay for troponin using single molecule array technology

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Background: Ultra-sensitive cardiac troponin measurement offers a promising new tool for early detection and monitoring of cardiovascular disease. With growing interest in exploring as an early indicator of adverse heart health trends, the ability to quantitate troponin in healthy control populations is emerging as a highly desirable assay capability. We report analytical data from a fully automated digital immunoassay for cardiac troponin I (cTnI) based on single molecule array (Simoa) technology with a limit of detection 2 logs lower than contemporary high sensitivity troponin assays.

Method: Reagents were developed for a paramagnetic bead-based ELISA for use in the Simoa HD-1 Analyzer. Anti-cTnI capture beads were prepared by covalent coupling of antibody to carboxy paramagnetic microbeads, detector antibody was biotinylated by standard methods, and an enzyme conjugate was prepared by covalent coupling of streptavidin and [[Unsupported Character - Symbol Font β]]-galactosidase. The HD-1 Analyzer first performs a 2-step sandwich immunoassay using 42 µL of serum or plasma sample, then transfers washed and labeled capture beads to a Simoa disc where the beads are singulated in 50-femtoliter microwells, sealed in the presence of substrate, and interrogated for presence of enzyme label. A single labeled cTnI molecule provides sufficient fluorescent signal in 30 seconds to be counted by the HD-1 optical system. At low cTnI concentration, the percentage of bead-containing wells in the array with a positive signal is proportional to the amount of cTnI present in the sample. At higher cTnI concentration, the total fluorescence signal is proportional to the cTnI in the sample. The concentration of cTnI is then interpolated from a standard curve (range to 300 pg/mL). Time to first result is 45 minutes. The assay was evaluated for sensitivity, recovery, linearity precision and normal range. Discrimination of healthy subjects from those with mild to moderate heart failure was also preliminarily assessed.

Results: Limit of detection (2.5 SD) was 0.010 pg/mL across 26 runs. Limit of quantification (20% dose CV from diluted serum samples) was 0.079 pg/mL across 16 runs and 144 determinations. Recovery of cTnI spiked into normal serum averaged 80.5%. Mean linearity was 89.5%. Precision per EP5-A guideline included two serum-based panels, 1 plasma-based panel and two cTnI controls assayed in replicates of three twice per day for five days using a single calibration curve. ANOVA gave CV's <10% for all levels. Serum cTnI values from 97 healthy control samples ranged from 0.072 to 8.40 pg/mL, with a mean and 99th percentile of 1.01 and 8.40 pg/mL. Serum cTnI values from 375 patients with mild to moderate heart failure (NYHA classification II and III) ranged from 0.440 to 1770 pg/mL, with a median of 15.1 pg/mL. The heart failure samples had significantly higher cTnI concentrations than the healthy subjects (p=0.0002). Evaluation of the predictive value of the cTnI concentrations is ongoing.

Conclusion: The results show the digital Simoa cTnI assay exhibited good general analytical properties and cTnI levels from healthy subjects were above the sensitivity limits. The assay represents a new enabling tool for ultra-sensitive cTnI measurement.

B-025

Development of an Enhanced Chemiluminescent High Sensitivity Troponin I assay* on VITROS® 5600 Integrated and VITROS® 3600 and ECi/ECiQ Immunodiagnostic Systems

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Background: The Joint European Society of Cardiology/American College of Cardiology guidelines state that cardiac troponins are the preferred biomarkers for the detection of myocardial injury, for risk stratification in patients diagnosed with acute coronary syndrome and for the diagnosis of myocardial infarction. Because of the demand for accurate precise measurement of low troponin levels, there is an increased need for assays with improved analytical performance. We are developing a rapid, fully automated high sensitivity assay for the measurement of cardiac Troponin I (cTnI) in human serum and plasma for use on the VITROS® Systems.

Methods: A prototype assay was developed which uses an immunometric technique in which the cTnI present in the sample reacts simultaneously with one biotinylated antibody and two horseradish peroxidase labeled antibodies. The antigen-antibody complexes are captured by a streptavidin coated well. Unbound materials are removed by washing. VITROS® Immunodiagnostic Products Signal Reagent is added and light emission is measured. The light signal generated is directly proportional to the concentration of cTnI present in the sample.

Results: The following results were generated using the prototype assay. The assay range is 1 pg/mL to 50,000 pg/mL. In a CLSI-EP-15-A2 precision study the results for four patient pools were: (mean cTnI pg/mL, with-in run %CV, with-in laboratory %CV, respectively): 12.3 pg/mL, 3.2%, 5.5%; 28.4 pg/mL, 1.4%, 1.8%; 60.6 pg/mL, 1.6%, 6.3% and 183 pg/mL, 1.5%, 4.4%. The LoB, LoD and LoQ (established according to CLSI-EP-17-A2) were 0 pg/mL, 1 pg/mL and 2.9 pg/mL (20%CV), respectively. The concentration at 10%CV was 6.5 pg/mL. The 99th percentile was determined by measuring cTnI in samples from 412 individuals with values within reference ranges for eGFR and NT-proBNP. The gender independent 99th percentile was 23 pg/mL. Correlations between the VITROS® High Sensitivity Troponin I assay and both a commercially available high sensitivity assay and the VITROS® Troponin I ES assay were obtained using 111 patient samples from a variety of clinical categories. The comparison of VITROS® Troponin I ES to VITROS® High Sensitivity Troponin I resulted in a slope of 1.00 pg/mL, an intercept of -20 pg/mL and a R of 1.00.

Conclusion:

In conclusion, the prototype VITROS® High Sensitivity Troponin I assay has a 10%CV at a concentration that is significantly lower than the 99th percentile (medical decision limit) and the assay has the ability to measure cTnI above the LoD in 93% of a reference population.

* Under Development

B-026

Relative contribution of high sensitivity cardiac Troponins I and T in cardiovascular risk stratification in patients with OSA after treatment with CPAP

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Background:

Obstructive sleep apnea (OSA) is a common condition caused by intermittent airway collapse during sleep that results in repetitive hypoxia, arousal, poor quality of sleep and excessive daytime sleepiness. OSA is a risk factor for various cardiovascular conditions and in recent decades, OSA was associated with increased cardiovascular mortality in patients with the severe form of the disease without treatment. Therefore, adequate treatment with CPAP (Continuous Positive Airway Pressure) may improve survival. With the advent of new high-sensitivity markers, there was an increase in the sensitivity of the method consequently increased interest from professionals in the use these high-sensitivity cardiac troponin T and I (hs cTnT and hs cTnI) in risk stratification of cardiovascular diseases since they are released in the cardiac lesions. The aim of our study was to evaluate the (hs cTnT and hs cTnI) methods and evaluate of cTnI conventional by two methods in patients with severe OSA before and after one year of effective treatment with CPAP.

Methods: 36 patients of the Sleep Institute in Sao Paulo, with moderate and severe OSA, 22 men, with mean BMI = 30.20 ± 9.12 Kg/m^2 and age = 65.4 ± 5.8 years, without other diseases were randomized and treated effectively with a year with CPAP and using average of 5 hours each night , nonsmoking and sedentary. Patients collected 10 ml of venous blood before treatment and after treatment with CPAP, which were frozen at -80ºC and was thawed on laboratory measurements. Between several biochemical parameters will be analyzed the hs cTnT by two methods and cTnI conventional by two methods too, before and after one year treatment to apnea with CPAP. The hs cTnT was quantified with a Electrochemiluminescence (hs cTnT) Elecsys ®-Roche/Elecsys) third generation method. The detection limit is 0.5 pg/mL. Other method was used hs cTnI immunoassay (Abbott/ARCHITECT system). It was measured cTnI ES conventional by chemiluminescence (Vitros ® - Ortho Clinical Diagnostics) 12 pg/mL is the limits of detection to this methods and cTnI by AccuTnIDx (Access/Beckman Counter) with detection limits of 10 pg/mL for this method. Paired samples statistics were performed for correlations and comparisons of results between methods before and after treatment and Wilcoxon test was performed to methods with significance differences.

Results: Based on the results presented there was a statistic significant effect of treatment with CPAP on the hs cTnT presented in the nonparametric statistical test Wilcoxon (Z=-1.955, p= 0.05 and Z=-1.634, p=0.04). However there was not significant difference of treatment with CPAP on the values presented by cTnI conventional dosages to both methods.

Conclusion: The hs cTnT and hs cTnI methods showed significance differences between before and after treatment with one year of CPAP.in patients with apnea. This method use monoclonal antibodies with high sensitivity and specificity for cardiac injury. However there is a need of definition about the real importance of these low levels found in the condition of obstructive sleep apnea for cardiac injuries. The cTnI ES and cTnI Dx did not show sufficient sensitivity on condition of OSA.

B-027

Investigation and analysis of the coefficient of variation of internal quality control of BNP and NT-pro BNP

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Objective To investigate the current status of internal quality control by statistical analysis of the Internal Quality Control (IQC) data of Brain natriuretic peptide(BNP) and n-terminal pro-brain natriuretic peptide(NT-pro BNP). **Methods** Web-based External Quality Assessment (EQA) system was used to collect IQC data of BNP and NT-pro BNP from 70 and 233 EQA participant laboratories nationwide. The data includes the coefficient of variation(CV) of IQC data under control in April 2014 and long-term cumulative data. Acceptable rates of CVs of two-lot internal quality controls in BNP and NT-pro BNP were calculated according to 2 criteria, namely 1/3TEa and 1/4TEa. The instrument the participant laboratories used to detect BNP and NT-pro BNP were sorted into 3 and 5 group and the passing rate of each group were calculated by the 2 criteria. **Results** 70 and 233 laboratories reported the data of level 1 IQC (lot 1) for BNP and NT-pro BNP, 25 and 98 of which reported the data of level 2 IQC(lot 2). The passing rate is different according to different criteria. For BNP, passing rates of CV in April of lot 1and Cumulative CV of lot 1 by the criteria of 1/3TEa and 1/4TEa are 90%, 75.71%, 81.43% and 64.29% respectively and the median CV is 4.55£¬4.41£¬5.25 and 4.4 accordingly. Passing rate of lot 2(80%¡«96%) are slightly higher than that of lot 1 and median CV(3.69¡«4.75) is slightly lower than that of lot 1. For NT-pro BNP, passing rates of CV in April of lot 1and Cumulative CV of lot 1 by the criteria of 1/3TEa and 1/4TEa are 93.99%, 85.41%, 85.84% and 78.11% respectively and the median CV is 4.11, 3.9, 4.61 and 4.42 accordingly. Passing rates of lot 2 are close to that of lot 1 and median CV(3.22¡«3.67) is slightly lower than that of lot 1. Statistical results show that instruments the participant mainly used were Roche(33/70), Simens(18/70) and Beckman(8/70) for BNP and Roche(117/233), BioMerieux (19/233), LEPU(19/233), Radiometer(15/233) and Johnson & Johnson(10/233) for NT-pro BNP. The passing rates of all the instrument group have few difference. **Conclusion** Most of the laboratories can meet the criteria of 1/3TEa and 1/4TEa. although laboratories should continue to do more internal quality control to further improve the quality of detection of BNP and NT-pro BNP.

B-028

Gender Wise Correlation of Serum Homocysteine and Gensini Scores with Anthropometric Indices in Coronary Artery Disease

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Background: Obesity is an emerging public health problem throughout the world especially in our country (India) where it is on the rise even during childhood. Obesity and elevated serum homocysteine levels both have been independently considered as risk factors for coronary artery disease (CAD). But, limited data available as to how these factors correlate gender wise with respective Gensini scores used for grading coronary artery disease.

AIM: To measure and correlate anthropometric indices with respective serum homocysteine and Gensini scores in male and female patients with CAD.

MATERIALS AND METHODS: Institutional Ethics committee permission was taken prior to the study. Total 70subjects (males and females) in the age group of 30-70years presented to Cardiology clinic who consented to participate were included. All of them had undergone angiogram evaluation and risk stratification using Gensini score as standard of care. Out of 70 subjects, 19 had no evidence of CAD on angiogram (controls) and remaining 51 (Male: Female= 42:9) were considered as cases. Subjects taking vitamin supplements, oral contraceptives, pregnant women were excluded from the study. Height and other anthropometric indices were measured to the nearest 0.1 cm, weight to nearest 0.5 kg in light clothing and without shoes. Body mass index (BMI) was calculated as weight (kg)/height (m^2).Two ml of fasting blood sample collected in plain vacutainers and serum was separated and stored at -70°c until use. Serum levels of homocysteine were determined by using commercially available ELISA kit. Data was analyzed using SPSS version 16.0.Correlation of Gensini scores and serum homocysteine with anthropometric indices was done using Pearson coefficient correlation.

RESULT: There was no significant difference in BMI among cases and controls, but homocysteine was significantly increased in cases. Homocysteine and Gensini scores were correlated with different anthropometric indices in cases also did not show any significant correlation. But when these comparisons were done gender wise, we found a significant correlation of waist to hip ratio with Gensini scores (p = 0.047) and homocysteine (p = 0.012) only in males.

CONCLUSION: Both homocysteine and Gensini scores had a significant correlation with waist to hip ratio only in male gender with CAD indicating that we have to use different strategies in males and females for risk stratification of coronary artery diseases using these markers.

B-029

Copeptin as an Early Detector of Acute Myocardial Infarction

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Background: Early identification of myocardial infarction (MI) in chest pain patients is crucial for early treatment and life saving. **Aim** of this work was to assess copeptin

for the diagnosis of MI and discrimination of patients with ST segment elevation myocardial infarction (STEMI) from non ST segment elevation patient (NSTEMI).

Methods: This study was conducted on 30 STEMI and 30 NSTEMI patients admitted to cardiac intensive care unit of Alexandria University Hospital; Egypt within 8 hours after chest pain. Another 30 healthy volunteers were considered as a control group all patients performed electrocardiograph (ECG) and blood sample was collected from all participants for assay of troponin I (TnI)'Ck-MB mass and copeptin by chemilumenescence' immumoturbidimetry and ELISA respectively.

Results: *There was a significant difference between patients and control groups as regards* TnI and CK-MB mass (P=< 0.001).No significant difference was detected as regards copeptin (P=0.13).Using the cut off levels for TnI = 0.05 ng/ml '5.7 ng/ml for CK-MB mass and 115 ng/ml for copeptin: the area under the ROC curve (AUC) showed that sensitivity' specificity' positive predictive value (PPV) and negative predictive value (NPV) for TnI and CK-MB mass were 100% with AUC=1. For copeptin the corresponding figures were 85%' 40%' 85% and 40% respectively with AUC=0.53. To discriminate between STEMI and NSTEMI groups' results for TnI were as follows: sensitivity=82.7%' specificity=66.4%' PPV=65.2% and NPV=81.6% at 2.5 ng/ml with AUC=0.77. For CK-MB mass these figures were 74.2%' 80%' 73%' 70.4% respectively at 5.3 ng/ml and AUC=0.75. For copeptin these figures were 60%' 58.9% & 53.2% and 52.1% respectively at 116 ng/ml and AUC=0.48

Conclusion: These results indicate that measuring copeptin did not add to the diagnostic accuracy of acute MI by TnI and CK-MB mass. Also, it did not add to the differentiation between STEMI and NSTEMI patients.

B-030

Levels of Galectin-3 in samples of patients with abnormal values of Brain Natriuretic Peptide.

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Acute cardiac conditions such as acute myocardial infarction and heart

failure (HF) are associated with significant morbidity and mortality. The

prognostic significance of natriuretic peptides (BNP and NT-proBNP) in patients with myocardial ischemia is well established, and their measurement is endorsed by the most important guidelines and recommendations for diagnosis and management of heart failure (HF). Numerous novel biomarkers, such galectin 3 (Gal-3), have been identified to predict outcomes and show potential in assessing prognosis beyond the established natriuretic peptides. In this study, we determined levels of Gal-3 in samples of patients with abnormal values of BNP and in control subjects and analyzed if there is a relationship between the values of BNP and Gal-3. Methods: Plasma Gal-3 concentrations were measured in 10 samples of patients with high BNP concentrations (>100 pg/ml) (Group I) and in 40 asymptomatic subjects, without a family history of cardiovascular disease, who had normal BNP levels (Group II). Gal-3 levels were determined using an automated test (VIDAS® Gal-3 kits, BIO MÉRIUEX, France) using the ELFA (Enzyme-Linked Fluorescent Assay) technique. Calibration of the assay was performed according to the manufacturer's recommendations and values were normalized to a standard curve. The McNemar test was used to test the differences between the results of the paires proportions of BNP and Gal-3. The tests are coded as 0 = "not altered" and 1 = "altered". The BNP cutoff value was 100 pg/mL. There were two Gal-3 cutoff values: 17.8 ng/mL (results of moderate to high risk of mortality and hospitalization) and at 25.9 ng/mL (results of high risk of mortality and hospitalization). Results: Levels of Gal-3 were 26.57 ± 11.20 ng/mL in Group I and 8.30 ± 1.80 ng/mL in the control group, respectively. Differences in levels of Gal-3 between the two groups were significant (independent samples t-test P < 0.0001). Using a Gal-3 cutoff value of 17.8 ng/mL, 7 out of the 10 patients of the Group I and Zero out of the 40 subjects of the Group II had high Gal-3 concentrations. In the Group I, 2 out 10 patients were classified as moderate risk (Gal-3 > 17.8-25.9 ng/mL) and 5 out 10 patients were classified as high risk (Gal-3 > 25.9 ng/mL). There was concordance between the proportion of altered BNP and Gal-3 results, using the cutoff value of 17.8 ng/mL and discordance when using the cutoff value of 25.9 ng/mL. Conclusion: An increased concentration of galectin-3 was found in all the patients with high BNP concentrations. The patients with BNP concentration above 100 were classified as moderate or high risk.

B-031

Characteristics of the New Beckman Coulter High-Sensitive Cardiac Troponin I Assay (hsTnI)

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BACKGROUND: In order to meet new IFCC guidance new Troponin I assays should exhibit increased sensitivity (LoB, LoD and LoQ), precise measurement of cTnI concentrations in the range seen in healthy individuals and the capability to accurately detect changes in cTnI concentration within this range.

RESULTS: The new hsTnI assay under development at Beckman Coulter exhibits superior sensitivity in comparison to other devices currently marketed in the US with an estimated LoB of < 0.3 pg/ml and 20% CV LoQ of 1.5 pg/ml. The estimated 99th percentile URL of a random healthy population is 21 pg/ml determined with < 3% intra-assay and 5% total imprecision. In addition, this new hsTnI assay is capable of accurately measuring 10 pg/ml changes in TnI concentrations. This hsTnI assay accurately measures TnI in comparison to a currently validated device (correlation between Access AccuTnI+3 and hsTnI within 5%) and exhibits < 5% bias between sample types (serum, plasma). The assay does not exhibit cross reactivity to cardiac TnT, cardiac TnC, skeletal TnI or skeletal TnT and is robust against common interferences (400 mg/dL hemoglobin, 40 mg/dL bilirubin, 3000 mg/dL triglyceride 60 mg/mL albumin, 1000 mg/dL fibrinogen, 28.8 U/mL heparin).

CONCLUSIONS: The new high sensitivity TnI (hsTnI) assay under development at Beckman Coulter is highly sensitive and sufficiently accurate to precisely measure TnI in > 85% of the normal population. This new assay also meets new IFCC guidance to accurately detect changes in cTnI concentration within healthy subjects.

B-032

Increased Incidence of Cardiac Troponin I Abnormalities in Women Utilizing a High Sensitivity Assay

S. A. Love, Y. Sandoval, S. W. Smith, K. M. Schulz, M. M. Murakami, J. Nicholson, F. S. Apple. *Hennepin County Medical Center, Minneapolis, MN*

Objective: To determine if sex-specific 99th percentiles with a high sensitivity cardiac troponin I (hs-cTnI) assay versus a single non-sex cut-off using a contemporary cTnI assay leads to more frequent increases in cTnI levels indicative of acute myocardial infarction (MI).

Methods: The data presented are the first results from our 'clinical trials.gov identifier: NCT02060760' study. Patients, 18+ years of age, presenting to the emergency department where providers used cTnI to

rule-in and rule-out MI, were included in the study. Serial cTnI measurements were obtained on clinical indication between February 4 and March 13, 2014. Clinical decisions were based on the contemporary cTnI results, with hs-cTnI measured simultaneously (both on the Abbott ARCHITECT $i1000_{SR}$ or $i2000_{SR}$). 99th percentiles were as follows: contemporary cTnI 30 ng/L (0.030 µg/L); hs-cTnI 16 ng/L for females and 34 ng/L for males.

Results: 792 patients presenting for MI rule-in or rule-out were enrolled, of which 45% were female. Over the course of serial cTnI measurements (0,3,6,9h) baseline and maximum values were examined by gender. At presentation mean (95% CI) values were: cTnI assay 128 (0-266) ng/L for males and 60 (31-89) ng/L for females; hs-cTnI assay 90 (0-190) ng/L for males and 45 (18-72) ng/L for females. Maximum values were also examined by gender: cTnI male 532 (126-938) ng/L, female 241 (52-430) ng/L; hs-cTnI male 457 (135-781) ng/L, 236 (29-444). The hs-cTnI assay resulted in a 15% decrease (p=0.01) in patients with at least one value greater then the sex-specific cut-off. The number of women with an increase above the 99th percentile cut-off was significantly different (p=0.003) vs. males. Further, the hs-cTnI assay sex-specific cut-offs resulted in a 29% decrease in males with an increased value and a 5% increase for females with an increased value.

Conclusion: Based on sex-specific hs-cTnI assay 99th percentiles we observed a significant decrease in the incidence of cTnI increases and a significant difference in increased rates between sexes. The increased incidence of cTnI increases for women using an hs-cTnI assay could have important implications for improving treatment and outcomes for women presenting with symptoms of acute coronary syndromes.

B-033

The relationship of Inflammatory cytokines (IL-6, IL-17a and TNFa) to co-morbidities of cardiovascular disease in a large community-based patient population.

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Background: Interleukins 6 and 17-A (IL-6 and IL-17a) and Tumor necrosis factor-alpha (TNFa) are inflammatory cytokines that may play a role in the pathogenesis of atherosclerosis. Previous studies have shown these cytokines to be relevant in many areas of cardiovascular disease (CVD) including acute myocardial infarction, heart failure and coronary artery disease. We determined plasma levels of IL-6, IL-17a and TNFa and identify the effect of co-morbidities on their levels in a large at CVD risk study population.

Methods: Immunoassays for IL-6, IL-17a and TNFa were developed using Single Molecule Counting technology and validated in a CLIA licensed, CAP accredited laboratory (Singulex). The 95%tile upper limit of normal (ULN) were 4.5, 1.9 and 2.5 pg/mL; and the precision profiles were 12% (2.3 pg/mL), 17% (5.3 pg/mL) 15% 92 pg/mL) for IL-6, IL-17A and TNF, respectively. Blood samples were measured for the cytokines, LDL, HDL and HbA1c in. >21,000 community-based patients at risk for CVD. Parametric and non-parametric analyses were performed in de-identified data using SAS v9.3 ($p < 0.05$ considered significant.

Results: The characteristics of the inflammatory cytokines in a population at risk for CVD are shown in the Table. All three cytokines showed higher concentrations in older patients as well as those with CV risk factors of pre-diabetes, diabetes and HDL dyslipidemia

Conclusions: In a population at risk for CVD, IL-6, IL-17 and TNFa concentrations are higher than those observed in healthy populations and coincide with risk factors for CVD.

Summary of cytokine results						
	IL6		IL17a		TNFa	
	Median	%>95th of ref. population	Median	%>95th of ref. population	Median	%>95th of ref. population
Entire cohort	1.3	8.9	0.4	4	2.5	32.2
Females	1.4	8.9	0.4	3.9	2.4	31.2
Males	1.3	5.1	0.4	4.2	2.5	33.4
	1.1	5.1	0.4	2.3	2.1	18.3
>= 50 y	1.5	10.6	0.4	4.7	2.6	37.8
HbA1c <=5.9%	1.3	7.3	0.5	3.4	2.4	28.4
HbA1c >5.9%	1.9	15.4	0.4	6.1	2.8	46.5
LDL <=129 mg/dL	1.4	10.2	0.4	4.7	2.5	23.6
LDL >129 mg/dL	1.3	6.7	0.3	2.7	2.4	29.6
HDL at target*	1.3	7.7	0.4	3.7	2.4	29.1
HDL less than target*	2.0	17.2	0.5	6.1	2.9	47.1
*HDL target	>=45mg/dL females	>=35 mg/dL males				

B-034

Validation of lithium heparin tube for Cardiac markers in a Clinical Laboratory and its benefits

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Background:

Accurate laboratory testing requires an understanding of the complex interactions between collection devices and blood specimens. Clinical laboratories must consider the pre-analytical challenges in laboratory testing. Proper blood collection and timely processing are critical pre-analytical steps required for the integrity of laboratory results. Although the influence of blood collection devices on laboratory tests is often overlooked, correct pre-analytical handling is essential. In this study, we discuss the use of plasma to chemistry analysis with an emphasis on heparin tube.

Objective:

Currently the clot activator with separator gel tube is the first option for biochemical analysis, the objective of the study is to evaluate the possibility and benefits of the introduction of lithium heparin tube for holding some biochemical studies.

Methods:

Twenty paired samples using the two tubes were carried out simultaneously. Both tubes were centrifuged before analysis with the difference that the clot activator tube with gel separator needs a time for the formation of clot before the centrifugation procedure. Samples were analyzed for CK mass and Troponin by two different methods: electrochemiluminescence (ECL), in the E411 Roche platform and fluorescence enzyme immunoassay (ELFA), in the Vidas 30 bioMerieux instrument.

Results:

For the ECL CK mass analysis, the Test F, T and the Pearson correlation showed up as expected. 5.14% of systematic constant was observed for level I and 0.82% for Level II that proved to be insignificant. Proportional systematic error was -3.37% with no impact on levels of clinical decision. The comparative test conducted with clot activator tube with gel separator and plasma from lithium heparin tube showed satisfactory results with total error obtained 7.39% and 8.17% for levels 1 and 2, respectively, less than the total allowable error of 30.6%. The troponin, F test and Pearson correlation showed results as expected. Constant systematic error was 4.06% for level 1 and 0.37% for level 2 which were considered not relevant. Proportional systematic error was -6.57% with no impact on levels of clinical decision. The comparative test conducted from gel x lytic plasma serum samples showed satisfactory results with total error obtained 7.63% and 11.32% for levels 1 and 2 respectively less than the total allowable error 48.9%

For ELFA , Ck Mass showed a correlated and Kappa index within the references, F test and Pearson correlation as expected. We observed constant and proportional systematic error of zero for the two levels. Comparative tests conducted between serum samples x lytic gel showed satisfactory results with plasma total error of 8.39% obtained for the two lower levels of total allowable error 27.91%.

Conclusion:

We observed that the use of both tubes had good performance in the evaluation of cardiac markers, CK Mass and troponin. The final result for the doctor had a shorter TAT, with high quality and efficiency, as the tube with plasma showed the benefit to be processed before the tube with the clot activator and separator gel.

B-035

Biomarkers of Cardiovascular Risk Assessment (High C-Reactive Protein, Homocysteine and Lipoprotein (a)) in First- Degree Relatives of Individuals with premature coronary disease

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Background: The use of biomarkers that can identify people about the risk of developing cardiovascular disease (CAD) is the subject of many studies. Several individual biomarkers have been linked to the risk of CAD including the levels of high-sensitivity C-Reactive Protein (a marker of inflammation), Homocysteine (a marker of endothelial function and oxidant stress) and Lipoprotein (a) that is involved in the adhesion of inflammatory cells and migration and up take of macrophage foam cells into the arterial wall. The aim of this study was to evaluate the profile of cardiovascular risk biomarkers (C-Reactive Protein, Homocysteine and Lipoprotein (a)) in first-degree relatives (FDR) of young individuals diagnosed with acute myocardial infarction (age < 45 years). **Methods:**It was a cross-sectional study that recruited 167 FDR of both sex of patients with premature coronary disease in period between 2010-2015. We measured high-sensitivity C- Reactive Protein, homocysteine and lipoprotein (a). Framinghan risk score was performed in among the patients. **Results:** The median age was 43,7±12,7 years. The laboratory analysis expressed in median showed homocysteine 9,7 (5,4 - 23,4)µmol/L; lipoprotein (a): 20 (2,9 - 106) mg/dL and high-sensitivity C Reactive Protein: 0,2 (0,01 -1,9) mg/dL. Sixty six percent of patients presented risk of developing cardiovascular disease in 10 years less than 10% by the criteria of Framingham score. **Conclusion:** There are few data available regarding these biomarkers of cardiovascular disease in FDR of patients with premature coronary artery disease. Despite the family history is an indisputable

cardiovascular risk factor was not observed in the evaluation of biomarkers such as homocysteine and lipoprotein (a) a significant change in this group of higher risk for cardiovascular disease. However when the biomarker of cardiovascular risk used was the high-sensitive C Reactive Protein most of these patients were classified as medium risk even those described as low risk by the Framingham criteria.

B-036

Endothelin Assay in Development for the Sgx ClarityTM Single Molecule Counting Platform Detects ET in Plasma and Discriminates Chronic Heart Failure from Healthy Donor Samples

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Background:

The Sgx Clarity System, a fully-automated in vitro diagnostics (IVD) platform that utilizes Singulex's Single Molecule Counting (SMCTM) technology, is in development. The new system will be a clinical diagnostics instrument which uses the same detection concept as the widely used and accepted Research Use Only ERENNA® instrument. The ERENNA has been shown to identify low-abundance biomarkers with unparalleled sensitivity, precision, and accuracy. The Sgx Clarity System utilizes a second generation scanning-based detection system which maintains the exquisite sensitivity of the ERENNA while improving upon the throughput, dynamic range, and usability of that system. Endothelin (ET) is a low abundance biomarker in plasma, and since its discovery in 1989, plasma ET has been studied as a biomarker for CVD risk stratification and for developing heart failure (HF). However, the very low endogenous concentration of the biologically active ET peptide in plasma has made such studies difficult, and to date, there is no IVD platform that tests for ET in plasma.

Objectives:

To assess the preliminary performance characteristics of the ET assay in development for the Sgx Clarity System, and to compare ET values obtained from the plasma of chronic heart failure (CHF) patients compared to those obtained from the plasma of apparently healthy donors (normals).

Methods:

44 CHF vs. normal donor plasma samples were tested with the Sgx Clarity ET assay on an Sgx Clarity prototype instrument. Protocols based on CLSI guidelines were followed for analytical performance assessment. Results were compared for statistical significance using the Wilcoxon rank sum test and were displayed in Box-and-Whiskers plots.

Results:

The ET assay had an LoB of 0.25 pg/mL and an LoD of 0.44 pg/mL as calculated using StatisProTM software. The 20% functional sensitivity was determined to be 1.0 pg/mL and the 10% functional sensitivity was 1.8 pg/mL as determined using precision profile analysis. Both Within-Run and Total Precision were ≤ 8% CV at concentrations of 1.2 pg/mL and above. The reportable range up to 25 ng/mL demonstrated > 5 logs of dynamic range, and the assay was linear down to the lowest concentration tested (0.3 pg/mL). When tested for hook effect, none was observed up to 500 ng/mL. No significant impact from common endogenous interferences was observed when tested at concentrations recommended in EP-7A2. ET was detected in 100% of normal and CHF plasma samples tested and a significant difference ($p<0.0001$) was observed between normal and CHF samples.

Conclusion:

The ET assay in development for the Sgx Clarity System demonstrates >5 logs of dynamic range, has sufficient sensitivity to detect ET in 100% of samples from apparently healthy donors, and effectively discriminates CHF from normals.

B-037

BNP Assay in Development for the Sgx ClarityTM Single Molecule Counting Platform Demonstrates Increased Clinical Sensitivity Relative to a Conventional BNP Immunoassay

X. Wang, S. Tjon-Kon-Sang, V. Torres, R. Livingston, L. Monsalve, J. Todd, J. Felberg, J. Bishop. *Singulex, Alameda, CA*

Background:

Singulex is developing the Sgx Clarity System, a fully-automated in vitro diagnostics platform that uses Singulex's Single Molecule Counting technology and has sensitivity equivalent to the widely used and accepted Research Use Only ERENNA® instrument while improving upon the throughput, dynamic range, and usability of that system. The BNP assay in development for the Sgx Clarity System uses the single epitope sandwich (SES) concept introduced by HyTest - where a capture antibody recognizing an epitope in the stable ring structure is paired with a detection antibody that binds only to the complex of the detection antibody-bound BNP molecule. The SES concept is hypothesized to confer a higher apparent stability of BNP in patient plasma, since much of the circulating BNP is truncated at the N- and C-termini, which are the epitope targets of most conventional commercially available BNP immunoassays.

Objectives:

To assess the preliminary performance characteristics of the Sgx Clarity BNP assay and to compare the clinical performance to a conventional BNP immunoassay.

Methods:

Forty plasma samples spanning the reportable range were tested on the Sgx Clarity BNP assay as well as the Siemens ADVIA Centaur BNP assay. Analytical performance studies followed protocols based on CLSI guidelines.

Results:

An LoB of 0.6 pg/mL, an LoD of 1.4 pg/mL, and a 10% functional sensitivity (LoQ) of 3.4 pg/mL were obtained. Within-Run Precision was 3 - 8% and Total Precision 4 - 10% with plasma samples from 4.5 to 1130 pg/mL BNP. The assay was linear throughout the reportable range, which extends to 5000 pg/mL, and there is no high dose hook effect up to100,000 pg/mL. No notable impact from common endogenous interferences and minimal cross reactivity was observed when other natriuretic peptides were tested. Passing-Bablok regression demonstrated good agreement between Sgx and Siemens methods (slope 1.09; Pearson correlation 0.97). Two samples, which had reported BNP concentrations of 220 and 297 pg/mL by the Siemens assay, had much higher results (1343 and 926 pg/mL) with the Sgx assay. A similar finding was observed in a subsequent study, with a small but significant percentage of discordant samples being observed. In all cases, the discrepant results were higher on the Sgx Clarity BNP assay. To further investigate this discrepancy, all samples were assayed for NT-proBNP (Roche). Those results showed clinical agreement with the Sgx Clarity BNP results relative to the established clinical cutoffs for the two molecules. This observation supports the hypothesis that some percentage of circulating BNP is not detected by conventional immunoassays whose antibodies bind to epitopes near the unstable termini of the BNP peptide. If true, the clinical relevance of this hypothesis requires further investigation.

Conclusion:

These results support the hypothesis that the Sgx Clarity BNP assay using the SES antibody concept may be more clinically sensitive than conventional BNP assays to circulating forms of BNP in patient plasmas.

B-038

A study of serum high sensitivity C-reactive protein and non-HDL-cholesterol as the marker for the risk for cardiovascular diseases.

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Background:

Hypertension (HTN) is a public health challenge globally and is one of the major risk factors for cardiovascular diseases. Approximately 1 billion of adults suffer from HTN worldwide with 333 and 639 millions in developed and developing countries respectively. In Nepal, the prevalence of HTN was reported to be 33.8%. HTN clusters with obesity and hypercholesterolemia with increased atherogenic Non-HDL-Cholesterol (Non-HDL-C). The early detection of the risk for cardiovascular disease (CVD) is essential to prevent the future cardiovascular events. High Sensitivity C-Reactive Protein (hs-CRP) is a sensitive marker of inflammation and tissue damage,

the concentration of which is associated with future development of atherosclerosis. Thus, this study was conducted to assess hs-CRP and lipid parameters in newly diagnosed cases of HTN and comparing the values with the controls.

Methods:

Forty seven newly diagnosed cases of hypertension and 47 age and sex matched healthy controls with prior informed consent were enrolled. The patients were clinically diagnosed as hypertensive according to the criteria of Joint National Committee (JNC-VII) on prevention, detection, evaluation and treatment of hypertension. Patients with chronic inflammatory diseases like rheumatoid arthritis (RA), osteoarthritis (OA), autoimmune diseases, tuberculosis and any previous history of diabetes/ stroke were excluded from the study. The age and sex matched healthy individuals without any history of hypertension, diabetes mellitus and chronic inflammatory diseases were enrolled as controls. The anthropometric measurements were done.

hs-CRP was measured by ELISA method and lipid parameters were estimated by spectro-photometric method in fasting blood sample. Non-HDL-C was calculated by subtracting high density lipoprotein cholesterol (HDL-C) from total cholesterol (TC). Data were analyzed by student 't' test, correlation was determined by Pearson correlation coefficient and P value was considered significant when $P<0.05$.

Results:

The result showed statistically significant increase in BMI (22.50 ± 2.59 Vs 24.41 ± 4.08), LDL-C (82.42 ± 20.41 Vs 104.32 ± 30.28), Non-HDL-C (104.72± 25.77 Vs 138.66± 38.80), TC: HDL-C (3.45 ± 0.76 Vs 4.36 ± 0.78) and hs-CRP (1.35 ± 1.20 Vs 3.07 ± 3.00) in hypertension as compared to controls ($P< 0.05$). However, the increase in TC and TG and the decrease in HDL-C were statistically not significant. Furthermore, hs-CRP had positive correlation with BMI, Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) and was statistically significant ($P< 0.05$) whereas correlation with TC was statistically not significant. The correlation of hs-CRP with HDL-C was negative and statistically not significant. Similarly, Non-HDL-C had positive correlation with SBP, DBP and TC and was statistically significant ($P< 0.05$), but correlation with BMI was statistically not significant. Non-HDL-C had negative correlation with HDL-C and was statistically not significant.

Conclusion:

Hypertensive subjects have significantly higher hs-CRP, non-HDL-C and BMI than that in controls. The levels of hs-CRP and Non-HDL-C are also correlated significantly with systolic as well as diastolic blood pressure. Hence, hs-CRP and non-HDL-C can be used as a marker for the risk for CVDs

B-039

Validation and Correlation study of the Values for the Beckman cTnI and TnI+3 Assay on the DxI 800 and Access-2 Analyzers.

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Background: Cardiac troponin (cTn) assays have been available in clinical laboratories for nearly two decades and considered a highly sensitive marker for myocardial damage. An elevation of cTn is used, together with other diagnostic criteria, to rule in/out a myocardial infarction (MI). Laboratories measure either cTnI or cTnT isoforms of troponin. Following a recall of cTnI reagents from the DxI Immunoassay analyzer in October 2010, Beckman Coulter (BC) recently re-introduced a Troponin-I (AccuTnI +3) assay for the DxI 800 and Access-2 analyzers.

Design: Method validation studies (correlations, linearity, inter- and intra-precision studies) were performed by comparing the following cTnI results (new reagent vs old reagent) on the following analyzers: 1. The Access-2 analyzers in the Emergency Department (ED) and the Main Laboratory (ML); 2. Access-2 in the ED and DxI 800 in the ML; 3. Access-2 ML and DxI 800 ML. A total of 115 patient specimens, presenting to our Emergency Department (ED) with history or evidence of cardiac disease or cTnI ordered following a review of patient's chart, were used for correlation studies. Specimens were spun, aliquoted, frozen within 24 hours at -20° C, and analyzed within 30 days of collection. Precision was performed using BioRad Cardiac Marker Plus Quality Control (Levels 1, 2, 3). Linearities were performed using BC calibrators.

Results:

Table. 1

Analyzers	No.	Mean x/y	Slope	Intercept	Correlation
Access-2 ED/ML	109	0.117/0.108	0.981	-0.008	0.9939
Access-2 ED/DxI800	115	0.12/0.111	0.791	0.015	0.9776
Access-2ML/DxI800 ML	115	0.131/0.111	0.775	0.009	0.9589

Linearities: 1) DXI: 0.000 to 67.240 ng/ml; 2) Access-2 ML: 0.000 to 96.857 ng/ml 3) Access-2 ER: 0.003 to 97.877 ng/ml

Conclusions:

The validation studies show that the new assay demonstrated good precision and correlation between the old and new reagents. The results also showed an extended reportable range (previously reported), allowing our laboratory to report results as low as 0.04 ng/mL (previously reported using the old reagents only down to 0.4 ng/mL). By increasing the sensitivity of the assay, earlier detection of an MI may be potentially achieved.

B-040

Matrix Metalloproteinase 9 as an Early Detector of Coronary Bare-Metal Stenting Restenosis

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Background : In-stent restenosis (ISR) is the major limiting factor of coronary bare-metal stenting (BMS). Establishing a reliable predictor for ISR risk would be an important factor for the decision making process to optimize patient management. Matrix metalloproteinase 9 (MMP9) was formed to be a predictor of cardiovascular mortality in patient with coronary artery disease (CAD). Aim of this work was to evaluate MMP 9 serum level to identify patient who are at high risk for development of ISR after BMS in a sample of Egyptian patients. Methods: Blood samples were collected prospectively from 60 patients previously diagnosed as stable CAD patients who were scheduled for elective BMS. Another 15 healthy volunteers were enrolled as a control group. All subject were evaluated for diabetic status'kidney function' liver function (including hepatic viral markers)' lipid profile & MMP 9 level was measured by ELISA . After 6 months the cooperative asymptomatic patients were subjected to coronary angiography & stress electrocardiogram (ECG). Results: Among our patients: 4 died within 6 months [Kaplan Meier survival = 93.3% with mean estimate = 5.652]. Fourteen patients were proved as clinical restenosis,(5 by angiography & 9 by positive stress test). Measuring the area under ROC curve ,the figure 139.9 µg/L was proved to be the best discriminating figure : sensitivity was 74.2% &specificity was 52.4% . The proved free asymptomatic patients were 43 patients (73.9%) with ≤139.9µg/L MMP9 while 12 (26.1%)had higher levels. Patients with clinical restenosis were 4 (=28.6%) with MMP9 ≤ 139.9 ug/L and 10 (= 71.4%) with higher levels This difference was statistically significant (P = 0.002) .Only diabetes mellitus among other risk factors (age, sex, hypertension dyslipidemia ,smoking &positive family history was significant between the two groups with &without restenosis . Conclusion the results suggest that serum MMP9 level may play an important role in the prediction of in-stent restenosis in patients with coronary bare metal stenting .

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Electrolytes/Blood Gas/Metabolites

B-041

Evaluation of GEM Premier 4000 Total Hemoglobin Test Accuracy Using Cyanmethemoglobin Reference Procedure and a Hospital Lab Reference Method

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Background: Whole blood total hemoglobin (tHb) measurement using Co-Oximetry methodology in hospital labs and at the point-of-care has become common practice for rapid evaluation of patients at-risk for bleeding or with suspected anemia. Reliability of tHb results is essential for patient care management and lack of inter-method harmonization of tHb results in hospital settings may cause confusion in tHb result clinical interpretation. Recently, differences in CO-Oximetry-derived tHb results using GEM Premier 4000 (Instrumentation Laboratory, Bedford, MA) versus Radiometer ABL 800 series analyzers (Radiometer, Westlake, OH) were identified and prompted enhanced efforts in tHb result harmonization. Cyanmethemoglobin (CNmetHb) based total hemoglobin (tHb) assay is recognized as a reference procedure by Clinical Laboratory Standards Institute (CLSI H15 A3) and International Council for Standardization in Hematology (ICSH). The GEM Premier 4000 tHb assay, similar to other CO-Oximetry analyzers, is traceable to CNmetHb procedure through its calibration reagents. CNmetHb procedure works well with hemoglobin standards and it has challenges with whole blood tHb measurements due to errors at different blood hemoglobin levels from turbidity or dilution issues from blood viscosity. Appropriate controls to minimize such errors in whole blood CNmetHb assay are implemented. The aim of this study was to evaluate GEM Premier 4000 tHb test accuracy in an industry setting compared with a CNmetHb reference procedure and in a hospital setting at 1 institution compared with Radiometer ABL 800 series analyzers.

Methods: At IL, lithium-heparinized blood was collected and the plasma to RBC ratio was adjusted to prepare five samples with increasing tHb concentration (range 3-21 g/dL). THb concentrations in the 5 samples were measured in triplicate using the CNmetHb reference procedure (CLSI H15A3) and GEM Premier 4000 analyzers. At the University of North Carolina (UNC) Hospital lab (Chapel Hill, NC), lithium-heparinized whole blood samples (n=110) sent to the lab for routine tHb testing were analyzed using Radiometer ABL 800 series analyzers, and residual whole blood from these samples was used for tHb measurement using GEM Premier 4000 analyzers.

Results: Linear regression analyses of tHb results yielded the following equations: [GEM 4000] = 1.001[CNmetHb] + 0.0947, (r2 = 0.9993, Range 3 -21 g/dL); [GEM 4000] = 0.9608[ABL 800] + 0. 4885, (r2 = 0.9868, Range 6.8 -16.7 g/dL). Bland Altman analysis of tHb results measured using GEM Premier 4000 compared with Radiometer ABL 800 series yielded a mean bias in 0.08 g/dL with a 95% confidence interval of ± 0.59 g/dL.

Conclusions GEM Premier 4000 tHb assay demonstrated excellent correlation compared with the CLSI and ICSH recognized CNmetHb reference procedure. Excellent correlation and good accuracy in tHb measurement was similarly observed between the GEM Premier 4000 and the Radiometer ABL 800 series tHb assays in a hospital setting. Taken together, these data indicate that using CO-Oximetry based tHb methods with calibration traceable to the CNmetHb reference procedure support inter-instrument harmonization of tHb results.

B-042

Evaluation of the Piccolo Xpress Implemented in an Ebola Bio-Containment Laboratory

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Background: The 2014 Ebola epidemic, the largest in history, revealed the need of medical institutions around the world to be able to provide medical care for patients potentially infected with highly contagious and dangerous infectious diseases. The University of Chicago Medicine (UCM) is one of fifty-five designated Ebola Treatment Centers in the United States by the Centers for Disease Control and Prevention. The challenge for UCM clinical laboratories is to provide quality laboratory results on highly virulent specimens with minimal risks to the medical technologists. The Piccolo Xpress (Abaxis, Union City, CA) is a small bench-top analyzer performs up to 14 chemistry tests on a single self-contained reagent disc. The complete test menu comprises of 31 different tests divided among 16 different panels.

Methods: The MetLac 12 (ALB, BUN, Ca, Cl-, CRE, GLU, K+, LAC, Mg, Na+, Phos, tCO2) and Hepatic Function (ALB, ALP, ALT, AST, DBIL, TBIL, TP) panels were evaluated and compared to the central lab's Roche Cobas 8000 chemistry analyzers. Precision studies were performed with low and high QC material (BioRad, CA) and linearity was assessed using linearity standards (Main Standards, ME; BRT, FL). Pooled patient samples were used in the interference studies and 51 plasma samples were used in the comparison study.

Results: The inter-assay precision for all assays varied from <0.01-16.4% CV for the low and high QC materials. The analytical measuring range for all analytes were linear (r2 = 0.9971-0.9999) over the testing range (ALB: 2.1-5.9 g/dL, BUN: 6-116 mg/dL, Ca: 4.4-13.6 mg/dL, Cl-: 89-135 mmol/L, CRE: 0.3-15.7 mg/dL, GLU: 28-701 mg/dL, K+: 1.9-7.3 mmol/L, LAC: 0.49-8.12 mmol/L, Mg: 0.6-6.5 mg/dL, Na+: 114-163 mmol/L, Phos: 1.2-14.8 mg/dL, tCO2: 11-39 mmol/L, ALP: 20-1758 U/L, ALT: 17-1400 U/L, AST: 16-1558 U/L, DBIL: 0.3-15.4 mg/dL, TBIL: 0.5-31.1 mg/dL, TP: 2.9-9.9 g/dL). Interference studies showed no significant interference for all analytes up to an H-index of 200 (except for LAC, DBIL, and TBIL) and an L-index of 212 (except for Phos and DBIL). Comparisons studies using Passing-Bablok linear regressions and Bland-Altman difference plots showed good overall agreement to the Cobas 8000 values except: ALB (y=0.85x+0.23), Ca (y =0.85x+1.38), Cl- (y=0.90x+14.10), Na+ (y=0.95x+6.90), tCO2 (y =1.20x-2.12), ALP (y=0.85x+0.16), ALT (y=0.89x+5.33), AST (y=0.93+8.80), and DBIL (y=0.56x+0.16). The calculated anion gap using the Piccolo Xpress values was significantly different from the central laboratory mainly due the positive biases in Cl- and tCO2. The reference ranges for ALB, Cl-, tCO2, AGAP, ALP, ALT, and AST required adjustment to account for the observed biases.

Conclusion: The overall analytical performance of the Piccolo Xpress is acceptable for use in a biocontainment laboratory. Despite the observed higher analytical imprecision and biases, the Piccolo Xpress has the additional advantages of having a small bench-top footprint, requiring a small sample volume in a self-contained reagent disc in ~13 minutes, minimal maintenance, and ease of operation.

B-043

Performance Evaluation of a New Fructosamine Assay to Measure Serum Glycated Protein on the High-Throughput ADVIA Chemistry Systems

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Background: Monitoring of glycemic status is important in diagnosis and monitoring of diabetes. The glycemic status of an individual can be assessed by measurements of fasting blood glucose, serum fructosamine (glycated protein), or glycated hemoglobin (HbA1c) for short term (daily), midterm (2-3 weeks), or longer (3-month average) periods. Fructosamine is formed by a nonenzymatic Maillard reaction between glucose and amino acid residues of proteins. During this glycation process, an intermediate labile Schiff base is produced which is converted to a more stable ketoamine (fructosamine) via an Amadori rearrangement. A new fructosamine assay [FRUC] has been developed for the measurement of serum or plasma fructosamine on the automated random access ADVIA® Clinical Chemistry Systems [Siemens]. The objective of this study was to evaluate the performance of this new assay on all ADVIA Chemistry Systems.

Methods: In the ADVIA Chemistry FRUC assay, sample is automatically pre-diluted (by 5x) and reacted with the first reagent (R1) for five minutes. The proteinase K in the reagent digests the serum glycated proteins. Fructosaminase and peroxidase in the second reagent (R2) then generates H_2O_2 and color, respectively, using the chromogen N-ethyl-N-sulphohydroxypropyl-m-toluidine (TOOS). The fructosamine concentration in a sample is determined from a linear calibration curve using Siemens ADVIA Chemistry Fructosamine Calibrator. The performance evaluation in this study included precision, interference, linearity, and correlation with a commercially available Diazyme glycated protein assay run on the Hitachi 717. Data were collected for all ADVIA Chemistry Systems (ADVIA 1200, ADVIA 1800, ADVIA 2400 and XPT*), which use the same ADVIA Chemistry FRUC reagent packs, calibrators, and commercial controls.

Results: The imprecision (total %CV) of the new ADVIA Chemistry assays with two-level commercial controls and six serum pools ranging from ~40 to ~750 µmol/L (n = 80) on all ADVIA Chemistry Systems was ≤2.9% (within-run) and 4.4% (total). The analytical range of the new assay is 30 - 1000 µmol/L. The assay correlated well with the Diazyme assay: ADVIA 1650 FRUC = 0.99 [Diazyme] - 13.1 (r = 0.995, n = 110; sample range: 40-737 µmol/L). The new assay demonstrated no interference (<10%) at a fructosamine level of ~150 µmol/L with unconjugated or conjugated bilirubin (5 mg/dL), hemoglobin (250 mg/dL), triglyceride (1000 mg/dL), albumin (6.1 g/dL), ascorbic acid (up to 10 mg/dL), glucose (2800 mg/dL), uric acid (50 mg/dL) and total protein - tested at approximately 490 µmol/L Fructosamine level - (8.4 g/dL). , Minimum on-system stability was 60 days with a calibration frequency of every 28 days.

Conclusion: The data demonstrates good performance of the FRUC assay on the high-throughput ADVIA Chemistry Systems from Siemens Healthcare Diagnostics.*Not available for sale in the U.S. Product availability may vary from country to country and is subject to local regulatory requirements.

B-044

Evaluation of Urine Performance on the VITROS® Cl- Slide Assay*

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Background

* The use of the VITROS Cl- Slide with urine specimens is in development

VITROS Chemistry Products Cl^- Slides (Chloride) quantitatively measure chloride (Cl-) concentration in serum and plasma using the VITROS 250/350/5,1 FS/4600 Chemistry Systems and the VITROS 5600 Integrated System. The VITROS Cl^- Slide is a multilayered, analytical element coated on a polyester support that utilizes direct potentiometry for measurement of chloride ions. Chloride is an essential electrolyte, and testing in urine is conducted to determine if there is an electrolyte imbalance. Testing is especially important in cases of persistent metabolic alkalosis where measured urine chloride levels are low.

Methods

We evaluated the accuracy of 81 patient urine samples (11 - 195 mmol/L) and 7 commercial Urine linearity fluids (1 - 316 mmol/L) diluted 1:1 with the VITROS Calibrator Kit 2, Level 1 on the VITROS 5,1 FS System compared to two commercial methods: titration using a Corning 926S Chloridometer and indirect potentiometry with the Chloride assay on the Siemen's ADVIA 1800 Chemistry System.

Results

The VITROS Cl^- Slides assay showed excellent correlation with both methods. VITROS 5,1 FS System = 0.989*Corning 926S + 3.08; (r) = 0.999 and VITROS 5,1 FS System = 1.001* ADVIA 1800 + 1.68; (r) = 0.997. Accuracy was also evaluated for 100 low chloride urine patient samples (5 - 50 mmol/L) run undiluted on the VITROS 5,1 FS System compared to the Siemen's ADVIA 1800 assay. The VITROS Cl^- Slides assay showed comparable correlation to the ADVIA 1800 assay as was observed in the previous assessment; VITROS 5,1 FS System = 1.053* ADVIA 1800 - 4.03; (r) = 0.987. A 5-day precision study conducted on the VITROS 350 and 5600 Systems with undiluted and diluted samples showed excellent precision with undiluted samples on both chemistry systems. Mean Chloride concentrations of 3.70 mmol/L, 9.99 mmol/L, 32.5 mmol/L, 97.1 mmol/L and 315.4 mmol/L resulted in within-laboratory percent coefficient of variation (%CV) of 2.0%, 0.81 %, 0.60%, 0.42%, and 0.67% respectively on the VITROS 5600 system.

Conclusion

The VITROS Cl^- Slides assay has exhibited good correlation with urine across a broad measuring range compared to commercial titration and indirect potentiometry methods. In addition excellent precision has been observed on the VITROS 350, 5,1 FS, and 5600 Systems with undiluted urine specimens.

B-045

Harmonization of ionized calcium levels in point-of-care blood gas analyzers

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Background: The measurement of ionized calcium has an important role in certain clinical settings such as primary hyperparathyroidism, cardiac and critical care, and patients undergoing major surgery. The aim of this study is to evaluate the differences of ionized calcium level among 6 manufacturers point-of-care blood gas analyzer and to check feasibility of harmonization mathematically.

Methods:A total of 6 kinds of point-of-care blood gas analyzer were used in this study for ionized calcium level measurement; Profile Critical Care Xpress (Nova Biomedical, USA), ABL 90 FLEX analyzer (Radiometer Medical), GEM Premier 3500 (Instrumentation Laboratory, USA), i-STAT System (Abbott Diagnostics), RAPIDPoint 500 Systems (Siemens Healthcare Diagnostics Inc.) and epoc Blood analysis System (Alere). Quality control materials provided by manufacturers were analyzed for 20 days to evaluate the precision according to the CLSI EP5-A2 guidelines. Ionized calcium levels were measured by each analyzer for 120 heparinized whole blood samples. The results were analyzed and compared with Deming regression and bias plot. Regression equation obtained from randomly allocated one group was applied for adjustment of results from another group.

Results: The total coefficients of variation (CV) of 6 instruments for the tested ionized calcium were less than 3.0%. Before adjustment, the slope, intercept and correlation coefficient of correlation equation were 0.881 to 1.124, -0.570 to 0.700, and 0.891 to 0.988, respectively. Adjustment of 6 analyzers made improvement in the slope, intercept, and correlation coefficient (1.011 to 1.054, -0.254 to -0.063, and 0.895 to 0.984, respectively). Three samples showed large bias (>10% of mean) before the adjustment, while all samples were within ±10% of mean after adjustment.

Conclusion:A majority of point-of-care blood gas analyzer showed excellent quality of performance of precision and correlation. This study demonstrates that harmonization may be accomplished technically by establishing adjustment to the overall mean values for a panel of patient samples.

B-046

Comparative precisions of intra-patient ICU blood gas results measured by paired GEM 4000's and Radiometer ABL800's

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Background: Over the last three decades, duplicate blood gas testing has been replaced by singlicate testing, usually by one of multiple blood gas instruments. Between-instrument variation can either falsely indicate trends or obscure real trends. We have developed a methodology that transforms sequential intra-patient results into biologic and analytic variation. We previously derived realistic biologic variation (s_b) parameters of blood gas analytes from ICU blood gas data (from two Radiometer 800 ABL Flex's[Copenhagen, Denmark]). We apply these results to derive the imprecision of two GEM 4000's (Instrumentation Laboratory, Bedford MA)

Methods: A repository provided arterial blood gas results generated by two GEM 4000's on ICU patients in 2012-2013 at Foothills University Hospital in Calgary, Alberta. As results were not linked to the analyzers' identity, sequential results appeared to originate from the same analyzer. For each analyte, we tabulated consecutive pairs of intra-patient results separated by time intervals of 0-2 ,2-4, 4-6, up to 16 hours. The average between pair variations were regressed against time with the y-intercept representing biologic variation and short term analytic variation, including between-instrument and between-cartridge variation: $y^2_{o\ GEM} = s^2_b + s^2_{aGEM}$. Using an equivalent equation for the Radiometer and simple algebra, the imprecision of the two GEM's can be calculated from: $s_{aGEM} = (y^2_{o\ GEM} - y^2_{o\ ABL} + s^2_{aABL})^{1/2}$.

Results: Over the two year period, approximately 60,000 arterial blood gases were analyzed. Regression graphs were derived from around 1800 patients with least 10,000 data pairs grouped into 2 hr intervals. The Table compares the directly measured s_{aABL}, the calculated s_{aGEM}, and corresponding sigmas, calculated from (biologic variation)/(analytic variation).

Conclusion: For a large number of GEM analytes, the effective analytical variation of GEMs used in tandem is on the order of the biologic variation, indicating a significant reduction in the clinical usefulness of the repeated analytes.

Test	S_{aABL}	S_{aGEM}	Sigma ABL	Sigma GEM
Chloride, mmol/L	0.44	0.52	1.8	1.5
Glucose, mmol/L	0.11	0.41	4.7	1.3
HCO_3, mmol/L	0.22	0.60	3.6	1.3
ionized Ca, mmol/L	0.0042	0.033	3.4	0.4
Potassium, mmol/L	0.0244	0.044	7.9	4.4
Sodium, mmol/L	0.38	0.72	2.2	1.2
pCO_2, mmHg	0.34	0.40	6.6	5.6
pH	0.00148	0.029	15.0	0.8
pO_2 ,mmHg	1.36	5.34	9.9	2.5

B-047

High Throughput Immunoassay for Kidney Function Biomarker Symmetric Dimethylarginine (SDMA)

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Symmetric dimethylarginine (SDMA) is a dimethylated derivative of arginine that results from intra-nuclear methylation and subsequent catabolism of proteins. SDMA is a sensitive and specific biomarker for kidney function and correlates well to GFR. Several recent studies have shown SDMA to be an earlier and more accurate marker than serum creatinine. In addition, studies have shown that SDMA is a better indicator of kidney function associated mortality in cardiac and stroke patients further establishing the value of this emerging biomarker. The current report describes a high throughput clinical chemistry immunoassay that has been developed and correlated to the gold standard LC-MS assay using samples from canine and feline models along with healthy and CKD human cohorts.

The LC separation was achieved using X-Bridge RP C-18 column and an ion pairing agent. The API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) was operated in Multiple Reaction Monitoring (MRM) mode with positive electrospray interface. The MRM transition for SDMA was observed at m/z 203.2 ---> 172.1. As part of the method validation, performance characteristics including sensitivity, carryover and interferences, matrix effect and recovery, linearity, accuracy and precision, ruggedness, stability, robustness and interfering substances were established. All performance metrics were within established FDA guidance.

The clinical chemistry immunoassay utilizes a SDMA-G6PDH conjugate and anti SDMA monoclonal antibody. The antibody is specific to SDMA and has no significant cross reactivity to arginine, monomethyl arginine and asymmetric dimethylarginine. The dynamic range of the assay is between 0 and 100µg/dL and within-run precision across the range is between 5 and 10%.

Accuracy was determined using 351canine and 280 feline and 160 human serum samples from healthy and CKD populations. All the samples were run on both the LC-MS assay and the clinical chemistry immunoassay (Beckman automated clinical chemistry analyzer) and the results were presented in the following table:

Slope Intercept R

Dogs 1 0.2 0.97

Cats 0.96 0.2 0.97

Humans 0.96 1.3 0.99

In conclusion, we have developed and validated a high throughput clinical chemistry immunoassay that correlates to the LC-MS and accurately quantifies SDMA in biological samples from dogs, cats and humans.

B-048

Validation of a Novel High Throughput Immunoassay for the Quantitation of Symmetric Dimethylarginine (SDMA)

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Symmetric Dimethylarginine (SDMA) is derived from intranuclear methylation of L-arginine by protein-arginine methyltransferases (PRMT) and released into the circulation after proteolysis. SDMA is eliminated primarily by renal clearance and is shown to be an accurate and precise biomarker for calculating estimated glomerular filtration rate (eGFR) in humans. Recent studies have also demonstrated its utility as an early and more sensitive biomarker than serum creatinine in assessing renal dysfunction. SDMA represents an emerging biomarker for diagnosing and monitoring chronic kidney disease (CKD). The objective of this study was to validate a new high-throughput, competitive homogeneous immunoassay to quantify SDMA in serum and plasma using a canine model.

The two-reagent system contains an anti-SDMA monoclonal antibody and a G6PDH-SDMA conjugate. Precision, dynamic range, and accuracy were determined following CLSI guidelines using Beckman automated clinical chemistry analyzers across multiple reagent lots. In the range of 10-20 µg/dL, within-run precision was ≤ 7%CV, and total precision was ≤ 10% CV. Dynamic range was shown to be 5 to 100 µg/dL. Accuracy, which was assessed by correlation to the gold standard liquid chromatography mass spectrometry method, showed a slope of 1.0±0.1 and an intercept below the assay limit of detection. No significant interference from lipemia or icterus was observed, and no significant interference from moderate levels (100 mg/dL) of hemoglobin was observed. Related compounds such as arginine, monomethyl arginine and asymmetric dimethylarginine had no significant impact on assay performance. The assay performance was acceptable on both serum and plasma sample.

This SDMA immunoassay demonstrates clinical utility as a novel diagnostic tool in measuring the promising chronic kidney disease biomarker SDMA.

B-049

Evaluation of Photometric Methods on the Siemens ADVIA® Chemistry XPT System

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Introduction

Siemens recently introduced the new ADVIA Chemistry XPT System which joined the ADVIA Chemistry family of analyzers: the ADVIA 2400 and ADVIA 1800 Clinical Chemistry Systems. The ADVIA Chemistry XPT System utilizes identical reagents as the previous analyzers while having a throughput of 2400 tests/hour. For this study we assessed five methods: Hemoglobin A1c_3 Automated Pretreatment (A1C_3), Calcium_2 (CA_2), Cholesterol_2 (CHOL_2), Glucose Hexokinase_3 (GLUH_3), and Creatinine_2 (CREA_2).

Materials & Methods

All studies were completed at a Siemens Healthcare Diagnostics laboratory. Precision was analyzed according to CLSI Guideline EP05-A2 and Method Comparison according to CLSI Guideline EP09-A3. Precision was evaluated on two ADVIA XPT systems with five replicates of commercial controls over ten days, two runs/ day. Method comparison was performed on two ADVIA XPT systems and one ADVIA 2400 Clinical Chemistry System.

Results

Hemoglobin A1c_3 Automated Pretreatment within-lab CVs in whole blood ranged from 2.2% to 2.6% across the concentrations tested.

Calcium_2 within-lab CVs in serum ranged from 1.0% to 1.4% across the concentrations tested. Within-lab CVs in urine ranged 1.2% to 1.6% across the concentrations tested.

Cholesterol_2 within-lab CVs in serum were 1.2% across the concentrations tested.

Creatinine_2 within-lab CVs in urine ranged from 2.7% to 3.2% across the concentrations tested.

Glucose Hexokinase_3 within-lab CVs in serum ranged from 0.7% to 0.9% across the concentrations tested. Within-lab CVs in CSF ranged 1.0% to 1.1% across the concentrations tested.

The method comparison table below shows Weighted Deming fit against the ADVIA 2400 Clinical Chemistry System.

Assay	Matrix	n	Slope	Intercept	r	Sy x	Range	Units
A1C_3	Whole Blood	75	1.00	0.13	0.990	0.030	4.50- 12.80	%
CA_2	Serum	130	0.99	-0.19	0.999	0.021	1.49- 15.22	mg/dL
CA_2	Urine	102	0.98	-0.17	0.999	0.031	2.16- 29.08	mg/dL
CHOL_2	Serum	99	0.98	0.61	0.997	0.033	28- 549	mg/dL
CREA_2	Urine	142	1.05	-0.05	0.998	0.040	3.47- 290.92	mg/dL
GLUH_3	Serum	97	0.99	-0.55	0.999	0.032	43- 659	mg/dL
GLUH_3	CSF	104	0.98	-0.55	1.000	0.019	16- 620	mg/dL

Conclusion

All methods demonstrated equivalent performance for both repeatability and within-lab performance as well as demonstrating agreement for method comparison testing on the ADVIA Chemistry XPT Systems versus the ADVIA 2400 Clinical Chemistry System.

*ADVIA is a registered trademark of Siemens Healthcare Diagnostics, Inc and System availability depends on local regulatory requirements.

B-050

Prevalence of clinically significant errors in sodium measurements due to ion exclusion effect using an indirect ion selective method

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Background: Indirect ion selective electrode (ISE) is the primary method used to measure serum sodium in clinical laboratories. Pseudohyponatremia can occur from the ion exclusion effect due to hyperlipidemia and hyperproteinemia. Reporting erroneous sodium values could impact patient management. Hyperlipidemia can easily be detected using the serum lipemia index on automated chemistry analyzers, while hyperproteinemia requires protein measurement for detection. **Objectives:** (i) Determine the relationship between serum total protein (TP) concentration and the change in sodium concentration observed between indirect and direct ISE methods, (ii) estimate the frequency at which sodium results measured by indirect ISE are clinically re-categorized due to abnormal TP concentration, and (iii) determine whether middleware rules that query test results for combined protein and sodium orders would be effective for error detection. **Methods:** Sodium concentration was measured using indirect ISE (Cobas 8000, Roche Diagnostics) and direct ISE (ABL 825, Radiometer) methods on residual serum from physician-ordered TP testing (Roche Biuret method; n=66, concentration range: 3.6-9.0 g/dL) or protein electrophoresis with confirmed monoclonal protein (n=49, concentration range: 9.5-15.4 g/dL). The difference in sodium concentration (Δ[Na+]) was calculated as follows: ([Na+]indirect-ISE – [Na+]direct-ISE). Retrospective sodium and TP orders and results from the Mayo Clinic (Rochester, MN) from 07/31/2013 to 09/24/2014 were analyzed. Specimens were stratified based on TP reference intervals: low TP (<6.3 g/dL, n=41), normal TP (6.3-7.9 g/dL, n=16), and high TP (>7.9 g/dL, n=57). The sodium reference interval is 135-145 mmol/L. **Results:** Δ[Na+] was inversely proportional to TP concentration (y=-1.22x+7.9, R2=0.835). When TP concentration was <6.3 g/dL the average difference(SD, range) in sodium concentration was 2.2(1.5, -2 to -4) mmol/L. This led to 17% of specimens with sodium concentration within the reference range (normal) by indirect ISE to measure low by direct ISE. The average difference(SD, range) was -5.6(3.3, -13 to 0) mmol/L when TP>7.9 g/dL, which led to 31.5 % of specimens with low sodium to become normal and 1.7 % considered normal to become high when measured by direct ISE. Only 12.8% of routine sodium test orders include an order for TP on the same collection. Of orders including both tests, 19.1% had low TP and 3.2% had high TP. Hematology/oncology and nephrology clinics accounted for 20.2% of low TP results, while general internal medicine and hematology/oncology clinic accounted for 36.6% of all high TP results. Only 5.1% of stat sodium orders include a TP test order; 41.1% had low TP and 1.8% had high TP. **Conclusions:** This study demonstrated that sodium measurement by indirect-ISE can give erroneous results in serum or plasma when TP concentrations are outside the reference interval. In our patient population, sodium is usually not ordered with TP so a middleware rule-based solution that queries TP results would not detect most cases of low or high TP. Health systems that use indirect ISE for sodium measurement need to be aware of the limitation of the method and the potential errors in sodium measurement and misclassification that may occur in patients with abnormal TP concentrations.

B-051

Ion chromatography as candidate reference method for the determination of chloride in human serum

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Background: Serum chloride is the major anion in human body which has to be kept within narrow limit to ensure the maintenance of electrolyte homeostasis in both intra- and extracellular compartments of the organism. The standardization of the measurement of serum chloride is of considerable interest for quality assurance in patient care. In this context, isotope dilution thermal ionization spectrometry (ID-TIMS) and coulometry are recognized as the traditional reference method principles for serum chloride. While, there is at least two independent measurement principles should be used to increase the reliability of the cerified value for reference materials. In this perspective, a simple, rapid, accurate and sensitive method based on ion chromatography, which could be recommended as candidate reference method, has been developed for the determination of serum chloride.

Method: Serum samples were diluted with 10 mmol/L KOH solution and chloride was measured by ion chromatography with a gradient elution procedure using a KOH eluent generator. The measurement accuracy and precision was calculated by analyzing IFCC-RELA samples. Furthermore, the proposed method was compared with inductively coupled plasma mass spectrometry (ICP-MS) by using 27 serum samples from individual patients.

Results: The calibration curve for chloride was linear in the concentration range from 0 - 15 mg/L with a correlation coefficient of 0.99995 under the optimum experimental conditions. The detection limit was found to be 3.5 µg/L. The measurement accuracy and precision is less than 0.8 % by analyzing 2012 and 2013 IFCC-RELA samples. The results were also comparable with the reference values obtained by the inductively coupled plasma mass spectrometry (ICP-MS), which were found to be in good agreement (see Figure 1).

Conclusion: The proposed method could be recommended as candidate reference method for the determination of chloride in human serum.

B-052

Comprehensive Correlation between Siemens Point-of-care and Central Laboratory Blood Gas Systems and ADVIA 1800 Clinical Chemistry System for Electrolytes and Metabolites

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Objective: Determine correlation between Siemens point-of-care (POC) and central laboratory blood gas systems versus the central laboratory ADVIA® 1800 Clinical Chemistry System in order to demonstrate harmonization across the diagnostic product portfolio.

Relevance: AACC's International Consortium for Harmonization of Clinical Laboratory Results has been working with a variety of stakeholders regarding harmonization among results from different methods and laboratories for the same measurand.[1] Malone states, "Harmonization means achieving comparable results among different measurement procedures···When lab measurement procedures give different results for the same specimen, patients may get the wrong treatment, because decision criteria are not appropriate for the procedure in use. In order to do this effectively, results need to be harmonized."

[1]. Malone B. AACC's Thought Leadership Series: Why Harmonization Matters.

*Not available for sale in the U.S. Product availability varies by country.

Methods:

Method comparison studies were performed with whole blood on the POC and central laboratory blood gas systems (RAPIDPoint® and RAPIDLab® Blood Gas Systems) and with plasma on the clinical chemistry system (ADVIA 1800 system) in accordance with the CLSI EP09-A3 guideline. Correlation statistics including regression types, slopes, intercepts, and coefficients of determination (r^2) were generated for the following comparisons:

- RAPIDPoint 500 Blood Gas System vs. ADVIA 1800 Clinical Chemistry System
- RAPIDLab 1265 Blood Gas System vs. ADVIA 1800 Clinical Chemistry System
- RAPIDLab 348EX Blood Gas System* vs. ADVIA 1800 Clinical Chemistry System

Results:

Regression statistics for each comparison across measured intervals for each measurand are shown in Table 1. The slopes for each measurand fell between 0.91 and 1.17, with $r^2 \geq 0.9679$.

Conclusion:

Harmonization at medical decision levels and average concentrations was demonstrated between Siemens POC and central laboratory blood gas platforms with whole blood and the ADVIA 1800 Clinical Chemistry System with plasma for the measurands evaluated.

Comparison	Measurand	n	Median Bias	Regression Type	Slope	Intercept	r^2	Interval
RAPIDPoint 500 Blood Gas System vs. ADVIA 1800 Clinical Chemistry System	Na^+ (mmol/L)	124	0.4	Deming	1.02	-1.2	0.9893	102.0–197.0
	K^+ (mmol/L)	100	3.89%	Weighted Deming	0.96	0.27	0.9979	1.10–9.60
	Cl^- (mmol/L)	124	-1	Deming	0.91	8	0.9916	66–147
	Glu (mg/dL)	100	3%	Weighted Deming	1.04	-2	0.9960	28–693
	Lac (mmol/L)	97	1.76%	Weighted Deming	0.97	0.19	0.9679	0.30–32.67
	nBili (mg/dL)	108	-0.3	Deming	1.14	-1.7	0.9974	2.3–24.7
RAPIDLab 1200 Blood Gas System vs. ADVIA 1800 Clinical Chemistry System	Na^+ (mmol/L)	123	0.9	Deming	1.05	-6.1	0.9865	102.0–197.0
	K^+ (mmol/L)	100	3.64%	Weighted Deming	1.02	0.09	0.9972	1.10–9.60
	Cl^- (mmol/L)	124	2	Deming	0.99	3	0.9908	66–147
	Glu (mg/dL)	100	5%	Weighted Deming	1.02	4	0.9985	28–693
	Lac (mmol/L)	95	-2.47%	Weighted Deming	0.93	0.27	0.9933	0.65–32.67
	nBili (mg/dL)	110	0.8	Deming	1.17	-0.8	0.9763	2.3–24.7
RAPIDLab 348EX Blood Gas System vs. ADVIA 1800 Clinical Chemistry System	Na^+ (mmol/L)	124	3	Deming	0.98	7	0.9851	102–197
	K^+ (mmol/L)	101	5.38%	Weighted Deming	1.03	0.13	0.9972	0.70–9.60
	Cl^- (mmol/L)	124	0	Deming	0.98	2	0.9901	66–147

B-053

Performance of the NephroCheck® for VITROS® Test** on the VITROS® 3600 Immunodiagnostic System

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Acute kidney injury (AKI) is a common disorder with potentially catastrophic complications that can lead to high morbidity and mortality rates. The NephroCheck for VITROS Test** (VITROS) quantitatively measures Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) and Insulin-like Growth Factor Binding Protein 7 (IGFBP-7) to generate an acute kidney injury (AKI) risk index (AKIRISK™ Score). We have evaluated the performance on the VITROS® 3600 Immunodiagnostic Systems. The test is linear across the range of 1.58 to 30.9 ng/mL for TIMP-2 and 20.6 to 647 ng/mL for IGFBP-7 yielding an AKIRISK™ Score range of 0.0325 to 20.0. Limits of Blank (LoB) were determined to be 0.52 ng/mL and 0.110 ng/mL for TIMP-2 and IGFBP-7, respectively. Limits of Detection (LoD) were determined to be 0.243 ng/mL for TIMP-2 and 1.994 ng/mL for IGFBP-7 resulting in LoB and LoD for the AKIRISK™ Score of 2.8×10^{-6} and 0.003 respectively. A 5-day precision study with samples at mean TIMP-2 concentrations of 1.26 ng/mL, 2.63 ng/mL, 9.67 ng/mL, and 10.6 ng/mL resulted in within-laboratory percent coefficient of variation (%CV) of 10.7%, 6.4%, 3.4%, and 3.7% respectively. Similar results were obtained for IGFBP-7 at concentrations of 35.1 ng/mL, 65.7 ng/mL, 138 ng/mL, and 202 ng/mL, resulting in within-laboratory %CV of 5.8%, 6.6%, 7.5%, and 8.0% respectively. The precision of the AKIRISK™ Score based on the two results were 11.5%, 7.9%, 9.0%, and 9.8% at AKIRISK™ Score of 0.04, 0.17, 1.34, and 2.14. The accuracy of the test was evaluated with 50 patient specimens against the Astute Medical NephroCheck® Test System (Astute) The following linear regression statistics were obtained: VITROS TIMP-2 = 1.153*Astute - 1.24; (r) = 0.960; VITROS IGFBP-7 = 1.069*Astute - 1.717; (r) = 0.984. The positive (PPA) and negative (NPA) percent agreement between the two assays were calculated based on the AKIRISK™ Score cutoff of 0.3 established on the Astute Medical NephroCheck® Test System, with AKIRISK™ Score greater than 0.3 being positive and AKIRISK™ Score less than 0.3 being negative. Compared to Astute, the VITROS AKIRISK™ Score had a 93.8% PPA and a 100% NPA. (** under development)

B-054

Asymptomatic severe hypophosphataemia in acute T-cell lymphoblastic leukaemia

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Introduction: Hypophosphatemia is a metabolic disorder that iscommonly encountered in critically ill patients.Hypophosphatemia is defined asplasma phosphate level below 0.80 mmol per litre(mmol/L), and can be further divided into subgroupsof mild (a plasma phosphate of 0.66 to 0.79 mmol/L),moderate (plasma phosphate of 0.32 to 0.65mmol/L) and severe (plasma phosphate of less than0.32 mmol/L).Phosphate has many roles in physiologicalfunctions, thus the depletion of serum phosphatecould lead to impairment in multiple organ systems,which include respiratory system, cardiovascularsystem, neurological system, muscular system,haematological and metabolic functions. The causes of hypophosphatemia include inadequate phosphate intake, decreased intestinal absorption, gastrointestinal or renal phosphate loss, and redistribution of phosphate into cells. Symptomatic hypophosphatemia associated with haematological malignancies has been reported infrequently. We report here a case of asymptomatic severe hypophosphatemia in achild withacute T-cell lymphoblastic leukaemia.

Case report:A 14-year-old Chinese boy initially presented with left lower motor neuron facial nerve palsy and was given oral prednisolone for two weeks. However, his symptom did not improve. After one month, he developed high grade fever and bilateral epistaxis. On physical examination, cardiovascular system was normal and lungs were clear. Abdomen examination revealed hepatosplenomegaly. However, he was noted to have bilateral submandibular and right inguinal lymphadenopathies. Central nervous system showed no other abnormality other than 7th nerve palsy.

His initial complete blood counts showed mild anaemiaand increased total white cellscount of183 x 10^9/L.His peripheral blood picture showed numerousblast cells. Bone marrow examination and immunophenotypingconfirmed the diagnosis of acute T Cell Lymphoblastic Leukaemia (ALL).

His serum biochemistry results were normal except inorganic phosphate and lactate dehydrogenase levels. The serum inorganic phosphate level was 0.1mmol/L and the level was low on repeated analysis.The laboratory notified the requesting clinician about the low phosphate level and enquired about any clinical signs and symptoms related to low phosphate level. The child had no symptoms related to low phosphate level. Since the serum phosphate level was very low without any symptoms for hypophosphatemia, lithium heparin sample was requested to rule out any interference. This confirmed very low serum phosphate level. The possible causes of low phosphate were ruled out and urine Tmp/GFR was normal.Chemotherapy regime was started and the serum phosphate levels started to increase. He was monitored for tumour lysis syndrome.

Hypophosphatemia in leukaemia was attributed due to shift of phosphorus into leukemic cells and excessive cellular phosphate consumption by rapidly proliferating cells. Several reports of symptomatic hypophosphatemia in myelogenous and lymphoblastic leukaemia in adults have been reported. To our knowledge this is the first case of severe asymptomatic hypophosphatemia in a child with ALL.

B-055

Bilirubin interference and bias evaluations of 7 routine creatinine measurement methods compared with ID-LC/MS

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Background:

Serum creatinine is measured in order to estimate glomerular filtration rate. For the measurement of creatinine, alkaline picrate reaction or Jaffe reaction is still widely used in clinical laboratories. However, the Jaffe method is interfered by bilirubin, resulting in falsely low creatinine level. Because enzymatic method is known to be free form the problem of bilirubin interference, reagent based on the enzymatic method can be alternative assay. The aim of this study is to compare enzymatic methods and kinetic Jaffe methods with isotope dilution-liquid chromatography mass spectrometry (ID-LC/MS) to estimate bilirubin interference.

Methods:

Forty clinical serum samples from 31 patients which had serum of total bilirubin concentration above 4.0 mg/dL were collected. The serum creatinine was measured using three enzymatic reagents: Pureauto S CRE-L (SEKISUI MEDICAL CO., LTD, Japan) and L-Type Wako CRE•M (Wako Pure Chemical Industries, Ltd., Japan) performed on Hitachi 7600 analyzer (Hitachi Co., Japan), and Stat Profile Critical Care Xpress (Nova Biochemical, USA) performed on Critical Care Xpress blood gas analyzer (Nova Biochemical). The serum creatinine was also measured with four kinds of kinetic Jaffe methods of Clinimate CRE (SEKISUI MEDICAL CO., LTD, Japan) using Hitachi 7600, SYNCHRON CREm (Beckman Coulter, Inc., USA) using Unicel DxC880i (Beckman Coulter), CREJ2 (Roche Diagnostics GmbH, Germany) using Cobas c 702 modules (Roche Diagnostics), and AU Creatinine (Beckman Coulter, Inc., USA) using AU680 chemistry system (Beckman Coulter) as well as ID-LC/MS. The total bilirubin values are plotted against percent bias, between serum creatinine values from each reagent and those of ID-LC/MS. In addition, the correlation between serum total bilirubin and percent bias was analyzed in three different ranges of serum creatinine, Low (1.1 mg/dL). Passing-Bablok regressions for method comparison between those 7 reagents and ID-LC/MS were also performed.

Results:

Pureauto S CRE-L, L-Type Wako CRE•M, SYNCHRON CREm and Stat Profile Critical Care Xpress reagents showed no significant serum bilirubin interference. However, Clinimate CRE showed significant negative serum bilirubin interference on the low and medium serum creatinine levels, while CREJ2 and AU Creatinine showed significant positive interference on the low serum creatinine level. Method comparison with ID-LC/MS using Passing-Bablok regression revealed that Pureauto S CRE-L and SYNCHRON CREm reagent had bias beyond the allowable total error at one or two medical decision levels.

Conclusion:

Three enzymatic methods evaluated were free from bilirubin interference while kineteic Jaffe methods showed negative or positive bilirubin interference except one method. However, one enzymatic method showed bias at medical decision level indicating the reagent was not traceable to ID-LC/MS. Therefore, to select an accurate method for creatinine, both traceability to ID-LC/MS and bilirubin interference should be considered.

B-056

Assessment of serum indices implementation on ADVIA Chemistry 2400 System

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Background: Use of hemolyzed, lipemic, and icteric samples can cause critical changes in the results of several laboratories analyzes. The increasing of laboratory examinations, combined with automated processes, reduces the possibility of manual inspection. So, it`s very important that an automated system provides this analysis quickly, accurately and in a standardized way. This study aims to compare the effectiveness of automated spectrophotometric detection with the visual inspection of lipemic and hemolyzed serum samples **Methods:** The study was conducted during the processing of 500 serum samples; 50 samples showed changes in one or more serum indices. For the automatic identification, it was used a specific and standardized protocol for the ADVIA 2400® Systems. At the manual inspection, three experienced laboratory analysts defined the graduation of interferences (According to Siemens Setting Up a Dedicated Serum Indices Method Rev. A, 2008-11 figures). For the comparability of visual reading with the automatic detection of lipemic and hemolyzed serum, it was accepted up to one level of difference for positive samples and no difference in negative samples. **Results:** For hemolysis, there was a complete correlation between the automation and manual classification in 90% of the samples. In 10 % there was a one-grade divergence. For Lipemia, 92% of samples showed no differences in evaluation. In 8% of the samples there was a one-grade difference. All negative samples for the visual reading were also confirmed as negative by the automated testing. **Conclusion:** According to this study, automated identification of serum indices performed by the ADVIA Chemistry 2400® System is considered highly reliable when compared to manual inspection. This eliminates subjective interpretations that may occur in ordinary visual reading. The automation of this process permits availability of the operators to perform high value activities, ensuring the release of the results, making clinical correlations and detecting the presence of interferents in a short time and with a high quality score.

B-057

Evaluation of Electrolyte Performance on the Siemens ADVIA Chemistry XPT System

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Introduction

Electrolytes play an important role in the human body. Analytical determination of electrolytes is a critical function in the clinical laboratory. The Siemens ADVIA® XPT Chemistry System is a floor standing, 2400 test per hour chemistry system engineered for continuous operation and timely, accurate results. We evaluated three electrolytes, sodium (Na), potassium (K), and chloride (Cl), on the ADVIA XPT system.

Materials and Methods

All studies were conducted on two ADVIA Chemistry XPT systems. Precision studies assayed QC material over 10 days. Correlation studies assayed both serum and urine samples over multiple days against an ADVIA® 2400 Clinical Chemistry System. Precision was analyzed according to CLSI Guideline EP05-A2. Correlation was analyzed according to CLSI Guideline EP09-A3.

Results

The method comparison table below shows Weighted Deming fits against the ADVIA 2400 Clinical Chemistry system.

Analyte	Matrix	n	Slope	Y-intercept	r	Syx	Range (mEq/L)
Cl	Serum	117	1.01	0.7	0.998	0.015	50-191
Cl	Urine	137	0.99	2.8	0.999	0.035	15-375
Na	Serum	102	0.98	2.4	0.996	0.012	101-196
Na	Urine	139	0.96	4.13	0.999	0.036	10-377
K	Serum	122	0.97	0.11	0.998	0.021	1.0-9.7
K	Urine	142	1.00	0.07	1.000	0.013	2.4-272.3

For Cl precision, repeatability and within-lab CVs in serum ranged from 0.2% to 0.4% and 0.3% to 0.6%, respectively, across the concentrations tested. Repeatability and within-lab CVs in urine ranged from 0.3% to 0.6% and 0.5% to 0.9% across all concentrations tested.

For Na precision, repeatability and within-lab CVs in serum ranged from 0.2% to 0.3% and 0.3% to 0.6%, respectively, across the concentrations tested. Repeatability and within-lab CVs in urine ranged from 0.3% to 0.8% and 0.5% to 1.1% across the concentrations tested.

For K precision, repeatability and within-lab CVs in serum ranged from 0.2% to 0.9% and 0.5% to 1.1%, respectively, across the concentrations tested. Repeatability and within-lab CVs in urine ranged from 0.4% to 0.6% and 0.7% to 0.9% across the concentrations tested.

Conclusion

The Na, Cl, and K assays all showed good repeatability, within-lab precision and correlation to ADVIA 2400 Clinical Chemistry systems when tested on the ADVIA Chemistry XPT System.

*System availability depends on local regulatory requirements.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Infectious Disease

B-058

Comparison of respiratory viral panel detection by Mediator probe and hydrolysis probe PCR

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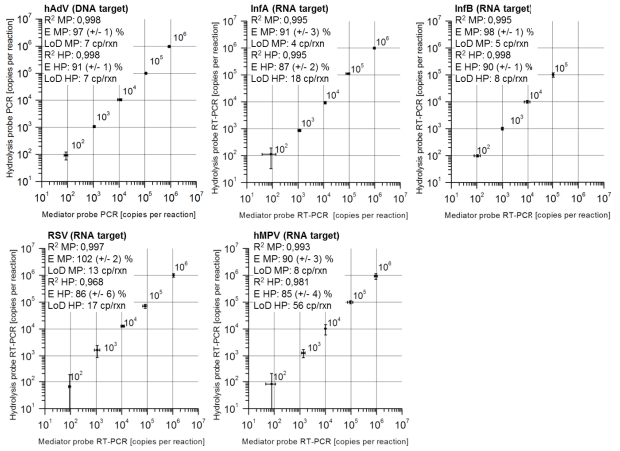
Background: Molecular diagnostics often uses hydrolysis probes (HP) for real-time nucleic acid sensing. However, each target sequence requires synthesis of specific dual-labelled HPs, which are expensive, especially when used at low batch sizes. Also, HPs must be individually optimized for signal generation efficiencies for each target sequence to be detected. We have published a novel approach, the mediator probe PCR (MP PCR) [B. Faltin et al.: Clin Chem (58) 2012] which overcomes these issues by using a labelled but universal reporter oligonucleotide (UR) as a biosensor for target-independent signal generation. It is triggered by unlabelled and thus cost-effective sequence-specific mediator probes. Compared to [Faltin 2012] we improved UR quenching efficiencies and reaction setup of MP PCRs to detect 5 different DNA and also RNA target sequences of viruses causing respiratory tract infections. HP based assays, which required 5 different dual-labelled probes were run as references.

Methods: MPs and the UR designs were adapted from [Faltin 2012] with the sequence-specific MP section equal to corresponding HP sequences. Nucleic acid standards from human adenovirus (hAdV), influenza virus A&B (InfA & B), human metapneumovirus (hMPV), and respiratory syncytial virus (RSV) were serially diluted enabling efficiency calculation and detection limit determination.

Results: Reaction efficiencies (E), correlation of DNA/RNA input and back-calculated output concentrations and the limit of detection with 95 % probability (LoD) were:



They correspond well to commercially available assays [L. Van Wesenbeeck et al.: J. Clin Microbiol. (51) 2013].

Conclusion: One UR was used for sensing 5 different DNA and RNA targets by MP (RT-) PCR. Even higher reaction efficiencies and lower detection limits as with the more expensive HP (RT-) PCRs could be reached. The method is especially recommended if many different target-specific probes are required at low batch sizes. In future, multiplexing degrees shall be increased using UR-microarrays.

B-059

Laboratory values from positive EBOV patients

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Background: The ongoing epidemic of Ebola Zaire (EBOV) in West Africa has resulted in a handful of patients with the disease to be treated in Western medical facilities. With these opportunities, more about EBOV disease course and treatment has been discovered. There is no published literature investigating routine laboratory values for individuals with EBOV.

Methods: Four patients with confirmed EBOV were admitted to the specialized isolation unit at Emory University between August and October 2014. All laboratory values were generated within the dedicated isolation laboratory using the following instruments: Chemistry analyzer (Abaxis Piccolo Xpress [ABAXIS, Inc, Union City, CA]), Blood-gas analyzer (GEM Premier 4000+[Werfen, Barcelona, Spain]), Automated urinalysis analyzer (CLINITEK Status [Siemens Corp., Munich, Germany)], and Hematology analyzer (pocH 100i [Sysmex Corporation, Kobe, Japan]). Testing was done daily in most instances; frequency increased and decreased depending on the status of the patient and the needs of the medical team.

Results: The patients presented upon admission with varying degrees of hypoproteinemia, hypocalcemia, elevated liver enzymes, and some electrolyte abnormalities. In the two patients with complete blood cell count results (CBC) on admission, platelet levels were mildly suppressed. Some, but not all of the laboratory values normalized by discharge. Notably liver enzymes were still elevated in three patients. Calcium, albumin, and total protein levels remained suppressed throughout most of the patients' recovery. All four patients showed decreased platelet counts during their hospitalization. Aspartate aminotransferase and alanine aminotransferase levels were significantly elevated (500- 1000 U/L) in two of the four patients. Alkaline phosphatase was elevated in three of the four patients. Red blood cell count (RBC) and hemoglobin levels fell during the course of disease in all four patients, with no evidence of gross hemorrhage, and these values remained low at discharge for all 4 patients. One patient with severe acute kidney injury had consistently elevated BUN and creatinine levels throughout their treatment.

Conclusion: The patients varied in symptom severity which was reflected in the laboratory values. Also, the patient with the least severe course of illness had more normal laboratory results except for the liver enzymes which were among the highest during hospitalization. These laboratory values are typical of patients dealing with acute viral infections and would be predicted in conditions associated with hemorrhage.

B-060

Evaluation of a Multiplex Array for the Simultaneous Detection of Ten Common STI Pathogens

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Background: Sexually transmitted infections (STIs) present a major public health concern worldwide with more than 1 million people acquiring an STI every day. Timely access to testing and treatment services can reduce the risk of onward transmission, however many STIs are asymptomatic and some display similar or overlapping symptoms, thus co-infections may remain undiagnosed. Minimum tests recommended in British Association for Sexual Health and HIV (BASHH) guidelines, include chlamydia, gonorrhoea and syphilis (*Treponema pallidum*); symptomatic women may also be tested for *Trichomonas vaginalis,* which causes vaginitis and

cervicitis in women and urethritis in men. *Trichomonas* infections are often asymptomatic and not tested for because prevalence is assumed to be too

low. In addition, wet mount microscopy, the routine diagnostic method for women, is insensitive and therefore *T.vaginalis* infection remains

underdiagnosed. New BASHH guidelines now recommend Nucleic Acid Amplification Tests (NAAT) for *T.vaginalis*. HSV testing is offered to both men and women in the presence of genital ulceration. Mycoplasmas and Ureaplasmas are not routinely tested for although *M. genitalium* is now accepted as an STI, being implicated in urethritis and cervicitis, but the previous lack of recognition has lead to inappropriate treatments and a significant rise in antimicrobial resistance. *U.*

urealyticum has also been associated with recurrent urethritis and, along with *M. hominis* and *M. genitalium* with some cases of pelvic inflammatory disease (PID).

In this context, the need for efficient means of detecting these infections has become increasingly important. This study reports the evaluation of a multiplex array on a biochip platform for simultaneous detection of ten common STI pathogens from a single sample. This approach increases detection capacity, with the potential of identifying more asymptomatic infections and co-infections.

Methods: Residual DNA extracted from a urine and urogenital swab anonymous sample cohort (n=869), obtained blind from the Regional Virology Laboratory (RVL, Belfast Health and Social Care Trust), were tested for the presence of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), Herpes simplex 1 and 2 (HSV1, HSV2), *Treponema pallidum* (TP), *Trichomonas vaginalis* (TV), *Haemophilus ducreyi* (HD), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH) and *Ureaplasma urealyticum* (UU) using the STI Multiplex Array on the Evidence Investigator analyser (Randox Laboratories Limited, Crumlin, UK). The protocol involves amplification of DNA using highly sensitive primers, followed by spatial separation and detection using biochip array technology. Assay results were compared against the RVL sample diagnosis and discrepant samples re-tested.

Results: Agreement with predicate assay(qPCR) was ≥94%. Analitical sensitivity was 100%, specificity ranged from 94% to 100% for all key targets. Of the 869 samples, 66% were negative, 27% positive for one infection and 7% harboured at least one additional infection. Results were confirmed by uniplex real-time PCR or another commercial assay.

Conclusion: The data indicate that this multiplex array assay detects simultaneously 10 common STI pathogens from a single sample without compromising sensitivity or specificity, furthermore it facilitates the identification of co-infections. This leads to increased diagnostic capabilities, which may allow tailored treatment, reducing broad spectrum antibiotic use and, in turn, the build-up of antibiotic resistance.

B-061

Detecting Acute HIV in a High Incidence Setting - Los Angeles and 4th Generation HIV Ag/Ab Testing

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Background:

In its 2013 report, the CDC tracked the rate of new HIV infections per capita (2011 data). Out of the top 25 U.S. cities with the highest rates, Los Angeles ranked 19th. This rate marks a need for the earliest possible detection mechanisms in such high prevalence areas. Historically, ~37% of individuals who are eventually diagnosed with HIV progress to AIDS within 6 months indicating that many are not caught before acute symptoms fade and do not seek testing until symptoms return in late stages.

During the acute stage, patients do not develop measurable antibodies to HIV. Consequently, testing these patients with third generation tests, which only detect antibodies, will yield a negative result. Only the 4th Generation HIV antigen antibody (Ag/Ab) Combo test and nucleic acid amplification tests (PCR/NAAT) are able to detect acute HIV infections.

Methods and Results:

Over a 15-month period at Cedars-Sinai Medical Center, 7 acute HIV-1 cases were detected by the Abbott ARCHITECT 4th Generation HIV Ag/Ab Combo test. An acute diagnosis constituted Combo positive, Western blot negative results, which were confirmed positive by PCR. Of the 7 acute cases, 6 presented to the ED with acute symptoms. Additionally, the false positive rate for the ARCHITECT Combo test was calculated at 0.08% (11 cases) for 12388 patients tested in the selected time period; Furthermore, the Combo assay detected 0.11% more cases (8 cases) than our previous BioRad Genetic Systems 3rd generation immunoassay.

Conclusion:

These cases demonstrate that it is imperative for first line HIV testing to be capable of detecting both acute and established HIV infection with accuracy, particularly in high transmission areas. By stopping new infections from occurring through early and accurate diagnosis, we can approach a future where rampant transmission becomes a thing of the past and the number of people living with HIV becomes relatively static.

7 acute HIV cases detected with 4th Generation Combo Assay at Cedars-Sinai (15 month timeframe)

Case	Patient	Presentation/Symptoms	Abbott ARCHITECT HIV Combo (4th Gen)	Western Blot	PCR Viral Load (copies/mL)
1	34 y/o Male	Presented to ED with fever, headache, chills	POSITIVE	NEGATIVE	156,000
2	34 y/o Male	Presented to ED with fever, rash, enlarged tonsils	POSITIVE	NEGATIVE	3,510,000
3	27 y/o Male	Presented to ED w/ fever/chills	POSITIVE	INDETERMI-NATE	5,600,000
4	49 y/o Male	Outpatient specimen (no further information)	POSITIVE	NEGATIVE	>10,000,000
5	37 y/o Female	Presented to ED with abdominal pain, diarrhea	POSITIVE	NOT TESTED	>10,000,000
6	25 y/o Male	Presented to ED with fever, muscle aches, and diarrhea	POSITIVE	NEGATIVE	1,760,000
7	52 y/o Male	Presented to ED with abdominal pain	POSITIVE	NEGATIVE	3,050,000
	y/o = year old				

B-062

A Novel Immunoassay that Distinguishes between Bacterial and Viral Infections Based on a Patient's Immune Response

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OBJECTIVES: Bacterial and viral infections are often clinically indistinguishable, leading to inappropriate patient management and antibiotic misuse. Effective use of infectious disease diagnostics has been hindered by long waits for results, high costs, inaccessible (or unknown) sites of infection, and the presence of non-disease causing colonizing bacteria that can lead to false positive results. An approach that has the potential to address these challenges relies on monitoring the host's immune-response to infection, rather than direct pathogen detection. Our goal was to develop and validate a novel assay that combines blood borne bacterial- and viral-induced host-proteins that can accurately distinguish between bacterial and viral infections. **METHODS:** We prospectively recruited 1002 hospitalized and emergency department patients with acute infection, and controls with no apparent infection (NCT01917461). For each patient, three independent physicians assigned a diagnosis based on comprehensive clinical and laboratory investigation that included PCR for 21 common pathogens. We quantitatively screened 600 circulating host-proteins and developed a multi-parametric signature using logistic-regression on half of the patients, and validated it on the remaining half. **RESULTS:** The cohort included 319 bacterial, 334 viral, 112 control and 98 indeterminate patients (139 were excluded based on pre-determined criteria). The cohort was balanced with respect to gender (47% females, 53% males) and included 56% pediatric patients (≤18 years) and 44% adults (>18 years). The best performing host-protein was TNF-related apoptosis-inducing ligand (TRAIL) (area under the ROC curve [AUC] of 0.89; 95% confidence interval [CI], 0.86-0.91), which was consistently up-regulated in viral infected patients. The signature with the highest precision included both viral- and bacterial-induced proteins: TRAIL, Interferon gamma-induced protein-10, and C-reactive protein (AUC of 0.94; 95% CI, 0.92-0.96). The signature outperformed routinely-used clinical parameters, such as white blood cell count (AUC of 0.64±0.04), absolute neutrophil count (AUC of 0.73±0.04), % monocytes (AUC of 0.64±0.04), % lymphocytes (AUC of 0.76±0.04), peak temperature (AUC of 0.51±0.04), pulse (AUC of 0.62±0.04), procalcitonin (AUC of 0.67±0.11), and an algorithm that combines these clinical parameters (AUC of 0.78±0.04). The signature was robust across various physiological systems (respiratory, urinary and systemic), times from symptom onset (0-12 days), and pathogens (56 species), with AUCs between 0.87 and 1.0. Finally, the signature's accuracy was not affected by the presence of potential colonizers and it was able

to provide accurate diagnoses even in cases where the infection site was not known or easily accessible. A kit called ImmunoXpert™ was developed, which measures the proteins in 99 minutes using an ELISA format, and computationally integrates the measurements into the final diagnosis. **CONCLUSIONS:** The present signature combines newly identified viral-induced with traditional bacterial-induced host proteins. It provides valuable information over standard laboratory and clinical parameters, which are routinely used in clinical practice today to facilitate differential diagnosis of infection etiology. Furthermore, assay run time can be further shortened as it is readily translatable to hospital-deployed automated immunoassay machines and point-of-need assay formats. This signature has the potential to significantly improve the management of patients with acute infections and reduce antibiotic misuse.

B-063

Validation and Correlation of VIDAS® IgGII and IgMII Lyme Antibodies vs VIDAS® Lyme IgG/IgM in the Laboratory Evaluation of Lyme Disease: Clinical Implications.

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Objective: To perform method comparison studies between the VIDAS® Lyme IgM II (LYM) and VIDAS® Lyme IgG II (LYG) assays versus the VIDAS® Lyme IgG/IgM (LYT) combination assay currently available for clinical use. A secondary goal was to determine if utilization of separate measurements of IgM and IgG Lyme antibodies done simultaneously would result in a reduction in Lyme western blot assays needed to determine a serologic diagnosis of Lyme Disease.

Methodology: VIDAS®LYT, LYG, and LYM assays are automated, qualitative, enzyme-linked fluorescent immunoassays (ELFA) for use in the detection of human IgG and IgM antibodies to *Borrelia burgdorferi* in serum or plasma. The LYT assay detects and reports total IgG/IgM antibodies whereas LYG and LYM assays are independent and separately measure and report IgG and IgM antibodies to *Borrelia burgdorferi*. The LYT assay requires 35 minutes to complete and the LYG and LYM require 27 minutes to complete.

Previously frozen serum samples from patients with signs and symptoms of Lyme disease were allowed to thaw to RT. Samples (N=114; 100ul) were analyzed using the LYT, LYG and LYM assays. All positives and/or equivocals in any assay were analyzed via standard IgG and IgM Lyme western blot (LYWB) analyses.

The sensitivities of the VIDAS®LYG, LYM assays versus the respective IgG and IgM LYWB assay results were estimated. The sensitivity of the VIDAS®LYT combination assay was estimated using the results of both IgG and IgM LYWB assays. Fisher's exact test was used to determine whether significant differences in sensitivities existed between the two independent assays and the combination assay.

Results: There was a high correlation between VIDAS® assays in terms of positives and negatives. The sensitivity of the LYG assay against LYWB was 90.4% and the sensitivity of the LYM assay was 73.8%. In comparison, the sensitivity of the LYT combination assay was 42.2%. Sensitivities of each of the two independent assays were significantly higher than the combination assay ($p<0.001$).There was a 23.3% reduction in the number of LYWBs needed to definitively determine Lyme diagnosis when separate measurements of LYG and LYM assays were performed compared to the LYT combination assay.

Conclusions: Per CDC guidance, laboratory diagnostic confirmation of Lyme Disease requires serologic analysis of Lyme IgG and IgM antibodies. Currently available testing in the clinical laboratory utilizes the reporting of total IgG/IgM antibodies followed by IgG and IgM western blot analysis to determine disease stage.

Utilizing the VIDAS® LYG and LYM separately provided enhanced specific laboratory diagnostic information earlier to couple with patient symptomology that was closer to that provided by LYWB. Utilization of separate IgG and IgM assays as first round diagnostic testing provides the ability to quickly separate acute from later stage or historical exposure. Having this information earlier decreases unnecessary and/or inappropriate treatment for previous infections, and provides earlier information aiding differential diagnoses in endemic areas of tick borne disease. The impact on quality of care via reductions in: time to definitive laboratory diagnosis, unnecessary treatments, long-term clinical symptoms and ultimate costs to patients and payors has yet to be determined.

B-064

CLIA-PCR: a High-Throughput PCR Technology for Molecular Screening, with an Application in Malaria Surveillance for Elimination

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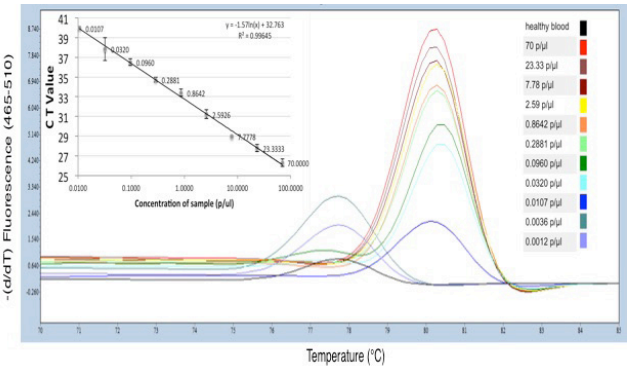
Background: sensitive and affordable methods for active screening of malaria parasites in low-transmission settings are urgently needed. Methods: we developed a novel molecular screening technology called Capture and Ligation Amplification PCR (CLIA-PCR), which achieved the sensitivity of reverse-transcription PCR yet eliminating the reliance on RNA purification and reverse transcription. In short, 18S rRNA of genus Plasmodium is released from blood and captured onto 96-well plates, and quantified by the amount of ligated probes that bind continuously to it. We first used laboratory-prepared samples to test the method across a range of parasite densities and pool sizes, then applied the method to an active screening of 3358 dried blood spot samples collected from three low endemic areas in China. Results: Plasmodium falciparum diluted in whole blood lysate could be detected at a concentration as low as 0.01 parasite/μl (Figure), and pool size of up to 35 did not significantly affect the assay performance. When coupled with a matrix pooling strategy, the assay drastically increased the throughput to thousands of samples per run while reducing the assay cost to cents per sample. In the active screening, CLIA-PCR identified 14 infections including 4 asymptomatic ones with less than 500 tests, costing less than 0.6 dollars for each sample. All positive samples were confirmed by standard quantitative PCR. Conclusions: CLIA-PCR, using dried blood spots with a pooling strategy, efficiently offers a highly-sensitive and high-throughput approach to detect asymptomatic submicroscopic infections with dramatically reduced cost and labor, making it an ideal tool for large scale malaria surveillance in elimination settings. More importantly, CLIA-PCR greatly reduced the complexity of PCR test with its ELISA-like workflow, offering the potential to significantly enhance the capacities of molecular laboratories with no need for additional equipment.



B-065

Prospective evaluation of two multiplex real time PCR assays for simultaneous detection of sexually transmitted pathogens

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According to the World Health Organization, sexually transmitted infections (STIs) are transmitted from person to person through sexual contact. It is estimated that worldwide, 448 million new cases of STIs are diagnosed each year. Molecular assays have been described for the detection of pathogens, and multiplex real-time PCR (M-qPCR), which uses multiple primers and probes, is a sensitive, rapid, and high-throughput approach for qualitative analysis of several infectious agents in the same

reaction. This technique has become a mainstay of research and clinical diagnostic applications. In this regard, the widespread prevalence of STIs and the public health costs associated with STIs management have led to the need for fast and reliable methodsfor diagnosing STIs. However, because STIs can be caused by polymicrobial infection methods to identify multiple pathogens in a single sample are necessary. The goal of this study was to develop and optimize a test for the simultaneous detection of five clinically important bacteria associated with STIs, including Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasmahominis, Mycoplasmagenitalium, and Ureaplasmaurealyticum, by Multiplex Real Time PCR (M-qPCR). A total of 10 clinical samples (vaginal swabs) from patients with presumptive diagnosis of infection with these bacteria were examined. Two M-qPCRs were developed: MI: N. gonorrhoeae, C. trachomatis, U. urealyticum; MII: M. hominis, M. genitalium, U. urealyticum. The primers and probes selected in this study were specific for conserved regions of the genes encoding glyceraldehyde 3 phosphate dehydrogenase (GAP/GAPDH) (M. hominis), MgPa surface protein (M. genitalium), urease B subunit (UreB) (U. urealyticum), and outer membrane protein (OMP1) (C. trachomatis), or for unique sequences within the cryptic plasmid (pJD1) (N. gonorrhoeae). The selected primers were consideredadequate and performed satisfactorily in silicoPCR as well as conventional PCR, generating specific products of the appropriate size for each examined infectious agent.All standard plasmids (positive controls) for each multiplex test amplified with Ct value less than 40, including the internal control (β-actin gene), which demonstrated that the extracted genetic material was of good quality. The reactions were able to detect the agents and according to the Ct values and the largest concentration of each point of the standard curve, the following detection limits were determined: 29.7 copies/mL for N. gonorrhoeae, 30.1 copies/mL for U. urealyticum, 29.9 copies/mL for C. trachomatis, 29.7 copies/mL for M. hominis and 30.4 copies/mL for M. genitalium.Among the clinical specimens evaluated, the multiplex reactions resulted in detectable Ct values (below 40) and at least one of the bacterial species was detected.These multiplex Q-PCRs using TaqMan 5´nuclease real-time PCR provided a novel, qualitative method for the rapid detection of diagnosis of STIs associated with N. gonorrhoeae, C. trachomatis, U. urealyticum,M. hominis, and M. genitalium. The results of this study were notably encouraging, and we believe that these methods indicate an advance in clinical laboratory medicine and its can be a valuable tool for routine laboratory diagnosis of infectious diseases

B-066

Prevalence Epidemiologic Study of HCV Genotype in HIV-HCV Co-infected Patients

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Background: According to the World Health Organization, it is estimated that 34 million people are currently infected with human immunodeficiency virus (HIV) worldwide and 25-30% of HIV-positive individuals are co-infected with hepatitis C virus (HCV). In Brazil, HCV was reported to be present in 10.3% of HIV cases in 2010 and among these cases HCV genotype 1 was predominant, followed by genotypes 3, 2, 4 and 5. Individuals between 40-44 years old had the largest number of co-infection cases inthat year. HIV and HCV share the same infection pathway sand the use of injection drugs, blood transfusion and sexual transmission are the primary means of infection. Different HCV genotypes vary in infectivity and pathogenicity influencing, therefore, the rate of progression to cirrhosis and cancer, disorders that have an increased risk in HIV-HCV co-infection. Furthermore, HCV genotyping is necessary for the selection of patients who will respond better to treatment of HIV-HCV co-infection. Objective: To investigate the HCV genotype prevalent in HIV-HCV co-infected patients. Method: The study is a prevalence epidemiologic study of HIV-HCV co-infected patientsdata with HCV genotype determined in Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil) in 2013. The inclusion criteria was the detection of HIV-RNA by polymerase chain reaction. 3,308 patients, 1802 male and 1506 female, mean age 50.6 ± 14.5, were included in this research. Results: 25(0.75%) of the patients evaluated were HIV-HCV co-infected. Among these, 16 had HCV genotype 1 (64.0%), 7 had genotype 3 (28.0%), 1 had genotype 2 (4.0%) and 1 had genotype 4 (4.0%). The median age of co-infected patients was 47 ± 9.60 years old, 23 patients were male (92.0%) and 2 were female (8.0%). Conclusions: Among the HIV-HCV co-infected patients, genotype 1 was prevalent, followed by genotypes 3, 2 and 4. The HCV genotype 1 patients usually have more rapid progression to AIDS than other genotypes. Further investigations should be conducted with more patients to elucidate if there is any interaction between HCV genotype and HIV-HCV co-infection, since HCV genotyping predicts the effectiveness of the treatment and, consequently, it becomes a key factor to support therapeutic decisions.

B-067

Serum Cytokines and Chemokines Pattern Characteristics in HBV Continuous Infection

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Background: Host immune responses are associated with hepatic inflammation and disease progression in HBV infected patients. To explore the effects of cytokines and chemokines on HBV continuous infection we detected serum cytokines and chemokines in different stages of patients infected with HBV.

Methods:123 HBV infected patients including 81 patients with chronic hepatitis B (CHB) and 42 patients with hepatoma, 70 subjects with HBV spontaneous clearance (SC) and 29 healthy controls (HC) were enrolled. Serum IL-1β, IL-6, IL-17, IFN-γ, IL-10, IL-8, IP-10, MCP-1 and MIP-1β were measured by Bio-Plex system and Bio-Plex Pro™ human cytokine reagent kits.

Results:(1) Serum IL-1β, IL-10, as well as IP-10 and MIP-1β, in CHB were significantly higher than those in HC and SC (P<0.05); IL-6 and IFN-γ levels in CHB were similar to those in SC, but higher than those in HC (P<0.05); IL-17 level in SC strikingly increased compared with HC and CHB (P<0.05). (2) In CHB serum IL-1β, IFN-γ, IL-17 and IL-10, as well as IP-10, MCP-1 and MIP-1β, were elevated compared with those in hepatoma. Discriminant analysis showed that elevated serum IL-17 and IL-1β would be more likely in patients with spontaneous clearance of HBV and CHB, respectively. (Fig. 1A) (3) The good correlation between serum IP-10 and ALT levels in CHB was found (Fig. 1B). Serum IP-10 levels in CHB with stable liver function was significantly decreased compared with CHB with unstable liver function (Fig.1C). (4) No striking relationship between serum cytokines or chemokines level and HBV-DNA load or HBeAg seroconversion was found.

Conclusion: Inflammatory cytokines and chemokines play two roles in HBV continuous infection and progression. Higher serum IL-17 contributed to HBV spontaneous clearance, elevated IL-1β could induce chronic inflammation in HBV infection and high IP-10 could strengthen the liver damage in CHB.

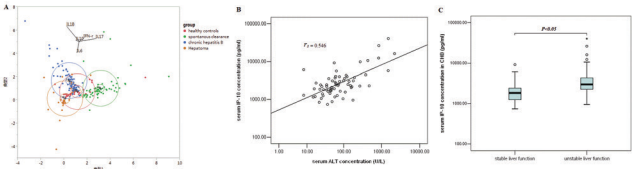

Figure 1. Serum cytokines or chemokines levels in hepatitis B progression. A. It showed canonical plots of serum cytokines levels in discriminating hepatitis B progression status. B. It showed the correlation between serum IP-10 and ALT levels in patients with CHB. (P<0.05) C. It showed the association of serum IP-10 concentration with liver function in CHB.

B-068

MULTI-ARRAY Assay to Discriminate Recent from Long-Standing HIV Infection

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In order to accurately assess and compare different prevention strategies, the rate at which new HIV infections are acquired in a population needs to be measured accurately. A simple laboratory test that indicates whether an HIV infection was acquired in the recent past (generally 4-12 months) would be very useful to estimate HIV incidence.

We demonstrated feasibility of several assay formats to separate recent from longstanding HIV infection. Using MULTI-ARRAY technology, we measured antibodies against the HIV proteins gp41, gp120, gp160, p17, p24, p55, p66, and nef in a multiplexed format using a very small sample volume (25 µL of a 1,000-fold diluted serum or plasma sample). We used the well-characterized “HIV Incidence/Prevalence Performance Panel” from SeraCare (part # PRB601), which contains plasma samples from 15 HIV positive donors that have been characterized either as “incident” (recent infection) or “prevalent” (longstanding infection) based on consensus results from nine tests. Our MULTI-ARRAY serology format for antibodies against gp120 and gp160 showed ~10-fold separation between the median signals for incident and prevalent samples (and another ~10-fold separation from apparently healthy controls). All samples in each of the three groups were completely separated from the other two groups. We also developed avidity assay formats for antibodies against gp41, gp120, gp160, and p66 that could accurately separate samples from patients with incident versus prevalent HIV infection. The assays were developed in a

96-well high-throughput assay format for the MESO® SECTOR S 600 Imager and the MESO QuickPlex® SQ 120.

We demonstrated feasibility for transfer of the assay format to a point-of-care (POC) platform. The POC assay is fully automated and simultaneously measures concentrations of antibodies against eight HIV proteins. Time to result is 25 minutes, and CVs are approximately 13%. The magnitude of the antibody response against gp120 and against gp160 accurately separates patients with incident HIV infection from patients with prevalent HIV infection, equivalent to the plate-based results.

In conclusion, we demonstrated feasibility for development of high-throughput and point-of-care assays to discriminate recent from longstanding HIV infection.

Acknowledgement of Source:

The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 Nef Protein (Cat# 11478).

B-069

HIV p24 Immunoassay with the Sensitivity of PCR Methods

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Patients who have recently been infected with HIV contribute disproportionately to the spread of the disease. Viral loads are high in the first few weeks after infection, and newly infected patients are unlikely to be aware that they are infected and can spread the disease to others. Therefore, early detection of acute HIV infection is of great importance for public health. PCR methods are the gold standard with respect to sensitivity; they can detect as few as 60 HIV RNA copies per mL of serum or plasma (30 virus particles per mL). However, PCR technology is complex and expensive, and therefore not suitable for all settings. Immunoassays are simpler and cheaper, but the detection limit of current, 4th generation p24 immunoassays is only about 10 pg/mL, or approximately 250 million capsid proteins per mL. On a per virus basis, these immunoassays are several thousand times less sensitive than PCR testing, despite the fact that there are about 2,000 p24 capsid proteins per virus.

A next-generation electrochemiluminescence assay format based on MSD's MULTI-ARRAY® technology was developed and its performance characterized. The detection limit for this novel p24 immunoassay was approximately 1 fg/mL, 10,000 fold more sensitive than current p24 immunoassays. A sensitivity of 1 fg/mL corresponds to less than 1 virus particle in our sample volume of 25 µL. The lower and upper limits of quantitation were 3 fg/mL and 38,000 fg/mL, respectively. Within-plate CV was 7%, and total CV 15%. Spike recovery and dilution linearity were between 80% and 120%. p24 was undetectable in the serum or plasma of 32 apparently healthy donors. A SeraCare p24 "Mixed Titer Panel" (12 samples) showed good correlation between our p24 assays and commercial p24 immunoassays. Two seroconversion panels were tested: SeraCare PRB948 (days 0 and 18, PCR negative; days 22 and 23, PCR positive) and PRB962 (days 0 and 2, PCR negative; days 7, 9, 14, and 17, PCR positive). In both cases, the MSD® p24 assay result was negative for all PCR-negative samples and positive for all PCR-positive samples, and infection was detected well before conventional p24 immunoassays.

In conclusion, we developed a next-generation p24 immunoassay that is 10,000 times more sensitive than the current limits of p24 ELISAs and comparable in sensitivity to PCR assays. The assay does not require specialized equipment and can be run on the MESO® QuickPlex SQ 120, and all MESO SECTOR® Imagers.

B-070

Evaluating the Utility of Matrix-assisted Laser Desorption Ionization Time of Flight (MALDI) Mass Spectrometry as a Rapid Diagnostic Tool in Urinary Tract Infections

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Background: Early identification of urinary pathogens will allow rapid, targeted therapy. Conventional urine cultures can take 24-48 hours to result. By contrast, Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI) can offer rapid identification of pathogens directly from urine specimens within 3-4 hours. We studied the efficacy of MALDI as a potential rapid diagnostic tool for the detection of urinary tract infections (UTI) by comparing it to conventional urine culture and urine chemical analytes.

Methods: Urine specimens less than 24 hours old were obtained by catheter from 14 spina bifida patients (SBP) and 41 non-spina bifida patients (NSBP) and analyzed by conventional urine culture and MALDI. The specimens were collected in BD Vacutainer® C&S preservative plus urine tubes and BARD infant catheter kits. For MALDI, 1 ml of urine was centrifuged at 5500 rpm for 30 sec to concentrate bacterial proteins and remove leukocytes. The supernatant was centrifuged at 14500 rpm for 5 mins. The bacterial protein pellet was washed with DI water, respun at14500 rpm for 5 mins and analyzed. MALDI results were compared to the culture and urine chemistry (nitrate, leukocyte esterase, and bacteriuria).

Results: For SBP, 4 of 14 specimens were positive with MALDI and culture, 3 were negative with both, and 7 were negative with MALDI and positive for culture. For NSBP, 6 of 41 were positive with MALDI and culture, 30 were negative with both, 1 positive with MALDI and negative with culture and 4 negative with MALDI and positive with culture. The sensitivity of MALDI direct urine identification was 48% while the specificity was 97%. The positive predictive value was 91% and a negative predictive value was 75%. Additionally, 30% of patients with MALDI and culture uropathogen isolation had urine chemical analytes indicative of infection.

Conclusion: This study suggests that using a two tiered approach of MALDI and urine chemistry analytes can be a reliable, rapid and specific tool for identifying UTI directly from urine specimens. Further study would include analyzing more urine specimens to improve sensitivity, creating a workflow process to confirm negative MALDI results, and determining overall cost savings.

B-071

Rapid Detection of Microbial Resistance to Lactam Antibiotics by LC-MS/MS

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Background:

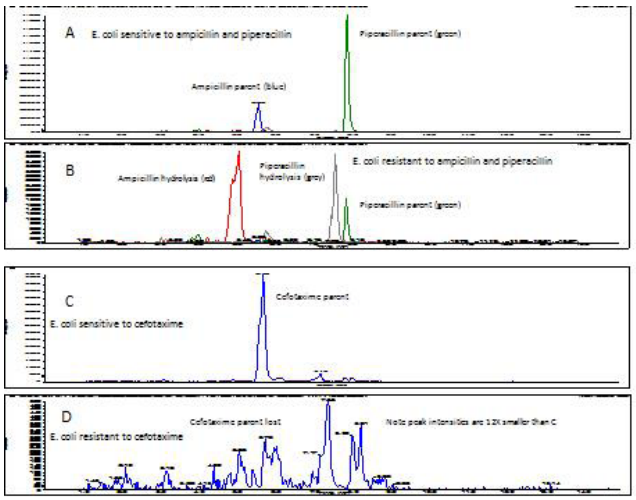
Bacterial sepsis and septic shock are major causes of mortality worldwide. In the US it is estimated that 250,000 patients a year develop life threatening infections with a mortality rate that varies from 28 to greater than 50% depending upon other underlying disease conditions and the severity of infection. MALDI-TOF mass spectrometry has revolutionized bacterial identification based on patterns of ribosomal protein expression. The determination of bacterial resistance to antibiotics by conventional turbidometric, spectrophotometric or disk diffusion methods which evaluate bacterial growth in the presence of antibiotics is still a relatively slow process which often requires 12-24 hours of incubation. This process often delays the administration of targeted antibiotics.

Methods:

Here we have adapted a rapid screening process for identification of bacterial resistance to antibiotics by utilizing LC-MS/MS to quantitate concentrations of parent drugs and also detect hydrolysis products which result from beta-lactamase activity. The susceptibility testing can be accomplished in time periods as short as 90 minutes which includes incubation of bacteria with antibiotics and LC-MS/MS analysis. The antibiotics can be multiplexed for incubation with bacteria to minimize analysis time.

Results:

We have evaluated 23 different strains of E. coli by this method including ATCC reference (3) as well as clinical isolates (20) and achieved complete concordance with traditional methods. To date the following antibiotics have been tested: penicillin, ampicillin, amoxicillin, cloxacillin, piperacillin/tazobactam, and cefotaxime. All incubations are conducted in the absence and presence of tazobactam which acts as a control. LC-MS/MS analysis was conducted on an AB SCIEX 3200 QTRAP® system utilizing positive electrospray ionization, with MRM detection. Chromatographic separation was performed using a C18 reverse phase column, with a linear methanol gradient. A sample chromatographic profile is displayed in Figure 1.



B-072

Preliminary validation of saliva samples as clinical matrix on the ReEBOV™ Antigen Rapid Test Kit for the point of care detection of Ebola Virus Disease

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Background: Ebola virus (EBOV) causes severe and often fatal viral hemorrhagic fever (Ebola Virus Disease; EVD). The 2014-2015 outbreak of EVD in West Africa is the deadliest of its kind, resulting in +9500 deaths and prompting an international emergency response. During the course of this outbreak, a need became evident for an easy-to-use, point-of-care rapid diagnostic test (RDT) that can be performed in any clinical facility or field laboratory to aid in the rapid triage of suspect EVD cases. To assist in this effort the Viral Hemorrhagic Fever Consortium (VHFC.org) led by Tulane University and Corgenix Inc. accelerated the development timeline of the ReEBOV™ Antigen Rapid Test Kit. This dipstick-format lateral flow immunoassay incorporates recombinant EBOV VP40 antigen and VP40-specific polyclonal antibodies to detect EBOV VP40 antigenemia in whole blood or plasma samples. We have also initiated validation studies for clinical testing of saliva samples in collaboration with Oasis Diagnostics Corporation (Vancouver, WA) and present those findings here.

Methods: In collaboration with the FDA, we developed an analytical validation protocol for the ReEBOV™ Antigen Rapid Test for whole blood and plasma samples. This protocol was used as a guideline for additional saliva sample validation studies, including Limit of Detection (LOD) using spiked recombinant antigen, mock sensitivity & specificity, interfering substances, cross-reactant reactivity, and prozone effect. Donor saliva (human) was collected using the Super•Sal™ Universal Saliva Collection Device (Oasis Diagnostics Corp.) In addition, the test was evaluated in comparison to qRT-PCR on in vivo saliva samples from EBOV Zaire (Makona)-challenged non-human primates.

Results: The LOD of the RDT is 4.8 ng/test for VP40 antigen spiked into saliva. Specificity was 100% (20/20; CI 83.6 - 100%) and mock sensitivity at LOD and above was 100% (40/40; CI 91.2 - 100%). Limited cross-reactant testing revealed no reactivity with several inactivated human viruses including Dengue virus, Junin virus, RSV, influenza, parainfluenza, mumps, and measles. No interference was observed for several drugs in common use. Aspirin appeared to cause a false-negative reaction; however, antigen-spiked samples from two donors under regular aspirin therapy (one low dose, one high dose) tested positive. For prozone effect, high levels of spiked antigen did not generate false-negative results. In non-human primates, the saliva of infected individuals tested positive 6 days after initial challenge. Conclusions: These preliminary validation studies have demonstrated the ReEBOV™ Antigen Rapid Test is capable of EBOV VP40 antigen detection in saliva samples. Available analytical validation presented here meets or exceeds comparable studies previously conducted with whole blood and plasma samples. Those studies contributed to the ReEBOV™ Antigen Rapid Test Kit being granted FDA Emergency Use Authorization and the WHO listing the test as eligible for procurement in February 2015. This product has not been authorized for the use of saliva samples in the diagnosis of EVD. However, these findings warrant further analytical and clinical validation efforts.

B-073

Fully automated digital immunoassay for p24 on the Simoa HD-1 Analyzer with the sensitivity of nucleic acid amplification for acute HIV infection

C. Cabrera, Y. Chen, L. Chang, D. H. Wilson. *Quanterix Corporation, Lexington, MA*

Background: Nucleic acid amplification techniques such as PCR have become the mainstay for ultimate sensitivity for detecting low levels of virus, including human immunodeficiency virus (HIV). As a sophisticated technology with relative expensive reagents and instrumentation, adoption of nucleic acid testing (NAT) can be inhibited in settings in which access to extreme sensitivity could be clinically advantageous for early detection of HIV infection. We report a simple low cost digital immunoassay for the p24 capsid protein of HIV based single molecule array (Simoa) technology. The assay exhibited over three logs greater sensitivity than conventional immunoassays, and comparable sensitivity to NAT for early detection of HIV infection.

Method: Reagents were developed for a paramagnetic bead-based ELISA for use in the Simoa HD-1 Analyzer. Anti-p24 capture beads were prepared by covalent coupling of antibody to carboxy paramagnetic microbeads, detector antibody was biotinylated by standard methods, and an enzyme conjugate was prepared by covalent coupling of streptavidin and [[Unsupported Character - Symbol Font β]]-galactosidase. The HD-1 Analyzer first performs a 2-step sandwich immunoassay using 144 µL of serum or plasma sample, then transfers washed and labeled capture beads to a Simoa disc where the beads are singulated in 50-femtoliter microwells, sealed in the presence of substrate, and interrogated for presence of enzyme label. A single labeled p24 molecule provides sufficient fluorescent signal in 30 seconds to be counted by the HD-1 optical system. At low p24 concentration, the percentage of bead-containing wells in the array with a positive signal is proportional to the amount of p24 present in the sample. At higher p24 concentration, the total fluorescence signal is proportional to the amount of p24 in the sample. The concentration of p24 is then interpolated from a standard curve (range 0-30 pg/mL). Time to first result is 69 minutes. The assay was evaluated for sensitivity, specificity, precision, recovery, linearity, and correlation to a NAT method across 24 early HIV infection serum samples (prior to seroconversion).

Results: Limit of detection (2.5 SD) was 0.0025 pg/mL across 10 runs. This corresponds to ~60 RNA copies/mL equivalents by NAT. Limit of quantification (20% dose CV of diluted serum samples) was 0.0076 pg/mL across 11 runs and 58 determinations. Specificity was 95.1% across 139 normal serum and plasma samples. Recovery of p24 spiked into normal serum averaged 84.1%. Linearity per CLSI EP6-A guideline averaged 102.2%. Precision per EP5-A guideline included three serum-based panels and two p24 controls assayed in replicates of three at two separate times per day for five days using a single calibration curve. ANOVA gave CV's <10% for all levels. Method comparison to NAT yielded 100% concordance with a R of 0.961. Samples ranged from 40 to 10 million RNA copies/mL as reported by NAT, most of which were non-reactive by 4th generation HIV combo immunoassay.

Conclusion: The results show the digital Simoa p24 assay exhibited comparable sensitivity to NAT, as well as good general analytical properties. The assay represents a potential alternative to NAT for early detection of HIV infection.

B-074

Analytical validation of the ReEBOV™ Antigen Rapid Test Kit for the point of care detection of Ebola Virus Disease

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Background: Ebola virus (EBOV) causes severe and often fatal viral hemorrhagic fever (Ebola Virus Disease; EVD). The 2014-2015 outbreak of EVD in West Africa is the deadliest of its kind, resulting in +9500 deaths and prompting an international emergency response. During the course of this outbreak, FDA and WHO approved qRT-PCR as a molecular diagnostic to detect EVD clinically, however with this technique a result is not available for 24hr or more and requires significant laboratory infrastructure and electricity. A need became evident for an easy-to-use, point-of-care rapid diagnostic test (RDT) that can be performed in any clinical facility or field laboratory to aid in the rapid triage of suspect EVD cases. To assist in this effort the Viral Hemorrhagic Fever Consortium (VHFC.org) led by Tulane University and Corgenix Inc. accelerated the development timeline of the ReEBOV™ Antigen Rapid Test Kit. This dipstick-format lateral flow immunoassay incorporates recombinant EBOV VP40 antigen and VP40-specific polyclonal antibodies to detect EBOV VP40 antigenemia in whole blood (capillary and venous) or plasma samples.

Methods: In collaboration with the FDA, we developed an analytical validation protocol for the ReEBOV™ Antigen Rapid Test. Validation studies included, Limit of Detection (LOD) using EBOV Zaire virus and recombinant VP40 antigen, specificity, interfering substances, nearest-neighbor & cross-reactant reactivity, prozone effect, reproducibility, and stability studies.

Results: The LOD of the RDT is 3.00E+04 PFU/test for EBOV Zaire virus and 1.88E+01ng/test for recombinant VP40 antigen when spiked into whole blood. Specificity is 97.5% (39/40; CI 86.8 - 99.9%) for fingerstick (capillary) whole blood and 94.9% (131/138; CI 89.8 - 97.9%) against a US normal serum panel. Nearest-neighbor testing demonstrated that the RDT is reactive to all known African strains of EBOV (Zaire, Sudan, and Bundibugyo) that cause human infection; Ebola-Reston and Marburg virus were non-reactive. Cross-reactant testing revealed no reactivity with several human viral, bacterial and parasitic pathogens including Dengue virus, HIV, influenza, and malaria. No interference was observed for several drugs in common use including anti-febrile medication. However, high levels of hemoglobin and rheumatoid factor did interfere with signal development and are listed as limitations of the assay. No prozone effect is observed with high levels of spiked antigen or high titer clinical samples. Reproducibility testing demonstrated +95% agreement by multiple readers at the LOD and above using both spiked antigen and pooled clinical samples. On-going accelerated stability testing indicates a kit shelf-life of 12 months.

Conclusion: These validation studies have demonstrated the ReEBOV™ Antigen Rapid Test is a sensitive, specific, reproducible, and stable test for the detection of Ebola virus VP40 antigen in whole blood and plasma. Based in part on these findings, the ReEBOV™ Antigen Rapid Test Kit was granted FDA Emergency Use Authorization and the WHO listed the test as eligible for procurement in February 2015. The intended use is for the presumptive detection of Zaire EBOV in individuals with signs and symptoms of EBOV infection in conjunction with epidemiological risk factors. This Point of Care RDT represents a breakthrough in the detection of EVD for this and future Ebola virus outbreaks.

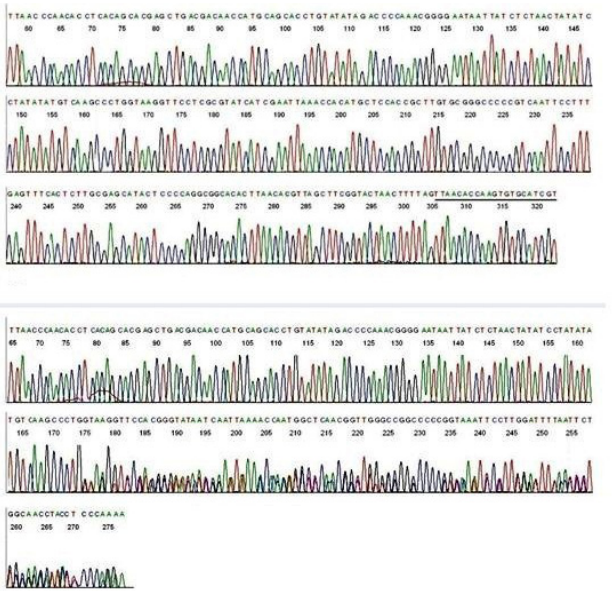
B-075

Direct DNA Sequencing Test for Lyme Disease Bacteria in Blood Samples

S. Lee. *Milford Molecular Diagnostics, Milford, CT*

Background Blood culture is not practical for the diagnosis of Lyme and related borrelioses. Various nucleic acid-based tests were developed for the detection of *Borrelia burgdorferi*. **Methods** A new pair of "genus-specific" primers, 5'-ACGATGCACACTTGGTGTTAA-3' (M1) and 5'-TCCGACTTATCACCGGCAGTC-3' (M2) was designed to amplify a 357-bp segment of the 16S ribosomal RNA gene (16S rDNA) of the *B. burgdorferi* sensu lato species and the correspondent 358-bp DNA segment of the relapsing fever borreliae, including *B. miyamotoi*. The crude DNA in NH_4OH extracts of the pellets spun down from patients' platelet-rich plasma or serum samples was the initial material for primary PCR amplification, followed by a same-nested PCR. The positive nested PCR products were used as the template for direct Sanger sequencing. **Results** Of 27 PCR-positive blood samples from clinically suspect "Lyme disease" patients, 19 contained *B. burgdorferi* sensu lato, 5 *B. miyamotoi*, 1 a mixture of *B. burgdorferi* sensu lato and *B. miyamotoi*, and 2 unnamed relapsing fever borreliae, including one with GenBank accession number KM052618. Of the 19 sequences of *B. burgdorferi* sensu lato isolates, 15 had a 100% ID match with a 16S rDNA signature sequence of *B. burgdorferi* senso lato, 3 had a single nucleotide mismatch against any closest sequence retrieved from the GenBank, and 1 showed more than one copy of 16S rDNA (Figure 1). **Conclusion** Direct DNA sequencing of the PCR amplicon of a highly conserved segment with hypervariable regions of the borrelial 16S rDNA may reveal greater strain diversity in the borreliae causing Lyme disease than previously estimated.

Figure 1 Upper: Signature sequence of *B. burgdorferi* sensu lato 16S rDNA. Lower: Many double base-calling peaks superimposed on each other downstream beyond the first 110 bases, indicative of more than one copy of 16S rDNA.



B-076

Performance of the VITROS ® Immunodiagnostic Products HIV Combo Assay*

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Objective: Assess the sensitivity, specificity and precision performance of the VITROS Immunodiagnostic Products HIV Combo Assay* on VITROS Systems with MicroWell capability. The assay is capable of simultaneously detecting both HIV antibodies (Ab) and p24 antigen (Ag) to enable earlier diagnosis of HIV infection.

Methods: Antibody detection in the VITROS HIV Combo Assay* is achieved using recombinant transmembrane envelope proteins for HIV-1 group M and O and HIV-2. p24 antigen detection is accomplished using monoclonal antibodies (MAbs) against HIV-1 p24. Biotinylated antigen or MAb are pre-bound to microwells coated with streptavidin. Sample is added to the coated wells in the first stage of the reaction and HIV analyte from the sample is captured by the biotinylated proteins. After washing, HRP conjugated envelope proteins and anti-p24 MAbs are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent.

All specificity and sensitivity testing was performed using one assay lot on a VITROS 3600 Immunodiagnostic System. Assay specificity was assessed using 2500 blood donor samples. Assay sensitivity was evaluated by running 8 commercially available seroconversion panels and 6 serially diluted patient samples. HIV-1 p24 antigen sensitivity was evaluated via serial dilution of AFSSAPS p24 standard. The assay was evaluated for total within lab precision over 20 days in accordance with CLSI

EP05-A2 using one VITROS 3600 Immunodiagnostic System and one VITROS ECiQ Immunodiagnostic System.

Results: Donor specificity was determined to be 99.84% (95% CI: 99.59% to 99.96%) for blood donors. When used to test 8 commercially available seroconversion panels the HIV Combo assay was reactive at the same bleeds as a commercially available 4th generation assay. During antibody dilution testing the VITROS HIV Combo Assay* generated reactive results at least one dilution earlier than a commercially available 4th generation assay when evaluating high and low titer HIV-1 group M and low titer HIV-2 antibody. The VITROS HIV Combo Assay* generated reactive results within 1 dilution for HIV-1 group O antibody as compared to a commercially available 4th generation assay. The assay detects AFSSAPS p24 antigen at 15.2ng/mL. Within Lab precision of the assay ranged from 5.7 to 14.1% near the assay cut-off.

Conclusion: The VITROS HIV Combo Assay* enables earlier detection of HIV infection and provides comparable sensitivity and specificity performance to a commercially available 4th generation assay.

*Under Development

B-077

Prevalence and Antimicrobial susceptibility profile of microorganisms isolated from lower respiratory tract infections in hospitalized patients of Belo Horizonte - Minas Gerais/Brazil.

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Background: Lower respiratory tract infections (LRTI) have a great importance because of their high frequency and associated morbidity, and show high prevalence rates in Brazil and in the world, being the most prevalent hospital infections, mainly in intensive care units. In this study, the susceptibility profile information of local bacterial isolates (Belo Horizonte area) was evaluated aiming to contribute with a better understanding of the main bacterial types causing LRTIsin our region. This knowledge can contributeto an orientated antibiotic therapy and, possibly, a reduction of bacterial resistance. Patients/Methods:The epidemiologic study analyzed data obtained from electronic records collected between October 2011 to September 2012 of 584 patients suspected to have LRTI. The LTRI samples were collected by tracheal aspirate, mini-bronchoalveolar lavage and bronchoalveolar lavage. We identified microorganisms and evaluated their antibiotic sensitivity using the automated system MicroScan WalkAway® (Siemens, Erlangen-Germany). This technique involves the broth microdilution antimicrobial to determine the minimum inhibitory concentration (MIC). The identification of strains with possible antimicrobial resistance phenotypes was performed according to the recommendations of CLSI 2011. Results: From the 584 patients' data analyzed, 381 had positive cultures. The most prevalent microorganisms were Pseudomonas aeruginosa (25.9%) and Acinetobacter baumannii/haemolyticus (20.5%) followed by Staphylococcus aureus (11.3%, S. aureus MRSA corresponding to 45%), Stenotrophomonas maltophilia (5.7%), Klebsiella pneumoniae (4.4%) and Escherichia coli (4%).Regarding the susceptibility profile, we found a high level of resistance to the antimicrobials evaluated. 26% of Pseudomonas aeruginosa isolates were resistant to ciprofloxacin and levofloxacin, and 22% toaztreonam; and we also found resistance to the class of cephalosporins and carbapenems class, with 15% resistance to imipenem and 16% to meropenem. For the strains of Staphylococcus aureusmethicillin-sensitive, we found that 21% of the isolates were resistant to clindamycin, 24% to erythromycin and 60% to penicillin and ampicillin. In addition, 93% of Staphylococcus aureus (MRSA) showed resistance to clindamycin and erythromycin, 52% to gentamycin; 56% to levofloxacin; 33% to moxifloxacin and 4% to tetracycline. Escherichia coli strains showed increased resistance to ampicillin (43%) and to tetracycline (33%). Over 50% of the Acinetobacter baumannii strains showed resistance to most of the antimicrobial evaluated, highlighting amikacin with 66% of resistance, cephalosporins with about 80%, quinolones and carbapenems with approximately 90% of resistant strains. Conclusion: Given the importance of knowing the regional prevalence of microbiota and delineate an antimicrobial susceptibility profile, this study is of great importance to standardize the treatment regimen of LRTI in hospitals of the evaluated region.

B-078

Association of Polymorphic Variants in TNF-Alpha, IL-6, Receptor FCγRIIA and Vitamin D Genes in Hondurans' with Dengue Infection.

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Dengue is an important problem of health public in tropical and sub-tropical countries. On the other hand, the response to dengue infection is influenced by the genetic background of the host.To evaluate the association of polymorphic variants in TNF-alpha 308, IL-6, Receptor FCγRII A and Vitamin D between individuals with different dengue disease presentation.The study was carried out in Tegucigalpa, Honduras, Central America. The study population consisted of 150 participants: 50 dengue fever (DF) and 50 dengue hemorrhagic fever (DHF) plus 50 asymptomatic controls (AC), all of them with proven dengue infection. The dengue classification was done following the WHO criteria. The selected single-nucleotide polymorphisms (SNPs) were carried out by a Real Time PCR methodology, using sequence specific primers, obtaining the allelic genotype for each investigated genetic marker. Comparisons between three population groups showed in DHF there was significantly more allelic frequency of TNFa-308 genotype GG (OR= 7.76, p= 0.0006) than in the AC, more over the frequency of this genotype is proportionally higher as disease severity progresses (AC: 67%, DF: 86% and DHF: 94%); genotype AA is absent in both DC and DHF. For FcgRIIA-131 H/R and IL-6-174 we observed no differences in the different population groups. As for the VDR-352, there is a higher frequency for the homozygous genotype TT(40%) in DF compare with DHF and AC (27 y 22% respectivetly), there is a tendendy for this polymorphism can be implied in protection to the infection severity for dengue virus for this genotype(TT) (OR=0.43, p=0.0597).

These results provide evidence for the first time in Honduran population about the genetic susceptibility to the infection for dengue virus. However further studies are still necessary.

B-079

Improved Detection of Histoplasma capulatum Antigen in Urine Specimens Utilizing Ultrafiltration and Commercially Available Enzyme Immunoassay Reagents

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Background: *Histoplasma* antigen detection in urine is useful for diagnosing and monitoring treatment of systemic histoplasmosis. Immuno-Myologic, Inc (Immy®, Norman, OK) has produced analyte specific reagents (ASRs) for a monoclonal based enzyme immunoassay for the qualitative /quantitative detection of this antigen. A literature search noted several studies where it was shown that the Immuno-Myologic assay demonstrated high specificity, but low sensitivity when compared to Mira Vista Diagnostics's proprietary Immunoassay, which some consider the industry's gold standard with regards to antigen detection. The aim of our study was to examine if ultrafiltration using Amicon Ultra-2 mL Centrifugal Filters would improve the detection and recovery of *Histoplasma* antigen.

Material and Methods: A total of 57 urine samples previously tested by Mira Vista Diagnostic were used for this study. 16 of the samples had antigen levels detected by Mira Vista at levels >0.4 ng/mL. The remaining 41 samples were below the established cutoff value and considered negative. The positive urine samples were tested neat with reagents from Immy to establish a baseline on a Dynex DSX 4-plate ELISA processing system. Following the analysis, these samples were concentrated according to the manufacturer's instructions for Amicon Ultra-2 Centrifugal Filter Unit with Ultracel-3 membrane (EMD Millipore UFC200324). The concentrated samples were then tested, and samples that had the antigen concentrated to 0.4 ng/mL or greater were considered positive. The results were than compared to Mira Vista's results.

Results: Correlation between Immy's ASRs and Mira Vista for the Detection of *Histoplasma* antigen increased from 56.3% (9/16) positive agreement to 93.8% (15/16) positive agreement following ultrafiltration. Negative agreement remained the same, but did fall slightly from 100% (41/41) to 97.6% (40/41). Overall agreement with Mira Vista increased from 86.0% to 96.5% neat versus ultrafiltration, respectively. The urine sample was concentrated from a starting volume of 2.0 mL down to 150 µL, representing, mathematically, a 13-fold increase in concentration, but for unknown reasons, the amount that it was concentrated varied from sample to sample. The amount of antigen recovered after ultrafiltration ranged from 4-times to 29-times the original concentration.

Conclusion: The monoclonal enzyme immunoassay from Immy provides a unique opportunity for laboratories to test urine for *Histoplasma* antigen. The ability of laboratories to accurately test for

Histoplasma antigen can lead to a faster turnaround time and provide the physicians with useful information to make an initial diagnosis. However, the low sensitivity of the assay could mean that patients with low levels of *Histoplasma capsulatum* antigen may go undetected. Our findings suggest that the concentration of the urine sample by ultrafiltration would lead to a better detection rate of the antigen using Immy's ASRs. This method offers an accurate and sensitive method for qualitatively detecting *Histoplasma* antigen.

B-080

Bloodborne pathogen contamination in the era of laboratory automation and Ebola

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Background:

Guidance from the Centers for Disease Control (CDC) on laboratory testing for persons under investigation for Ebola virus disease has stated that routine laboratory testing can be safely performed using automated laboratory instruments by adhering to bloodborne pathogen practices. Many laboratories use total laboratory automation (TLA) systems to perform routine clinical testing wherein specimens are centrifuged, decapped, and transported uncapped on a conveyor belt to downstream test analyzers. TLA systems come with a range of safety features standardly available; furthermore, decontamination procedure recommendations from manufacturers has been variable during the 2014-2015 Ebola outbreak. To develop evidence-based protocols for handling high risk pathogens in the clinical laboratory, we sought to investigate the levels of contamination by common bloodborne pathogens Hepatitis B (HBV) and Hepatitis C viruses (HCV), of a TLA system occurring through routine clinical use as well as immediately after processing high-titer samples.

Methods:

In order to ensure that our clinical HBV and HCV assays, a laboratory-developed test (HBV) and Abbott RealTime HCV, were capable of nucleic acid detection from environmental specimens, we assessed the recovery of viral nucleic acid from swabs of non-porous surfaces (glass slides). We observed linear recovery of viral nucleic acid over a range of concentrations with a recovery of 75% and 46% for HBV and HCV, respectively. Following a risk assessment, environmental swabs were then performed at key locations along a representative TLA system, taken during routine clinical use. Contamination was further assessed immediately after running a small number of high-titer HCV specimens (mean 5.8 x 107 IU/mL); to distinguish this contamination from baseline contamination present prior to the experiment, clean glass slides were placed at key locations and swabbed for the presence of HCV, as above.

Results:

Of 79 baseline swabs performed on the TLA system, 10 were positive for HBV and 8 for HCV. Viral nucleic acid was consistently detected from swabs taken from the distal inside surface of the decapper discharge chute, with areas adjacent to the decapper instrument and the centrifuge rotor also positive for HBV or HCV nucleic acid. Of note, contamination was occasionally detected on exposed surfaces in areas without protective barriers between samples and personnel. After running known HCV-positive samples, at least one additional site of contamination was detected on an exposed area of the line after the decapper and next to a barcode reader.

Conclusions:

Together, these data indicate that a low level of viral contamination of automated clinical laboratory equipment occurs during clinical use and suggests a need for better risk-mitigation procedures when handling highly infectious agents such as Ebola virus. At our institution, we have coupled engineering controls and modified procedures with increased communication between laboratories and clinicians via on call Laboratory Medicine residents to increase safety of lab personnel while maximizing the test menu offered for patients with high risk pathogens.

B-081

Association of HLA-DP/DQ Polymorphisms with transplant etiologies and liver function recovery in liver transplant recipients

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Objectives: Recently genome wide association study showed that the genetic variants of human leukocyte antigen (HLA)-DP/DQ loci were strongly associated with a risk of hepatitis B virus (HBV) infection. The aim of this study was to investigate whether the HLA-DP/DQ loci was associated with liver transplant aetiologies and the liver function recovery in Chinese liver transplant recipients.

Methods: A total of 243 liver transplant recipients were enrolled into this study. The aetiologies of all those recipients were liver Cirrhosis (LC), hepatocellular Carcinoma (HCC), progressive HBV hepatitis and non-HBV related disease (including alcoholic hepatitis,hepatitis C or hepatic echinococcosis). Three single nucleotide polymorphisms (SNPs) HLA-DP (rs3077 and rs9277535) and HLA-DQ (rs7453920) were studied in all recipients by high-resolution melting (HRM) curve analysis. We dynamically detected the liver functions (including liver enzyme and coagulation index) before and after transplant.

Results: We found that the protective A allele of HLA-DQ (rs7453920) in LC (A=4.4%) and HCC (A=6.25%) recipients was much lower than non-HBV relative recipients (A=16.7%). In addition, the direct bilirubin levels were lower in AG and AA genotype than those with GG genotype before and after transplant, especially on the 14th day after surgery (17.80 vs. 5.35, P=0.038,figure1). No significant correlation was found in HLA-DP (rs3077 and rs9277535) with HBV infection and liver function recovery.

Conclusions: HLA-DQ (rs7453920) was associated with transplant aetiologies as well as prognosis after liver transplantation. The A allele of rs7453920 served as a protective character in both HBV infection and liver function recovery after liver transplant.

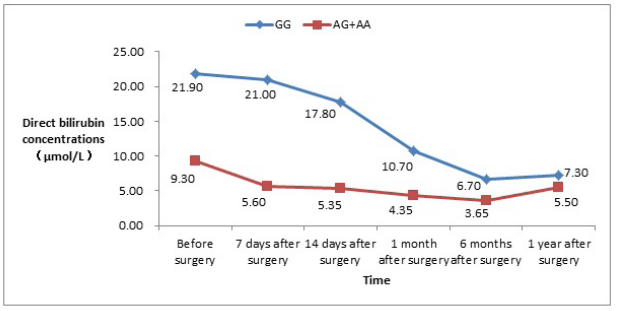

Figure 1. The association between the HLA-DQ rs7453920 and recipients' dynamic direct bilirubin concentration

B-082

Serum markers and microbial etiology of bacteremia

J. D. Santotoribio, A. García-de la Torre, C. Cañavate-Solano, F. Arce-Matute, S. Pérez-Ramos. *Puerto Real University Hospital, Cadiz, Spain*

Background: The microbial etiology of bacteremia determines the choice of adequate therapy for severe infections. The clinical manifestations of gram-negative and gram-positive bacterial infections are similar while biological markers may serve as a guide for the early diagnosis of the nature of a pathogen. The purpose of the study was to assess an association between the serum levels of lactate, procalcitonin (PCT) and C-Reactive Protein (CRP) and the microbial etiology of bacteremia.

Methods: We studied the role of these serum biomarkers in patients with gram-positive and gram-negative bacteremia. The PCT was analyzed by immunoassay in B.R.A.H.M.S.-KRYPTOR®, lactate and CRP was measured in DIMENSION EXL - SIEMENS® and blood culture was made in BACTEC-9240® blood culture system (Becton Dickinson). The program used for the data processing and statistical analysis was SPSS®.

Results: Our study included 77 patients, the median age was 64.5 years old (inter-quartile range (IQR): 53-71). Twenty-eight patients (36.4%) had bacteremia due to gram-negative bacteria and 49 (63.6%) due to gram-positive, with 38 isolations

Staphylococcus spp were the most frequently isolated bacterium followed by Enterobacteria (11%), Escherichia coli (9.1%), Pseudomonas aeruginosa (9.1%) and Streptococcus pneumoniae (6.5%).

No statistically significant differences were found between gram-negative and gram-positive bacteremia according to the CRP levels (p>0.05). PCT levels were significant higher in the gram-negative bacteremia 6.23 ng/mL [IQR: 1.5-33.53] vs. 2.27 ng/mL [IQR: 0.48-27.6] in gram-positive, however lactate levels were significant higher in the gram-positive bacteremia 3.08 mmol/L [IQR: 1.65-4.85] vs. 1.09 mmol/L [IQR: 1.23-4.5] in gram-negative group. E. coli had the highest PCT value 27.06 ng/mL [IQR: 8.62-137.2] and S.pneumoniae had the highest lactate level 4.4 mmol/L [IQR: 1.5-5.7].

Conclusions: PCT and lactate showed diference between gram-negative and gram-positive bacteremia, it may be useful in differentiating the pathogenic bacteriemia and supposed the etiology before obtaining blood culture results.

B-083

Development and Validation of Robust Assay for Detecting Trypanosoma cruzi Parasites for Clinical Trial of Treating Asymptomatic Chronic Chagas Disease

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Background:

Chagas disease is caused by *Trypanosoma cruzi* (*T. cruzi*) infection. About 8 million people are estimated to be infected, primarily in Mexico, Central and South America. Left untreated, 40% of chronically infected patients will develop serious heart and digestive problems, with an associated 10 to 20% mortality for patients with severe cardiopathy. Current treatments with antiparasitic therapy such as benznidazole (BNZ) have limited efficacy in chronically infected patients and can cause serious side-effects. In 2011 Merck sponsored a Phase 2 proof-of-activity study of comparing posaconazole (POS), a triazole antifungal drug, to BNZ, in treating asymptomatic chronic Chagas disease. For primary trial objective, qualitative PCR results would be used to measure efficacy of treatments.

Methods:

PAXgene Blood DNA Tubes were selected over the traditional complicated method of collecting whole blood specimens from Chagas disease patients, which includes mixing 10 ml of blood with 10 ml of Guanidine Hydrochloride-EDTA followed by 10 minute boiling. PAXgene method allowed participating clinical sites in Latin American countries to follow a simple protocol to collect and handle the specimens and to ship them at room temperature. However, PAXgene protocol required development of new methods to release parasitic DNA from blood and to extract DNA. Zymo Research's Quick-gDNA Blood MiniPrep kit was found to be able to efficiently release and extract *T. cruzi* DNA from PAXgene blood, without organic denaturants or Proteinase K digestion. After published PCR assays shown to have suboptimal sensitivity, we developed custom-designed TaqMan based qPCR assays to detect and quantify minicircle kinetoplast DNA (kDNA) from two strains of *T. cruzi*, representing the two lineages of *T. cruzi*. Linearized plasmid containing a sequence from *Arabidopsis thaliana* was spiked into the lysed blood before DNA extraction as internal control for evaluating DNA extraction efficiency and qPCR inhibition by potential blood heme contamination.

Results:

DNA extraction efficiency with PAXgene blood from healthy volunteers was high and consistent, with average efficiency of 93% with 7% CV. qPCR amplification efficiency for both *T. cruzi* strains, K98 representing Lineage I and CL-Brener (CLB) Lineage II, were > 90%. Assay precision for parasitic loads was good for both strains, with K98 ranging from 8% CV for 10 ppm (parasite per ml of blood) to 22% for 0.25 ppm, and CLB from 22% CV for 10 ppm to 27% for 1 ppm. The assay sensitivity, in term of limit of detection, which is the lowest ppm at least 95% of technical replicates can be detected, was 0.025 ppm for K98 and 0.05 ppm for CLB. Assay accuracy, in terms of measured ppm divided by known spiked-in ppm, were 103%, 99%, and 76% for 10, 1, and 0.25 ppm respectively for K98, and 123%, 97%, and 101% for 10, 1, 0.25 ppm respectively for CLB.

Conclusion:

A robust assay combining simple DNA extraction procedure with optimized TaqMan qPCR assay was validated for PAXgene blood specimens containing *T. cruzi*. The assay has high accuracy, sensitivity and precision and is being utilized to support the clinical trial for chronic Chagas disease treatment.

B-084

An Evaluation of Rapid Molecular Tuberculosis Identification with the Automated GeneXpert MTB/RIF System

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Background:

Tuberculosis (TB) is one of the major communicable diseases around the world and an estimated 9 million people were diagnosed based on the report from World Health Organization (WHO). With 12,338 new cases and 626 TB-related deaths in 2012, TB remains the highest incidence and mortality rate among all airborne contagious diseases in Taiwan. Conventional methods, sputum acid-fast bacilli (AFB) smear microscopy and culture of TB bacteria on liquid or solid media, can be inaccurate and time-consuming. The study aimed to determine the accuracy of the GeneXpert MTB/RIF system, a commercially available nucleic acid amplification technology, in diagnosing TB.

Methods:

A total of 469 sputum samples were collected in a medical center from November 2013 to November 2014. Lowenstein-Jensen (L-J) medium, Mycobacterium Growth Indicator Tube (MGIT), and GeneXpert MTB/RIF assay were performed to identify TB. Additionally, culture based conventional drug susceptibility test (DST) was administered to measure the efficacy of this molecular method.

Results:

The sensitivity and specificity of the GeneXpert MTB/RIF were 96.7% (59/61) and 98.0% (400/408) respectively. The negative predictive value (NPV) was 99.5% (400/402) and positive predictive value (PPV) was 88.1% (59/67). The MTB/RIF assay also detected 1 rifampicin resistant specimen and 3 susceptible specimens from the 469 cases, and the results were confirmed by drug susceptibility testing. Besides, the turnaround time for TB confirmation test decreased from 2-3 weeks to 2-3 hours in this microbiology laboratory.

Conclusion:

In conclusion, the GeneXpert MTB/RIF system exhibits high sensitivity and specificity for detecting tuberculosis and has the potential to apply to other human infectious pathogens. The study shows that the laboratory requires an efficient and accurate tool for identifying communicable disease. Timely confirmation of communicable diseases can lead to earlier treatment for the suspected individual, decrease the chance of transmission in the clinical settings, and provide more effective public health interventions.

B-085

Evaluating the Agreement between Architect-Abbott Chemiluminiscent Assay and Immunofluorescense Assay for Detection of Chagas Antibodies

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Background: Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite, Trypanosoma cruzi (T.cruzi). Chagas disease occurs mainly in Latin America. However, in the past decades it has been increasingly detected in the United States of America, Canada, many European and some Western Pacific countries. This is due mainly to population mobility between Latin America and the rest of the world. In our country the prevalence of Chagas disease is 4%. The diagnosis of Chagas disease can be made by observation of the parasite in a blood smear by microscopic examination. However, a blood smear works well only in the acute phase of infection when parasites are seen circulating in blood. Diagnosis of chronic Chagas disease is generally based on detection of anti-T.cruzi IgG antibodies testing with at least two different serologic tests. Blood screening is vital to prevent infection through transfusion and organ transplantation and, diagnosis of infection in pregnant women is essential. Objective: The purpose of this study was to estimate the agreement for determining anti-T. cruzi antibodies between the Architect Chemiluminiscent Assay (Candidate method) with a well-established Immunofluorescence Assay (Comparative method) in a group of individuals who were attended in our Laboratory in Argentina. Materials and methods: Serum samples were obtained from 41 healthy volunteers: 29 women and 12 men (aged 24-61 years) and from 22 individuals previously characterized as positive for anti-T.cruzi antibodies by other serologic tests (agglutination, ELISA), 10 women and 12 men (aged 23-65 years). Anti-T.cruzi antibodies were determined by

CMIA Chagas ARCHITECT i1000SR Immunoassay Analyzer (Abbott Diagnostics) and Immunofluorescence Assay (Biocientífica S.A, Argentina).To estimate the agreement between the candidate method and the comparative method the CLSI guidelines EP12-A2, Vol. 28 N°3 was used. To compare the results obtained by the two methods the EP Evaluator® program was used, the percentage of agreement was calculated with a confidence interval of 95% and Kappa (κ) of Cohen coefficient was assessed on the scale of Landis and Koch. Results: The 41 healthy volunteers were negative by both methods, from the 22 individuals previously characterized as positive, 19 were positive by both methods and 3 were positive by CMIA Chagas and negative by Immunofluorescence Assay. Overall Agreement was 95.2% (86.9 to 98.4%); Positive Agreement 100.0%; Negative Agreement 93.2%; Cohen's Kappa 89.2% (77.2 to 101.1%). Conclusions: Our study, although small, suggested the use of a chemiluminescent immunoassay for anti-T.cruzi antibodies detection. The agreement between the results was assessed as 95.2%, Cohen's Kappa 89.2%, which indicates a high agreement. This way Immunofluorescence Assay could be replaced by the fully automated method as an initial test in the diagnostic algorithm of Chagas disease. The main advantages of CMIA Chagas ARCHITECT Immunoassay are: no experienced technician required, traceable and reliable alternative to more time-consuming procedures. In summary, the CMIA Chagas ARCHITECT Immunoassay characterized here is suitable for routine analysis in the clinical setting, is simple and rapid for anti-T.cruzi antibodies detection in serum or plasma.

B-086

Distribution of microorganisms detected in blood culture according to the presence or absence of neutropenia in cancer patients.

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Background:

Infection remains an important cause of morbidity and mortality in patients with cancer, and neutropenia has been recognized as a major risk factor for the development of infections in this population. The presence of neutropenia and other factors such as comorbid illnesses, presence the mucosa damage, catheters, and antibiotic use influence the spectrum of bacterial infection among cancer patients. Recognition of the epidemiological bacterial infection is important to guide appropriate empiric therapy.

Objective:

To evaluate the distribution of microorganisms detected in blood culture according to the presence or absence of neutropenia at the time of blood culture collected in cancer patients.

Methods:

A retrospective analysis of the records from the microbiology laboratory was performed to identify patients with blood cultures requested from July to September 2014. The study population was inpatients and outpatients from a private cancer hospital in São Paulo. Blood cultures were processed by the BacT/ALERT 3D® Microbial Detection System; positive bottles were smeared in common media plates and identified by Vitek MS® MALD-TOF, an automated microbial identification system that uses mass spectrometry technology to provide identification results in minutes. A positive blood culture was defined as the recovery of a microorganism(s) from one or more bottles from a blood culture set. The neutrophil count cell was checked at the same date that the blood culture was collected. Neutropenia was defined as an absolute neutrophil count (ANC) less than 500/mm^3.

Results:

Throughout the study period, a total of 5,499 blood cultures were obtained from 2,569 patients; 209 (8%) blood cultures were positive, 5 were polymicrobial. The microorganisms recovered from blood cultures included gram-positive (65%), gram-negative (34%), and yeast (1%). Coagulase-negative staphylococci (CoNS) was the most frequent isolated microorganism in neutropenic and non-neutropenic patients. *Escherichia coli*, *Klebsiella* spp. and Viridans streptococci were more common among neutropenic than non-neutropenic patients, although the difference were not statistically significant.

Conclusion:

Gram-positive were more common than gram-negative bacteria during the study period independent of the presence or absence of neutropenia. There was no significant differences in microorganism distribution between the groups of patients with neutropenia versus patients that did not had neutropenia at the time of blood culture drawn.

B-087

Analysis of cases of co-infection between HIV and syphilis in Brazil in 2014

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Background: Syphilis has been a public health problem known for centuries, while AIDS is a relatively new. Syphilis and AIDS co-infection is a complex clinical interaction and yet not well understood, because Syphilis significantly increases the risk of contracting HIV, and in turn, HIV can change Syphilis natural course.

In Brazil has been observed a substantial increase in the number of STD cases, such growth is mainly observed in cases of HIV and Syphilis, typically affecting similar patient groups, where coinfection can occur. Co-infection represents a potential risk for public health, as patients who practice unprotected sexual intercourse, with potential to increase the rate of new cases for both diseases.

This study aims to analyze the data of a large laboratory in Brazil, were patients were screened for both diseases.

Methods: Using the database of a large clinical laboratory we assessed 591626 patients results of HIV and 127946 patients results of syphilis during the year of 2014 from all the regions of Brazil.

All HIV test were performed by the methodologies of electrochemiluminescence immunoassay - ECLIA (HIV Combi PT COBAS®, Roche) and chemiluminescent microparticle immunoassay - CMIA (HIV Ag/Ab Combo Architect®, Abbott) both tests detect the HIV-1 antigen and total antibodies to HIV-1 and HIV-2, simultaneously, as a combo test. All syphilis test were performed by the methodology of chemiluminescent microparticle immunoassay - CMIA (Syphilis TP Architect®, Abbott) and confirmed by Fluorescent Treponemal Antibody-Absorption - FTA-ABS (FTA-ABS IFA Hemagen Diagnostics, Inc. VIRGO® Products Division).

Results: In all analyzed tests were verified 9790 HIV positive results and 40 534 positive cases of syphilis. Among the total number of patients we observed that 14 908 patients had a correlation of HIV, and syphilis tests. Of these, 1794 are syphilis positive, 140 are HIV positive and 125 have co-infection. In these 125 cases of co-infection 87.2% are males.

Conclusion: Analyzing at the correlation of positive cases, we realize that the incidence is low if compared to low number of co-infected patients, given the number of Syphilis cases diagnosed in Brazil.

This data shows that 94% of patients with positive syphilis are in risk patients. If we consider the total number of patients in this study, we can conclude that 34101 patients are at HIV infection risk, as syphilis infection, indicates that intercourse was done with no protection.

The absolute predominance of co-infected men (87.2%) may suggest that co-infected women are being under diagnosed because the clinical presentation of syphilis in men be more apparent (or easily detected) than in women.

B-088

A novel multiplexed qPCR assay for the detection of 10 bacterial and viral causes of meningitis.

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Background: Meningitis is an infection of the central nervous system that can have serious clinical outcomes. Viral meningitis caused by Enterovirus is very common, usually self-limiting and does not require treatment. Bacterial meningitis is associated with high rates of mortality and high frequency of severe sequelae, with patients requiring prompt treatment with antibiotics. Although the vast majority of cases of meningitis are viral, most patients with suspected meningitis are admitted to hospital and treated with antibiotics until a diagnosis of viral or bacterial meningitis has been made. The diagnosis of meningitis infection by culture is time consuming, lacks sensitivity, requires high-level technical expertise, and in the case of bacterial meningitis culture may return false-negative results especially after the initiation of antimicrobial therapy. Early identification of causative bacterial and viral pathogens may be important for prompt and proper treatment of meningitis and for prevention of life-threatening clinical outcomes. We have developed a panel of multiplexed qPCR assays to assist with the rapid detection of bacterial and viral agents commonly associated with meningitis.

Material and Methods: Multiplexed qPCR based assays were developed using MNAzymes for detection and differentiation of the most common causes of viral and bacterial meningitis in Cerebrospinal Fluid (CSF) samples. The assays consisted of four panels; (1) Bacterial panel 1 to detect N. meningitidis, S. pneumoniae and H. Influenzae, (2) Bacterial panel 2 to detect Group B streptococcus, E. coli and L. monocytogenes, (3) Viral DNA panel to detect Herpes Simplex Virus type 1 (HSV1), Herpes Simplex Virus type 2 (HSV2) and Varicella zoster (VZV), and (4) Enterovirus Viral RNA. Each of the panels additionally included an internal control to verify adequate sample processing of the target(s) and to monitor the presence of assay inhibitors. Performance characteristics tested for each assay panel included specificity, sensitivity, inclusitivity, linearity, impact of interfering substances and reproducibility. MNAzyme qPCR utilises multiple partial DNA enzymes or 'partzymes' that are inherently inactive, but when they combine in the presence of a target, they form active MNAzymes which cleave universal probes leading to signal generation indicating the presence of the target(s). MNAzyme qPCR possesses superior specificity and multiplex capacity compared to many other real-time chemistries.

Results: The four multiplex panels showed robust specificity, sensitivity, reproducibility with no inhibition of internal control signal. The R2 for all targets ranged from 0.97 to 0.99 and efficiencies ranged from 95% to 109%. No inter-panel cross-reactivity was observed with any components and no cross-reactivity was detected using a wide range of non-target organisms. The limit of detection (probit regression analysis, 95 % probability) ranged from 8 - 50 copies for each target organism. Signal detection was not affected by the presence of high levels of potentially intering substances found in CSF.

Conclusion: MNAzyme qPCR provides a flexible and unique approach to qPCR that is specific, sensitive, rapid and easily multiplexed. The assays developed are useful for rapid identification of important meningitis pathogens.

B-089

Clinical characteristics and drug-resistance profile of urinary tuberculosis in Southwestern China

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Objectives: Urinary tuberculosis is the second most common form of extra-pulmonary tuberculosis, however, less is known about the drug-resistance of urinary tuberculosis in China. Our study pays attention to the drug-resistance gene profile of urinary tuberculosis patients in Southwestern China in order to improve tuberculosis diagnostics.

Methods: A prospective, cross-sectional study of 193 patients with urinary tuberculosis was evaluated in a duration from January 2009 to March 2014. Abundant data including presenting symptoms, laboratory investigations, radiologic findings and drug-resistance gene patterns were analyzed. Real-time PCR was performed to detect urine mycobacterial DNA. PCR-positive samples were then subjected for drug resistant test using the GenoType® line-probe assays. Urine smear microscopy was carried out with Ziehl-Neelsen acid-fast staining method. The serologic biochemical markers were performed with the automated Biochemistry Analyzer Modular P800. Blood routine examination was conducted by using the fully automated hematology analyzer XE-5000TM.

Results: The most common symptoms of urinary tuberculosis were urinary irritation symptoms (61.1%) and lumbago (49.2%). High proportions of microscopic hematuria (63.2%) and microscopic proteinuria (45.6%) were also seen. The positive rate was 66.3% for mycobacterial DNA PCR, otherwise 13.1% for culture and 9.8% for smear. The total rate for the drug-resistant tuberculosis was 39.7%, containing 20.7% for multidrug resistance tuberculosis (MDR-TB), and the most prevalent mutation sites were katG S315T1, rpoB S531L and gyrA D94G.

Conclusion: Southwestern China has a serious epidemic of drug-resistant urinary tuberculosis, a substantial number of new and previously treated urinary tuberculosis cases harbor MDR-TB. We suggest that real-time PCR for mycobacterial DNA identification and drug-resistant test as routine assays for suspected urinary tuberculosis patients.

B-090

A first comparison study between two Fungus (1-3)-β-D-Glucan Assays for the diagnosis of invasive fungal diseases in China

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Background:

The study aims to evaluate the performance of the newly developed (1-3)-β-D Glucan Assay (G Test, Dynamiker Biotechnology (Tianjin) Co., Ltd.) with a side-to-side comparison with Fungitell® assay (Associates Cape Cod, Inc.) (ACC) in detecting invasive fungal diseases (IFD) using clinical serum specimen.

Methods:

21 clinically suspected IFD patient serum samples were collected from The First Affiliated Hospital of Guangzhou Medical University and a specific number was assigned to each sample. (1-3)-β-D Glucan detection was performed using ACC's Fungitell assay and Dynamiker's G Test. Fungitell kit is an FDA-cleared and CE-Marked diagnostic kit specialized for the detection of IFI. G test was recently approved by the CFDA authority and became available in the China market. Fungitell assay uses a positive cut-off value of 60-80pg/ml, as opposed to G Test which has a positive cut-off value of 70-95pg/ml. Moreover, the concentrations for the five standards of Fungitell assay are 31.25, 62.5, 125, 250 and 500pg/ml, while G Test's standard concentrations are 37.5, 75, 150, 300 and 600pg/ml respectively. The results were compared and analyzed using statistical tests.

Results:

Fungitell assay detected 11 positives (samples 1-9, 19 and 21), 9 negatives (samples 11-18, and 20) and 1 inconclusive result (sample 10). On the contrary, G test detected 11 positives (samples 1-10, and 21) and 10 negatives (samples 11-20). Positive results of G test that match with Fungitell assay are samples 1-9 and 21, while in samples 11-18 and 20 negative results were obtained from both kits.

Conclusion:

The test results demonstrated that the performance of Dynamiker's G Test is highly consistent with that of Fungitell assay with the total coincidence rate of over 90%.

Test results comparison between the two reagent kits.		
Reagent Kit	ACC Fungitell® assay	Dynamiker Fungus (1-3)-β-D-Glucan Assay
Cutoff value	60-80pg/mL	70-95pg/mL
Sample No.	Test Results	Test Results
1-9	+	+
10	+/-	+
11-18	-	-
19	+	-
20	-	-
21	+	+

B-091

Analyzing Amino Acids Profile in Influenza Virus Infection to Identify Potential Biomarkers for Clinical Applications

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Background: Influenza A viruses are major pathogens that cause respiratory infections and have considerable impact on human health yearly. It is not clear why some influenza viruses cause severe, even fatal diseases, while others cause relative mild respiratory infections. From our previous publication, we found that the CCR2+ monocyte plays a crucial role in the pathological outcome of highly pathogenic H1N1 influenza A virus infection. We also observed excessive accumulation of CCR2+ inflammatory monocytes in severe infection, which is correlated with type I interferons (IFNs) prolong production. Production of Type I IFNs in limiting viral replication is the first line of defense against virus infection. A lot of molecules are involved in type I interferon dependent immunity including toll like receptors (TLRs), myeloid differentiation factor-88 (MyD88) and interferon receptor α/β receptor (IFNAR). We have observed that amino acids concentrations in mice broncho alveolar fluid (BALF) are correlated with severity of influenza infection when using different pathogenic strains of viruses. The objective of this study is to investigate the amino acids profile in the IFNs related signaling pathway in innate immunity upon high pathogenic influenza infection by using knockout mouse models.

Methods: C57BL/6 (wild type) and three strains of knockout mice, CCR2 (-/-), MyD88 (-/-) and IFNAR (-/-), were used. Mice were anesthetized with Isoflurane and then infected by intranasal applications of 200 PFU of recombinant influenza A H1N1 virus A/PR8/34 (PR8). Infected mice were sacrificed on day 6 after infection. BALF was obtained by flushing airway three times with 0.5 mL sterile PBS and spun down to remove cellular debris. BALF amino acids were quantitated by ultra-performance liquid chromatography tandem mass spectrometry. Data were analyzed using a web-based server MetaboAnalyst.

Results: A significant difference was observed between these different strains of knockout mice when we analyzed the amino acids profile. The disease severity of knockout mice after infection was evaluated by the infiltration of leukocytes in pulmonary tissues. We observed that amino acids concentrations positively correlated with the disease severity. The uncontrolled viral replication leads to accumulation of CCR2+ cell in wild type mice, which help amplifying the inflammatory signal and lead to fatal outcomes of high pathogenicity virus infections. Interestingly, the BALF amino acids profile of PR8 infected CCR2 knockout mice is indistinguishable from naive mice. Obviously, CCR2+ monocytes are important cells involved in regulating the amino acids metabolism upon influenza virus infection. Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) enable a clear differentiation of different strain of knockout mice samples. The results suggested the regulation pathways for these molecules may not be the same.

Conclusion: The amino acids profile holds the potential to be diagnostic markers for highly pathogenic influenza virus infection. These findings provide insight into disease pathophysiology and can serve as the basis for developing disease biomarkers for influenza infection.

B-092

The Improvement in Blood Volume to Positively Impact on Bacteremia Diagnosis

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Background:

Blood culture volume is the most important variable in detecting bacteremia. However, the majority of hospitals in Taiwan do not meet the criteria for ideal volume during collection. The object of this study is to evaluate the relationship between blood volume and contamination rate, and furthermore time for positive by using the BD BACTEC FX blood culture system.

Methods:

Using BD BACTEC Blood Volume Monitoring system (Becton Dickinson, Sparks, MD) we know the average blood volume between 01/2013 and 06/2013 is 3.9ml; the ratio of 6ml or above blood among total collection is merely 10%, and the contamination rate is over 3%. All indexes are not fit in with acceptable criteria. From 07/2013 to 08/2013 we issued a questionnaire toward nursing department to understand their cognition in blood culture collection. To go on, we arrange a series of activities to correct their misunderstanding, including lecture and hands on training, mark blood drawing line on each blood culture bottle, video talking about standard operational procedure to perform blood culture collection was made for every new coming nurse, also to issue on intranet, randomly inspect their clinical practice, report of unusual indexes and collector's name to nursing department monthly. The nursing department also holds racing activity to promote low contamination concept. The positive rate and contamination rate along with blood volume were monitored monthly.

Results:

Start from June 2013 to December 2014, we initiated blood volume improvement plan to set 6ml blood per bottle as target. With implantation of educational program, continuous blood collection devices and automatic blood volume monitoring software, our 6ml achieve rate reach 100%, the blood volume increase from 3.9ml to 7.2ml; contamination rate decrease to 2.19%, average time to positive shorten for 3 hours.

Conclusion:

With our study we demonstrated that the education program is effective by significant volume increase, the collection of adequate blood volume is correlated with decreased contamination rates and faster recovery time of blood culture. By continuous educate phlebotomists on this concept, patients with bacteremia can be rapidly and correctly detected, benefiting both patients and hospitals.

B-093

Evaluation of maternal and congenital syphilis in six maternity hospitals in São Paulo, Brazil.

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Background:

Congenital syphilis (CS) is a major public health concern, even after the implementation of intervention protocols in several countries. Many of these women are not tested for syphilis or not treated adequately; about 40% of such pregnancies will end in miscarriage, stillbirth, or perinatal death, and surviving newborns are at risk for congenital malformations. The serological diagnosis of syphilis is based on nontreponemal tests (Veneral Disease Research Laboratory, VDRL, or Rapid Plasma Reagin RPR), which detect antibody to cardiolipin, and treponemal tests (such as fluorescent treponemal antibody-absorption, FTA-ABS, or hemagglutinin assay, TPHA), which detect antibodies specific to *T. pallidum*. The diagnosis of CS is based on laboratorial serology of women during the first and third trimester of pregnancy, and at the time of delivery. All infants potentially exposed to syphilis in utero based on the maternal serology should undergo serological testing for syphilis, regardless of maternal treatment. Asymptomatic infants whose mother received appropriate treatment should be followed monthly until their nontreponemal antibody disappears. If there is no documented successful maternal treatment during pregnancy or presence of signs or symptoms of CS, infants should complete clinical and laboratorial evaluation.

Objective:

To determine the frequency of positive syphilis tests among women and their infants at the time of delivery in six maternity hospitals in São Paulo, Brazil.

Methods:

A retrospective analysis of laboratorial records were performed to identify serological tests of syphilis among parturient and their infants attending in maternity hospitals in São Paulo, Brazil, during 2014. Parturient syphilis were tested by serological syphilis screening at the time of delivery; positive screening test was confirmed by RPR and, if RPR negative, by TPHA. In screened infants born to women with any previous positive serology for syphilis; positive screening test was confirmed by RPR plus FTA-ABS IgM (since maternal IgM does not cross placenta, its detection is indicative of active infection).

Results:

During the study, 1,628 mother/infant pairs with serological tests for syphilis were analyzed. A total of 67 (4.1%) women were diagnosed with syphilis infection. 60 (90%) of infants born to syphilis-infected women had positive screening test, and 18 (30%) were RPR positive. There were two cases (3%) of mother-to-child transmission of syphilis confirmed by positive FTA-ABS IgM.

Conclusion: Syphilis continues to affect large numbers of pregnant women. Our data are in agreement with an estimated 2% of seroprevalence of syphilis among pregnant women in São Paulo state, Brazil. To reduce cases of CS, it is important to ensure syphilis testing and treatment into routine antenatal programs. Besides, neonatal diagnosis with appropriate treatment and follow-up is mandatory, since the sensitivity of IgM serological tests have been reported been approximately 80% with a specificity of 90%.

B-094

Human Papillomavirus type 16 E7 attenuates AKT signaling and the downstream effector p70 S6 Kinase

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>**Background:** Papillomaviruses are small DNA tumor viruses that are the causative agents of squamous cell cancers in humans. Two of the viral proteins, E6 and E7, are required for the establishment and maintenance of human cancers. High risk E6 and E7 degrade p53 and Rb, respectively, which eliminates cell cycle checkpoints and drives cancer progression and viral replication. While the role of E6 and E7 in targeting p53 and Rb has been intensively studied, how E6 and E7 manipulate cellular signaling cascades to promote the viral life cycle and cancer development is poorly understood. The AKT signaling pathway is central to cell proliferation and overall cell survival, and is often upregulated in cancers.

Results: We hypothesized E6 and E7 could be activating the PI3K/AKT pathway in order to promote proliferation, anti-apoptosis, and overall survival of the cell. To our surprise, we observed the opposite. E7 proteins with high affinity Rb binding sites decreased phosphorylated AKT (pAKT), the active form of the kinase. This

result was unexpected as pAKT levels are typically increased in transformed cells, including cervical cancer. Repression of pAKT by E7 was independent of the Rb and p130 degradation function of E7, but could be mapped to a novel domain in the C-terminus. E7 also decreased the activation of p70 S6K and 4EBP1, suggesting that E7 may induce a switch in the protein translation pattern of the cell. We hypothesize this phenotype promotes an environment primed to replicate and amplify viral DNA.

Conclusions: Decreasing pAKT, pp70 S6K, and p4EBP1 suggests that E7 is attenuating AKT activity in order to putatively alter protein translation in the cell. Previously, E7 has not been shown to alter AKT pathway in this manner, suggesting a novel function of E7. We hypothesize this decrease in AKT activity is to increase IRES dependent translation of particular cellular and viral proteins, which is the subject of further investigation. This translation shift could promote viral genome amplification and potentially an increased sensitivity to chemotherapeutics, due to the decreased AKT signaling. Our observed decrease in pAKT in primary keratinocytes is contrary to the observed increase in cervical cancers, but suggests the activation of AKT signaling could be acquired during the transition from initial infection to invasive carcinoma.

B-095

Development of Simultaneous Detection Lateral Flow Immunoassay Kit for GAS and ADV

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Background: Diagnosis of pharyngitis using a lateral flow immunoassay kit (LFIA) for group A streptococcus (GAS) antigen is widely used. About 15 - 30 % of cases in children of acute pharyngitis are caused by GAS. On the other hand, viruses (such as adenovirus (ADV)) are most common causes of acute pharyngitis.[1)] GAS pharyngitis and ADV pharyngitis is similar clinical features, but treatments is different. A simultaneous antigen detection of LFIA for GAS and ADV can assist the accurate diagnosis and reduce the physical burden of patients with minimizing specimen collection number of times. However, for the problem that the extraction process is different between GAS and ADV, these kit is not making a practical use as yet. We have developed a new simultaneous antigen detection of LFIA for GAS and ADV.

Method: A simultaneous antigen detection of LFIA for GAS and ADV kit consisted of four main components and one extraction buffer: extraction pad, conjugate pad based on colloidal gold, membrane pad, absorbent pad and a buffer including surfactants. The sensitivity and specificity of this kit were evaluated. As a positive control, inactivated GAS antigen and inactivated ADV antigen were diluted with running buffer mixing throat swab obtained from normal adults. As a negative control, three kind of viruses, bacteria two kinds are prepared. This kit is performed by the addition of 100µL of running buffers. This kit was read after 10min. We measured the intensity of the GAS detection line and ADV detection line, respectively by using densitometer.

Results: The analytical sensitivity of the kit when using inactivated GAS and ADV were in the range of 5 x 10^5org/mL to 2 x 10^7org/mL and in the range of 1ng/mL – 100ng/m, respectively. Intra CV is less than 15 %. No cross-reactivity in 5µg/mL influenza A virus, 5µg/mL influenza B virus, 5µg/mL RSV, 2 x 10^7 CFU/mL staphylococcus aureus, 2 x 10^7 CFU/mL streptococcus pneumoniae was observed.

Conclusions: Collectively, these data suggest our developed kit deserve inclusion in the diagnosis of pharyngitis. To our best knowledge, this is a first report of a simultaneous antigen detection of LFIA for GAS and ADV. This kit make high sensitivity, high specificity, simple to use and user friendly. We believe that this kit will be a useful tool for a rapid diagnostics of acute pharyngitis.

1) Bisno AL. Acute pharyngitis: etiology and diagnosis. Pediatrics 1996; 97:949-54.

B-096

Independent Verification of the Dynex M² Multiplexed Assay System Performance in the Democratic Republic of Congo Using Dried Blood Spots

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Background: A Dynex Technologies, Inc. Multiplier Flex System powered by M²® technology, a multiplexed chemiluminescent immunoassay platform, was utilized as the processing system for an MMRVT immunity assessment in support of the 2013 Democratic Republic of Congo Demographic Health Survey (DRC-DHS). In the course of that study 9978 dried blood spots (DBS) from children aged 12 - 59 months were processed. A multiplexed bead cytometry assay for Measles, Rubella and Tetanus was configured at the University of North Carolina and 754 result-blinded DBS independently processed in order to assess the validity of the M² results.

Method: For the M² system, polystyrene beads coated separately with antigen to Measles, Mumps, Rubella, Varicella-Zoster Virus and Tetanus were immobilized within 54-well M² assay strips with 10 beads per well. Three separate within-well positive control beads were coated with horseradish peroxidase, total human IgG, and polyclonal goat anti-human IgG. Two negative control beads were coated with MRC-5 and E6 cell lysate. Positive control DBS were made using a 5-donor pool of normal defibrinated serum. Negative control DBS were made from pooled normal IgG-stripped serum. For the bead cytometry system, antigen to Measles, Rubella and Tetanus were bound to carboxylated fluorescent microspheres using EDC - Sulfo-NHS attachment chemistry. 0.25" DBS punches were extracted into 1ml of PBS, 0.5% tween20, 5.0% dried milk and processed on each platform according to established ELISA processing protocols.

Results: Assay response for each system was calculated as negative-subtracted ratio to within-plate positive control. Pearson regression analysis provides R^2 values of 0.45, 0.61, and 0.55 for Measles, Rubella and Tetanus, respectively, with $p < 0.0001$. Cohen's kappa coefficient of 0.45, 0.93 and 0.69 for these assays indicate moderate, excellent and substantial agreement between platforms. Using the UNC data set as reference, M² sensitivity was shown to be 84.7, 98.5 and 88.7% with specificity of 65.4, 97.3 and 90.2%.

Conclusion: Independent assessment of equivalent DBS show a very good but assay-dependent levels of agreement between this flow cytometry and corollary Dynex M² panels. These data support the conclusion that the Dynex Multiplier Flex system powered by M² technology is a very robust system with excellent sensitivity and specificity. The use of this system in conjunction with DBS processing offers a cost-effective easy to use automated multiplexed immunoassay processing system in challenging environments

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B-097

Workflow characteristics of two random access molecular diagnostic instruments for Chlamydia trachomatis/Neisseria gonorrhoeae testing

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Background: Next Generation Molecular Diagnostic instruments are becoming increasingly flexible to provide workflow advantages to the clinical laboratory. One of these features, readily available on Clinical Chemistry systems but only recently recognized in molecular diagnostics, is Random Access, allowing laboratory technologists to load patient samples in a random and continuous manner without batch constraints, improving operational efficiencies and overall costs. These next generation Molecular Diagnostic instruments provide full automation to reduce labor and manual errors, consolidation of menu to run multiple assays at the same time and speed to release results quickly. Two instruments on the market that can deliver all these workflow benefits are the Panther System (Hologic) and the GeneXpert

Infinity-80 System (Cepheid). The purpose of this study was to evaluate both instruments to determine which could deliver the broadest workflow advantages.

Methods: Workflow characteristics of each automated platform were determined based on testing 192 urine specimens with the respective manufacturer's *Chlamydia trachomatis/Neisseria gonorrhoeae* **(**CT/NG) assay. Utilizing Nexus, a company that specializes in time-motion studies, the hands-on and automation times were precisely measured by recording the start and end times of each step. Data was also collected for 96 urine samples within the 192 test run to compare the time required for lower and higher volume testing. The following parameters were compared for each platform: 1) hands-on time, 2) required return visits during processing, 3) time to first and final results and 4) instrument footprint.

Results: The GeneXpert Infinity-80 required more labor time for 96 tests (1 hour 52 minutes) and 192 tests (3 hours 44 minutes) than the Panther, which required 23 minutes of labor time at 96 tests and 33 minutes at 192 tests. Sample preparation and loading time were significantly longer on the Infinity-80 due to the manual pipetting of each sample into the GeneXpert cartridge prior to loading into the instrument. This required the operator to be vigilant and continuously working for the first 3 hrs and 44 minutes vs 27 minutes on the Panther System when running 192 tests. The Infinity-80 was quicker to first result (1hr 28 min) than the Panther system (3hrs 30 min) and could complete 96 samples faster (4 hours 3 minutes), while the Panther completed 192 samples faster (6 hours 54 minutes). The GeneXpert Infinity-80 has a larger footprint at 9 feet wide x 2.9 feet deep, whereas the Panther is 4 feet wide by 2.6 feet deep.

Conclusion: While both instruments offer random access sample loading, there are differences in the amount of labor, time and space required to operate each system. In clinical settings requiring relatively large sample throughput, such as demonstrated here with batch testing ≥ 96 samples, the Panther offers the advantage of reduced hands on time required for technicians to prepare samples. In contrast, the quicker time-to-result per sample may render the GeneXpert Infinity-80 more desirable in clinical labs having lower sample volumes.

B-098

Comparison of DiaSorin HBeAg and anti-HBe ELISA Methods: Generation of Combinable Data Using Automated Dynex DSX versus Manual Pipetting

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Background: As a goal for the laboratory, manual method assays are desired to transfer to an automated pipetting platform, thereby increasing efficiency and productivity. Therefore, Hepatitis Be Antigen (HBeAg) and Antibody to Hepatitis Be Antigen (anti-HBe) analyses by Enzyme Linked Immunosorbent Assay (ELISA) methods manufactured by DiaSorin S.p.A. were validated on the Dynex DSX platform, to be considered as a substitute for the existing qualitative manual ELISA platform using the BioTek ELx800 Gen5 Secure data analysis software.

Objective: The manufacturer states that if an automated instrument is used other than the instrument characterized in the package insert, the user is responsible for establishing their own assay performance characteristics. This study purpose was to make qualitative comparisons, evaluate precision, and to determine acceptance criteria for interchangeable use between automated Dynex DSX and manual HBeAg and anti-HBe assay performance.

Method: A correlation was performed with 90 serum samples for both analytes, evenly split between positive and negative results. Acceptability criterion within the EP Evaluator [Release 9 Semi-Quantitative Method Comparison] was defined as passing the Test for Symmetry and Cohen's Kappa >75%. Precision experiments were performed using 3 samples having low positive reactivity near the borderline cutoff. The precision samples were tested in 20 replicates for 3 days, for a total of 60 data points for each of the 3 samples, and assessed using the signal to cutoff ratio. Acceptance criteria for the precision experiments were: 1) Both assay processes generated precision data within ±15% agreement, or; 2) The automated DSX platform validation-derived precision values were less than manual pipetting historically-derived precision values. All experiments, whether processed on the DSX platform or pipetted manually, were read on the BioTek ELx800 using Gen5 Secure data analysis software.

Result: The correlation for HBeAg demonstrated 98.9% agreement with respect to qualitative interpretation, with a passing Test for Symmetry and Cohen's Kappa value of 97.8%. The intra-assay precision mean was 8.8%CV for the DSX and 7.6%CV for the manual method. The inter-assay precision mean was 11.5%CV for the DSX and 7.7%CV for the manual method. The historically-derived inter-assay precision was 16.3%CV. The correlation for anti-HBe demonstrated 94.4% agreement with respect to qualitative interpretation, with a passing Test for Symmetry and Cohen's Kappa value of 89.4%. The intra-assay precision mean was 9.4%CV for the DSX and 10.3%CV for the manual method. The inter-assay precision mean was 9.9%CV for the DSX and 10.4%CV for the manual method. The historically-derived inter-assay precision was 9.7%CV.

Conclusion: The correlation and precision experiments for HBeAg and anti-HBe analyses by ELISA demonstrate acceptable combinability between samples that are processed by automated DSX platform or manual pipetting means.

B-099

Assessment of the Elecsys Anti-HCV II assay in three new seroconversion panels

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Background: Approximately 170 million people are chronically infected with the hepatitis C virus (HCV) and many more are undiagnosed. The availability of treatments that can cure most patients means that screening and diagnosis of HCV infection is a priority. The recently developed Elecsys Anti-HCV II assay has enhanced sensitivity (100%) and specificity (in routine samples 99.66/99.64% and in blood donor samples 99.84%) compared with comparator assays. In previous seroconversion panels, the Elecsys Anti-HCV II assay showed excellent seroconversion sensitivity. However, HCV is a rapidly evolving virus, with many different variations across genotypes, therefore, it is important that assays are continually assessed against the available seroconversion panels to ensure ongoing sensitivity and specificity.

Methods: Three seroconversion panels were recently released by SeraCare: PHV924 (collected over 88 days, genotype 2b), PHV925 (collected over 27 days, genotype1a) and PHV926 (collected over 14 days, genotype 3a). The Elecsys Anti-HCV II assay was performed with these seroconversion panels using the cobas e 601 platform. Results with competitor assays were recorded by SeraCare and are available with the seroconversion panel information pack.

Results: The signal/cutoff ratio for the Elecsys Anti-HCV II and competitor assays on each bleed date, for each seroconversion panel are presented in the figure. For panel PHV925 and PHV926 the Elecsys Anti-HCV II was positive strikingly earlier than the competitor assays. In panel PHV924 all assays were positive in the same bleed, however, there is a long period of time between donations 3 and 4 for this panel. The Elecsys Anti-HCV II assay also had a greater signal/cutoff ratio compared with the other Anti-HCV tests.

Conclusion: These data generated with the new seroconversion panels confirm that the Elecsys Anti-HCV II assay has excellent seroconversion sensitivity across HCV genotypes, allowing early detection of HCV infection.

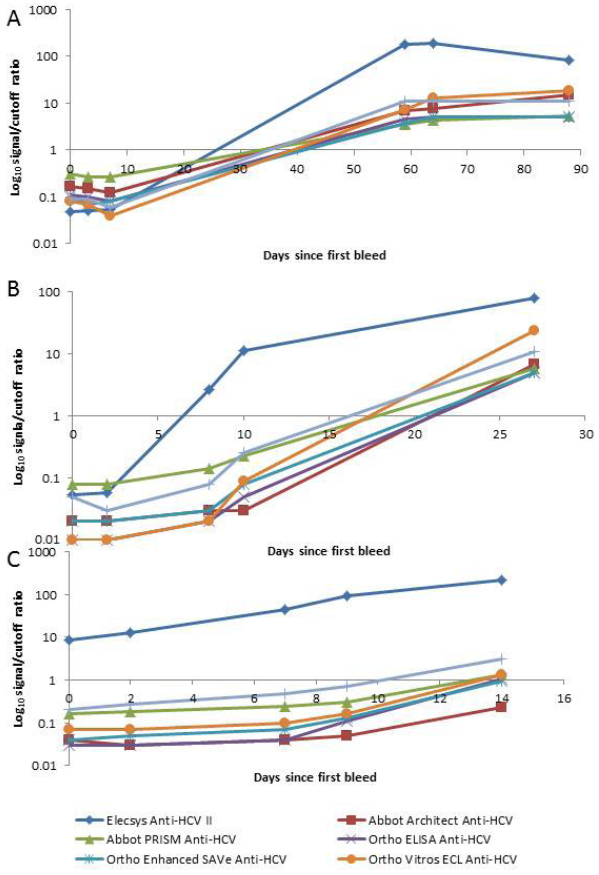

Figure. The log_{10} signal/cutoff ratio for each bleed day of the seroconversion panels A) PHV B)PHV925 and C)PHV926, for several anti-HCV assays. Values above 1 are positive results.

B-100

HIV-1 genotyping test by DNA sequencing: a new 'in-house' method

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The use of highly active antiretroviral therapy for the treatment of HIV has led to an increase in survival of HIV-infected individuals. However, due to a combination of extremely high level of virus production and a high mutation rate, HIV resistance to antiretroviral drugs is increasing, and can make such therapy ineffective over time. Therefore, close monitoring of HIV resistance is required and has been shown to improve antiretroviral treatment. Resistance testing may be performed by direct measurement of virus susceptibility to drugs, or through analysis of the sequence of genes encoding antiretroviral targets (genotyping). Genotypic resistance testing is currently recommended to aid the choice of new drug regimens following treatment failures. Aiming the development of an efficient and cost-effective assay the Research and Development (R&D) group and Genetics division of Hermes Pardini Institute (Belo Horizonte-MG/Brazil) established a new in-house assay. This genotyping method is based on DNA Sanger-based sequencing of the viral genome coding for protease (PR) and reverse transcriptase (RT), which has mutations associated with resistance. Three sets of highly conserved primers were designed to have a wide coverage, producing three overlapping fragments. Different HIV-1 subtypes and known drug resistance genotypes of the Hermes Pardini database were used for the validation. All sequencing reactions were performed using the BigDye Terminator kit (Applied Biosystem) on 3730 DNA Analyzer (Applied Biosystem). Sequences alignment against the HXB2 reference genome and analyses of the electropherograms were performed with the SeqScape software package version 2.5.The genotyping was performed by HIV Drug Resistance Database program (http://sierra2.stanford.edu/sierra/servlet/JSierra?action=sequenceInput) of Stanford University and the results were compared with the genotyping previously determined by commercial TRUGENE kit (TRUGENE™ HIV-1 Genotyping kit/OpenGene DNA Sequencing System - Siemens). Results of the present in-house assay were 100% concordant with the results obtained with the TRUGENE, revealing the accuracy and specificity of our genotyping assay. Sequencing of three new molecular targets of HIV drug therapy - integrase, gp 41 and gp 120 - are being now added to these assay to improve the genotyping and consequently the clinical management of the patients.

B-101

In-house real-time quantitative PCR assay for the diagnosis and monitoring of hepatitis B virus and hepatitis C virus

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Acute and chronic infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) lead to significant mortality and are a major public health problem worldwide. Over 350 million people are chronically infected with hepatitis B virus, and more than 170 million people areinfected with HCV worldwide.According to the World Health Organizationis estimated that 30% of infected patients develop liver cirrhosis and/or hepatocellular carcinoma (HCC).Diagnosis of hepatitis B and C infection isusually done by detection of anti-HBV/or anti-HCV antibodies against recombinant HBV or HCV proteins using enzyme immunoassay (EIA) and chemiluminescence immunoassay in the patient's serum. However, these markers have their own limitations hampering diagnostic accuracy. In order to overcome these problems, some studies reported in the last few years have demonstrated the development of molecular assays for detectionof nucleic acid-based markers related to hepatitis-causing viruses. The aim of the study was to develop anin house real time quantitative PCR (qPCR) assay to quantify the viral load of hepatitis B virus and hepatitis C virus in patient´s serum by using specific primers and TaqMan minor groove binder (MGB) fluorescent probe technology.Nucleic acids were extracted from serumusingQIAamp Virus Spin MiniElute Kit and Reverse transcriptionof viral RNA was performed withHigh Capacity cDNA Reverse Transcription Kit. The amplification reactions wereperformed in a 7500 Real Time PCR System, using the TaqMan detection system with predetermined concentrations of primers and probes,based on the amplification of a conserved region of the HBV and HCV genomes. For standardization and validation of the assay, an international panel of HBV/HCV and standard plasmids was used. A correlation coefficient of 0.98 and 0.96 for HBV and HCV, respectively, was obtained from Ct values and the concentration of DNA or RNA copies. The standard curve showed a linear relationship from 102 to 108 copies/mL of serum, with a coefficient of determination (r2) of 0.99and efficiencies of 90 - 100% for both HBV and HCV. In 116 clinical samples with a range of viral loads, the detection limit was of 103 copies/mL serum for both virus. The results suggest that the assay is suitable for viral quantification of low and high viral loads and that the amplification efficiency is stable over a range of input copy numbers. In conclusion, we developed a novel qPCR assay based on the TaqMan MGB system that is rapid, sensitive, and accurate. This assay, validated with both standards and clinical samples, provides an ideal system for routine diagnosis, monitoring of therapy and to confirm indeterminate serological results, especially in immunosuppressed patients

B-102

Prevalence and antimicrobial susceptibility profile of microorganisms isolated from blood cultures of hospitalized patients from Belo Horizonte/MG, Brazil.

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Background: The bloodstream infections (BSI) are the most relevant infections related to health care due to their high prevalence, high morbi-mortality rates and associated costs. They are among the most frequent and cause the most serious infectious complications of hospitalization and medical care. Regarding the severity and importance of the process, the correct diagnosis of bloodstream infections is extremely important and results in increased patient survival. Thus, the blood culture has significant predictive value when examinaning these infections. Methods: This study was carried out on samples of blood fo 1.394 patientes suspected to have bloodstream infection who underwent examination in laboratory from February 2011 to January 2012.The variables evaluated were the frequency of positivity in the

samples and the sensitivity profile of microorganisms to antimicrobials agents. Three blood samples from each patient were collected by nursing staff and immediately inoculated into blood culture bottles for automated system BACTEC® (BD, New Jersey/USA). Regarding this system, the growth of microorganisms is detected based on the automatic detection of CO2 production in the culture medium bottles with fluorescent sensors. The identification and antibiogram were evaluated by the automated system MicroScan WalkAway® (Siemens, Erlangen-Germany). Results: 225 (16.1%) patients had positive blood culture for microorganisms. 553 microorganisms were isolated from the positive cases. Among patients with positive blood cultures, the mean age was 66.7 years(18-96 years old). The male/female ratio was 1.3 (55.8% male). The microorganisms most frequently isolated during this period were Staphylococcus aureus (n = 103; 18.6%), coagulase-negative Staphylococcus (n = 78; 14.1%), Escherichia coli (n = 60; 10.8%), Pseudomonas aeruginosa (n = 50; 9.1%), Acinetobacter baumannii (n = 39, 7.1%) and Klebsiella pneumoniae (n = 38, 6.9%). Fungi represented approximately 5.5% of the isolates. About the Staphylococcus aureus in blood cultures, 22% were Staphylococcus aureusmethicillin-resistant (MRSA). Regarding these, none was resistant to daptomycin, linezolid or vancomycin. However, among Enterococcus, 41% were resistant to vancomycin but none was resistant to daptomycin and 8% were not sensitive to linezolid. In relation tothe production of beta lactamase extended spectrum (ESBL), 20% of the strains of Escherichia coli (12/60), 38% of Klebsiella spp (16/42) and 39% of Proteus spp (12/31) produced this enzyme. Conclusion: The BSI prevalence data and its etiology should be the more regionalized and updated as possible, so that prevention actions can be more effective and concrete. This study contributed to the knowledge of the microbiological panorama of a large hospital in Belo Horizonte/Brazil, providing very important information for rational use of antimicrobial strategies and reduction of bacterial resistance.

B-103

Development and Evaluation of a Serological Chikungunya Antibody Detection Assay

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Background: Chikungunya (also named breakbone fever) is a highly emerging disease in many tropical settings with great socioeconomic impact. Causative agent for this disease is a single-stranded, enveloped RNA-Virus that belongs to the genera Alphavirus of the togavirus family (Togaviridae). In general the viruses are not transmitted from human to human but transmissions from infected pregnant women to unborn children have been proved. The symptoms of Chikungunya include fever which can reach 39°C (102,2°F) a petechial or maculopapular rash usually involving the limbs and trunk, and arthralgia or arthritis affecting multiple joints which can be debilitating. The symptoms can also include headache, conjunctival injection and slight photophobia. The fever typically lasts for two days and abruptly comes down. However other symptoms, namely joint pain, intense headache, insomnia and an extreme degree of prostration last for a variable period, usually for about five to seven days. But patients have complained joint pain for much longer time period depending on age of the patient. It has been observed that the severity of the disease as well as its duration is less in younger patients and pregnant women. Heavy damages to somebody's health or death are rare. Alphaviruses rarely appear in Europe but can be noticed as import or travel associated infection.

Methods: The aim of this work was to develop an serological assay to detect IgG and IgM antibodies against Chikungunya and to evaluate in endemic outbreak settings.

Results: An IgG-capture and IgM-capture ELISA was developed. Both take advantage of native antigens produced with a proprietary technique which was exclusively developed for this serological antibody detection assay. In house measurements as well as external evaluations in many endemic regions of the world conducted by well know tropical institutes revealed excellent clinical sensitivity and specificity as well as high positive and negative predictive values (all above 95%). Data from the current outbreak in the Caribbean will be discussed.

Conclusion: Therefore the newly developed ELISA seems to be a superior tool to diagnose past and acute Chikungunya infection in common and outbreak settings all over the world. It will assist diagnosis of travel returners with unknown fever as well as military in endemic operation area.

To further improve Chikungunya diagnostic a Lineblot is currently under development as tool for conformation of ELISA results as well as for small labs with limited lab equipment.

B-104

Multiplex sensitive type-specific detection of 14 high-risk HPV strains in a single closed-tube reaction

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Human papillomavirus (HPV) is one of the most common sexually transmitted infections (STI). Some, but not all, types of HPV can cause cervical cancer (high-risk HPV types or HR-HPV). Since early stage cervical carcinomas are nearly 100% curable, early detection is very important. Accurate molecular diagnosis is needed to inform patient management and follow-up treatment. We have developed a unique technology (Multiplex Probe Amplification or MPA) for sensitive type-specific detection of 14 HR-HPV types in a single closed tube reaction. In this study, we analyse the analytical and clinical performance of this novel HPV molecular diagnostics test.

Validation of the MPA HR-HPV assay was initially conducted using plasmids bearing type specific HPV sequence. Data showed that 100% of HPV plasmids were detected and genotyped correctly by MPA real-time PCR assays. Serial dilution of cell line DNA was also performed to evaluate the MPA real-time PCR sensitivity which showed that the assay can detect at a concentration of 10 copies per reaction. A pilot clinical evaluation of the MPA HR-HPV assay was conducted by investigation of HPV genotypes in 30 clinical samples. A comparison of MPA results with commercially available Luminex based genotyping assay showed 76.6% full concordance. Current work is looking at clinical evaluation of MPA HR-HPV assay on a set of 500 clinical samples from a screening population and its comparison with other commercially available clinically validated HPV assays.

In conclusion this study shows that the MPA HR-HPV assay is efficient in combining screening and genotyping of HPV-DNA. The current commercially available probe-based methods are limited to detection of only one target sequence per fluorescence channel. MPA technology overcomes this limitation, allowing 14 targets to be detected and quantified in a single closed-tube reaction. We believe that the MPA HR-HPV assay may offer a simpler and more cost effective means to identify women at risk and optimise treatment strategies.

B-105

Incidence rates of compulsory notification of reportable diseases in three different states of Brazil in 2013 and 2014 according to laboratory tests results

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Backgroud:

In Brazil, reportable diseases are responsibility of the Board of Health Surveillance of the Brazilian Federal Ministry of Health. The occurrence of new cases of a disease (transmitted or not) or injury (unusual or not), that can be prevented and controlled by health services, indicates that the population is at risk and may pose threats to health and need to be detected and controlled still in its early stages. The compulsory notification is the notification of the occurrence of individual cases, clusters of cases or outbreaks, suspected or confirmed, which shall be communicated to health authorities by health professionals or any citizen, aimed at the adoption of the measures relevant control. We present here a summary of the notifications for five main diseases in three Brazilian federation states during the years of 2013 and 2014.

Methods:

According to the federal standards for notification of reportable diseases data from three states were collected in this study: São Paulo, Paraná and Goias. The laboratory tests used for reporting the main diseases were: non treponemal antibodies tests and treponemal antibodies tests (syphilis), Western blot test (HIV), anti-HBc IgM and HbsAg (hepatitis B), Anti-HCV (hepatitis C) and Dengue IgM antibodies tests (dengue fever). Each state had its own data and all information from the years 2013 and 2014 were unified into a common file.

Results:

São Paulo had an average over ten thousand notification per year whereas Paraná counted three thousand and Goiás had a close rate of almost three thousand notification per year. The five main reported disease considering the 3 states were, Syphilis 14588 cases, HIV 4619, Hepatitis C 2727, Hepatitis B 2148, Dengue fever 1333.The number of tests processed for these five different diseases during this two years period were:

Syphilis - 1,289,076; HIV - 10,994 Western blots, realized for 1,238,128 samples; Hepatitis C - 1,339,793; Hepatitis B - 1,324,068; and Dengue fever - 15,384.

Conclusion:

Syphilis was the most reported disease in the three states, with the highest positivity rate, followed by HIV, Hepatitis C, Hepatitis B and Dengue fever. The physician that receives the tests results ad integrate them with the clinical data is the most reliable source to notificate the reportable diseases. But large labs as DASA, that have access to millions of samples, can provide very useful information to the sanitary and surveillance authorities.

B-106

Blood culture candida isolates in tertiary public hospitals in southeast brazilian region

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Background: Candida is an opportunistic pathogen that affects high-risk patients who are either immunocompromised or critically ill and an increasing cause of bloodstream infection (BSI). Candida spp is currently between the forth and the sixth most common nosocomial bloodstream isolate in international studies and is associated with almost 80% of all nosocomial fungal infections. Candida albicans is the main cause of candidemia, but other species with more reduced susceptibility to antifungal agents has emerged as common pathogens. Importantly, identification of Candida isolates helps in selection of effective antifungal therapy.

Objective: The aim of this four-month observational retrospective tudy was to evaluate the distribution of Candida species of candidemia from eleven tertiary public hospitals in Brazil.

Methods: from October 2014 till January 2015 all cultures originated from blood specimens were processed in accordance with NCCLS - National Committee for Clinical Laboratory Standards - and incubated in Bactec (Becton Dickinson Inc.). Positive samples were submitted to identification including Gram stain and sowing in chromogenic medium (chromIDTM Candida, bioMérieux) for fungal isolation and identification of Candida albicans and other medically relevant candida species. The statistical analysis was performed with SPSS v18.0 software (IBM).

Results: During the study period were analyzed 2,710 samples and blood cultures positive for fungi was observed in 5.5% (149/2710) of the samples. The average time for positivity was 27 hours and the majority of patients were concentrated in two age groups (p<0,001): infants to 1 year (37.6 %) and above 60 years (24.2 %). Candida non-albicans was the more frequent isolates (59.1%) without significant distribution among month (x2= 2,132; p=0.547).

Conclusion: our study confirmed data from previous studies that demonstrated high prevalence of BSI by Candida spp in Brazil caused by species other than C. albicans, and shows that Candida non-albicans was the main isolated agent in nosocomial fungal infections. Use of chromogenic medium allowed a rapid identification that is important to effectiveness of fungal therapy.

B-107

Investigation of epidemiological links of four Mycobacterium abscessus (sensu stricto) strains by Multilocus Sequence Typing

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Background: Between 12 and 21 July 2013 , four rapidly growing mycobacteria (RGM) strains were isolated in our hospital. To investigate epidemiological links of the four strains, we conducted a molecular analysis of the four isolates.

Methods: Four isolates were identified by sequencing analysis of partial hsp65 and rpoB gene sequences. Minimum inhibitory concentrations (MICs) of antibiotics were determined by E-test method. Additionally, four isolates were analysed by multilocus sequence typing (MLST).

Results: The four isolates were identified as Mycobacterium abscessus (sensu stricto). Identical sequence types (ST1) and antimicrobial susceptibility patterns were identified for the two isolates from blood samples of a neonate who was later diagnosed with catheter-related bacteraemia. Two isolates from bile samples of two adult patients had identical multilocus sequence typing (ST49) and antimicrobial susceptibility patterns and the patients were lack of distinct clinical manifestations, suggesting a pseudo-outbreak.

Conclusion: Our report comfirms that multilocus sequence typing is a very robust technique to validate molecular epidemiological links among Mycobacterium abscessus isolates.

B-108

Different Characteristic of Cryptococcal Meningitis between HIV-infected and HIV-uninfected Patients in Southwest China

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Background: Cryptococcus is the major pathogen which causes the fungal meningitis. The previous studies about cryptococcal meningitis have mostly focused on the HIV-infected patients.In China, especially in the southwest area,cryptococcal meningitis in HIV-negative patients is found more common than in HIV-positive patients.In order to provide the earlier diagnosis clues of cryptococcal meningitis with different immune state to the clinicians,we compared their clinical features and laboratory data of cryptococcal meningitis.

Methods: Demographic data,clinical manifestations and laboratory data from inpatients in our hospital were collected from June 2009 to June 2014.Continuous data were presented as mean and standard deviation or median and inter-quartile range. Continuous variables were calculated by the Student's *t* test or Wilcoxon rank sum tests.Categorized variables were analyzed by the Chi-squared test.A p value <0.05 was considered statistically significant.

Results: There were total 85 patients with cryptococcal meningitis were identified,Among them, 53 (62.4%) were HIV-negative patients.There were no significant differences in ages between the two groups (38.56 vs. 42.03,P=0.112). Comparing with the HIV negative group,cryptococcal meningitis occurred in male (90% vs. 67.9%,P=0.017) more frequently in HIV-positive group.On the other hand,HIV-negative patients tend to have at least one of the underlying disease (37.7% vs. 15.6%,P=0.025).Two group showed no significant differences in initial symptoms,most of the patients were acute onset. Headache (96.2% vs 96.9%) was the predominant symptoms of admission to the hospital.Compare with the HIV-positive patients,HIV-negative patients had higher numeration of leukocyte in blood (median, 9.15 vs. 5.37,P<0.001),higher numeration of leukocyte(median,80 vs. 5,P<0.001) and protein (median,0.9 vs. 0.55,P<0.001) in the cerebrospinal fluid (CSF).More HIV-negative patients with increased CSF/serum albumin ratio (Q_{ALB}) (85.7% vs. 55.5,P <0.001),at the same time intrathecal immunoglobulin G (IgG) synthesis in the HIV-negative group were significantly increased (median,52.81 vs. 6.9,P <0.001).

Conclusions: There are some differences between the two groups, comparing with HIV-negative patients,lower leukocyte and protein in the CSF,lower local synthesis of IgG level in the central nervous system demonstrated its low level of immune response in HIV-positive patients.These characteristics should be noticed to avoid missed diagnosis.High intensity of inflammation of HIV-negative cryptococcal meningitis patients should be differentiated with other septic meningitis.Different drug dosage and course of treatment should be used in the two group patients in consideration of the different state of the a blood-CSF barrier (BCB) permeability to achieve a better outcome.

B-109

Seroprevalence of HIV I and II in blood samples processed in a clinical pathology reference laboratory in Brazil between 2008 and 2010.

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Background:The epidemic of infection with human immunodeficiency virus (HIV) is a global, dynamic and unstable phenomenon, and thoroughly discussed by the scientific community and society in general. This disease is considered the first great pandemic of the second half of the twentieth century and was first described in 1981. In Brazil, the first cases of AIDS were reported in 1987. The transmission, as far as we know, is through contact of mucous with body fluids, such as blood or blood products, semen and breast milk.

Objective: We aimed to statistically analyze the serological results of HIV I and II processed at Hermes Pardini Institute (IHP) in the 2008 - 2010 period and describe the epidemiological characteristics of HIV-positive patients.

Method:This was a retrospective study, carried out through consultation of laboratory test results stored in IHP web LIS. All results of an HIV I and II obtained and released from January 2008 to December 2010 were compiled. Epidemiological data such as gender, age and region of the country of HIV-positive patients were statistically analyzed and compared with national data from the Ministry of Health (MH) - Epidemic Update 2011.

Results: We evaluated 816,922 results from all over the country between 2008 and 2010. There was a predominance of patients from the Southeast region (59.5%), home of the laboratory, followed by the Northeast (21.1%), North (8.2%), South (7.8%) and Midwest (3.4%). The annual rates of seropositivity for anti-HIV I and II were 1.52% (2008), 2.32% (2009) and 1.11% (2010). Among HIV-positive cases, 8.2% were children (under 20 years), 88% adults (between 20 and 60 years) and 3.7% elderly (over 60 years). There was a male predominance in adults and the elderly patients. In the pediatric population, the positivity was higher in women. In the Southeast and Midwest, serology was mostly positive in men while in the north, northeast and south, the positivity was higheramong women. Comparing the results from IHP with the data from the MH, there was epidemiological similarity concerning gender and age of seropositives.

Conclusions: The findings of this study allow us to infer that the epidemiological profile of seropositive for anti-HIV I and II antibodies assisted by IHP reflect the Ministry of Health data regarding the sex and age of the Brazilian population. The differences found in relation to regionalities may be caused by the IHP assistance coverage throughout the national territory.

B-110

Distribution of nearly 100,000 positive urine culture performed in Brazilian private hospitals

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Background:

Urinary tract infections (UTIs) represent the second most often observed infections in outpatients, and the most frequent infection among inpatients. UTIs are more frequent in female than male patients, particularly between 20 to 60 years-old. Complicated urinary tract infection occurs in women or men of all ages with functional or structural abnormalities of the urinary tract. The UTI diagnosis is based on medical history, urinalysis and the bacteriological urine culture with identification of the causative agent. The most common bacterial cause of uncomplicated community-acquired UTI is *Escherichia coli*. In nosocomial UTIs, the most frequent pathogens are *Proteus spp*, *Klebsiella spp*, *Enterobacter spp*, *Serratia spp, Pseudomonas spp*, *Enterococcus spp*, and *Candida spp*.

Objective:

To evaluate the culture of urine samples from patients attending 10 private hospitals in São Paulo, Brazil, and analyzed the microorganisms distribution according to sex and age.

Methods:

The study included samples from patients attending ten private hospitals of São Paulo, the largest city of Brazil. Retrospective analysis of all urine samples collected in 2013 and 2014 from inpatient and outpatients for bacteriological cultures were performed. All urine samples were smeared in the hospital where they were collected and, afterwards, sent to analysis in a central microbiology laboratory to be processed. Positive urine culture was defined as culture with >100,000 CFU /ml.

Results:

During the timeline of the study, 18,698 (19%) of a total of 97,962 urine cultures were positive; the proportion of positive culture among women was higher (23%) than among men (12%) ($p<0,001$). The percentage of positivity was 7% among < 1 year-old patients, 10% among 1 to 19 year-old ones, 23% among patients 20 to 60 year-old, and 26% in over 60 years-old. The female-to-male rate was higher in 20 to 60 years-old patients (6,5), than 1 to 19 year-old (4.5), < 1 year old (1.5) and over 60 years-old patients (2.0).

Gram-negative bacteria represented 85% of total of isolated microorganisms and *E. coli* was the most prevalent isolated microorganism. The most prevalent microorganism among female were *E. coli* (62%), *K. pneumoniae* (9%), *P. mirabiis* and *S. saprophyticus* (5% each). Among male, the most prevalent microorganism were *E. coli* (35%), *K. pneumonia* (14%), *P. aeruginosa* (13%), and *P. mirabilis* (9%). *C. albicans* was responsible for 2% of total UTIs, with no difference between sex.

Conclusion:

Although females are more prone to infection than males, the female-to-male rates are lowest in the extremes of age. The percentages of positive urine culture increase by age, from 7% among young than1-year-old to 26% among older than 60 year-olds. The distribution of the microorganisms varied according age and sex. The distribution of strains are in agreement with recent studies where Gram-negative microorganism being represented by the *Escherichia coli*.

B-111

Epidemiology of Respiratory Virus among Patients Attending Private Hospitals in São Paulo, Brazil.

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Background:

The respiratory viruses are the main cause of acute respiratory infection and are responsible for high levels of morbidity particularly in children, elderly people, and persons with comorbidities and immunosuppressive conditions. Most respiratory viruses present with similar symptoms, making a diagnosis difficult without laboratory testing. Although rapid antigen testing can offer quick results, the specificity and sensitivity of this testing vary greatly. Molecular biology techniques are capable to detect a panel of respiratory virus with a higher sensitivity.

Objective:

The aim of this study is to evaluate the distribution of respiratory viruses isolated during 2014 in patients from São Paulo hospitals and the seasonality pattern.

Methods:

Records from respiratory virus panel test results performed in 2014 were evaluated. Respiratory viruses were investigated from nasopharyngeal swabs of patients attending private hospitals in São Paulo city, Brazil. The exams were indicated based on attending physician decision in order to establish the etiology of respiratory infections. The test utilized was RT-PCR Microarray CLART® Pneumovir detecting 18 respiratory virus: Influenza A (H3N2, H1N1, H1N1pdm 2009, Influenza B and Influenza C, Parainfluenza (PIV) 1, 2, 3 and 4, Syncycial Respiratory Virus (SRV) A and B, Rhinovirus (HRv), Adenovirus (Ad), Bocavirus (HBoV), Metapneumovirus (HMPV), Coronavirus (CoV), and Enterovirus (Echovirus, Cosackievirus A and B).

Results:

Respiratory virus was detected in 1,689 (66%) of 2,569 nasopharyngeal swabs samples analyzed. A total of 528 samples identified more than one respiratory virus from the same patient specimen: 436 detected two, 79 detected three, 12 detected four, and one sample detected five respiratory viruses. A total of 2,323 respiratory viruses were identified. The most frequent respiratory virus detected were SRV (A and B, 27%), HRv (24%), HBoV (12%) and HMPV (9%). Results for both gender and all age groups were similar for all respiratory virus detected. The positivity of the total samples was higher in the fall (35%) and lower in the summer (17%) period, a difference not statistically significant. All respiratory viruses were also detected more frequently in the fall, with a difference not statistically significant, compared to other seasons.

Conclusion:

During the study period, the majority of patients submitted to laboratory investigation for respiratory infection, in private hospitals, had at least one respiratory virus detected in nasopharyngeal swabs. Non-influenza respiratory viruses are more important and the present study highlight the contribution of molecular methods used to detect respiratory viruses in the epidemiological knowledge and management of patients with respiratory disease.

B-112

Genotypic resistance profile of nucleos(t)ide analogue-treated chronic hepatitis B patients in Western China

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Objectives: Chronic hepatitis B (CHB) infection remains a worldwide health problem and it is highly prevalent in China. There weren't insufficient data reflecting whether different HBV genotypes affect the incidence of antiviral drug-resistant mutations. In this study we systematically investigated CHB patients in Western China to determine the prevalence of HBV genotypes, clinical characteristics and drug-resistant mutations as well as the possible relationships among them.

Methods: A total of 639 Chinese patients with CHB infection were enrolled. HBV genotypes and reverse transcriptase mutations were determined by direct sequencing.

HBV serological markers were evaluated by Enzyme-linked immunosorbent assay and HBV DNA was quantified by real-time PCR.

Results: Genotypes B, and C were detected with percentages of 64.0% and 36.0%, respectively. No significant difference referring the serological index was observed between two genotypes (P > 0.05). In this cohort, 47.6% of them harbored nucleos(t)ide analogues resistance, and lamivudine-resistance was the highest (44.0%), followed by telbivudine-resistance (38.2%). The most common pattern was M204I alone (31.91%, 97/304), then followed by L180M+M204M (12.5%, 38/304) and L180M+M204I (6.58%, 20/304). L180M+M204I, A181T and N/H238T appeared more common in genotype C (p=0.026, 0.014, 0.018, respectively), while M204I and N236T pattern occurred more frequently in genotype B (p=0.003, <0.001, respectively). There was no significant difference between mutant models and the levels of HBV-DNA in total cases, however, among the patients with the mutant of L180M+M204V, patients with genotype B had higher HBV-DNA levels than those with genotype C.

Conclusion: Western China has a serious epidemic of drug resistance that nearly half of CHB patients harbor complicated nucleos(t)ide analogues-resistant mutations. HBV genotype does not influence the pre-treatment viral factors or viral-host interaction but imply a potential effect on modulating resistance development under nucleos(t) ide analogues-treated pressure. Drug-resistance test before treatment is important to approach an optimal CHB therapy.

B-113

Correlation between Antifungal Treatment and Galactomannan Antigen in Adult Hematologic Patients at Risk for Invasive Aspergillosis

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Background:

To analyse the correlation between antifungal treatment and galactomannan antigen in adult hematologic patients at risk for invasive aspergillosis (IA) together with the results of serial serum Aspergillus galactomannan (GM) antigen testing.

Methods:

In a retrospective study for patients at high risk of aspergillus pulmonary infection, serum GM testing was used to detect GM concentration 2-3 times/week during the periods of high risk for IA. High-resolution CT was performed in case of abnormal chest X-ray and/or persistent fever after 5 days of antibiotic treatment. IA was classified as either “proven” or “probable” in accordance with the definitions stated by EORTC-MS.

Results:

A total of 82 hematological patients were diagnosed of “proven” (n=1),and “probable” IA(n=28) , and “possible” (n=23) and “No” IFI(n=30) .The sensitivity of the Dynamiker’s GM test was 84.6%, and the specificity 81.3%. The false positive rate was 18.8%, the false negative rate 15.4% and the diagnosis rate 82.8%. This group of 82 patients received prophylaxis fluconazole treatment at a median of days 19.7 (range 9- 26).

Conclusion:

Dynamiker’s serum GM test could be taken 2-3 times/week in adult hematologic patients at risk for IA. The GM value was correlated to the amount and the fungal load in patients. The GM test is also earlier than the conventional CT or chest X-ray scan.

B-114

Cytomegalovirus and Neonatal Screening: A protocol adaptation of ELISA kit for the detection of IgM antibodies in total blood collected on filter paper.

C. M. M. Oliveira, I. Bendet, M. C. Silva, D. S. Poleto, S. L. V. Argolo, C. F. d. Pereira. *DASA, Niterói-RJ, Brazil*

Congenital CMV infection, which occurs in 0.2 to 1% of live births worldwide, may result from transplacental acquisition of either a primary or recurrent maternal infection. Clinically apparent disease in the neonate is much more likely to occur after a primary maternal exposure, particularly in the first half of pregnancy. In some higher socioeconomic groups in the US, 50% of young women lack antibody to CMV, making them susceptible to primary infection.

This study is aimed to evaluate the use of a commercial kit with the ELISA methodology for the detection of IgM antibodies for cytomegalovirus (CMV), in blood samples from newborns, collected on filter paper. With this objective, we performed the adaptation of the original protocol of kit ETI-CYTOK-M reverse PLUS Diasorin, indicated for the qualitative determination in human serum or plasma of IgM antibodies for CMV. The modification consisted of extracting 2 spots of filter paper containing blood of a newborn, directly to each microplate and making the elution of the total blood as a previous step before the original procedures described on the test datasheet.

The validation process used 40 samples, 11 with expected positive results, and showed a 95% match with a Kappa coefficient of 0.88. This ELISA Kit was adopted in the routine neonatal screening in filter paper. Of the 13,011 samples of total blood on filter paper, from different regions of Brazil, between January and December 2014, 12,968 (99, 66 %) were not reactive and 43 (0.33 %) were reactive. We conclude that the ELISA kit ETI-CYTOK-M reverse PLUS Diasorin, with modified protocol, for qualitative determination of IgM antibodies for CMV in blood collected on filter paper, is appropriate as an initial method of research for CMV infection in neonatal screening.

B-115

Influenza Virus in Long-Term Care Facilities

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Background: Influenza is an infection caused by influenza virus; they are three types A, B, and C; both A and B can be responsible for an epidemic disease. Influenza is a serious condition in Log-Term care Facilities where most of the residents are elderly, frail, disable, and are on multiple medications. Preventing transmission of influenza include: vaccination, testing, infection control, and appropriate treatment.

Design: 1,545 nasal swabs were collected from residents in Long-Term Care Facilities from November 2014 to February 2015. Tests were done using Binax rapid influenza diagnostic test (RIDT); the assay is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect influenza type A and B nucleoprotein antigens in respiratory specimens; the assay has 83% sensitivity and 96% specificity for influenza A, and 69% sensitivity and 100% specificity for influenza B. Statistical analysis was done using Analyse-it.

Results: 148 swabs were positive for influenza A and 5 swabs were positive for influenza B; we notice an increase in percentage positive test over the period tested with the highest prevalence in February. Only one patient needed hospitalization and no dearth has been reported.

	November	December	January	February
Total	6	224	926	389
Influenza A	0.0%	8.5%	9.3%	11.1%
Influenza B	0.0%	0.0%	0.4%	0.3%

Conclusion: influenza activity has been noted since December; the increase in the percentage positive results were higher in February may be due to testing patient with suspected influenza whereas in January it was done to screen the residents when one patient is presenting with influenza-like symptoms. Residents in Long-Term care Facilities has lower hospitalization and complication compared to national published data; which might be due to the higher vaccination rate to all residents and workers against the flu every year, early detection and administration of antiviral chemoprophylaxis to all residents as soon as they have an outbreak will prevent the complication, and the availability of infection control to prevent the spread of the illness.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Lipids/Lipoproteins

B-116

The role of lipid free apolipoprotein A-I self-association in the formation of high density lipoproteins

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Background: Apolipoprotein A-I (apoA-I), the main protein constituent of high density lipoprotein (HDL), forms a dimeric antiparallel structure that both confers nascent HDL stability and provides a scaffold for the lipid phase. While earlier studies suggest a compact, helical bundle structure for the lipid free apoA-I in solution, the mechanism of how monomeric lipid-poor apoA-I self-associates to form nascent HDL is not known. Here, we present kinetic analyses of monomeric and oligomeric apoA-I by employing both hydrogen-deuterium exchange mass spectrometry (HDX-MS) and functional lipidation assays to determine the effects of self-association on apoA-I dynamics and HDL formation.

Methods: Concentration dependence of apoA-I self-association is assessed by crosslinking SDS PAGE gel by using bissulfo-succinimidyl suberate (BS^3) as the crosslinker. Different concentrations of lipid free apoA-I was subjected to HDX at 0 °C in an ice/water bath at a pH of 6.8 (pD = 7.2) in PBS containing 90% deuterium oxide (D_2O) for various time intervals. After a proteolytic digestion using either pepsin or fungal protease XIII, the solution was injected into the LC-MS system consisting of binary LC20 HPLC pumps (Shimadzu, Kyoto, Japan) and an LTQ Orbitrap Velos Mass Spectrometer (Thermo Scientific, Waltham MA). The deuterium incorporation of the digested peptides were identified by manually analyzing the MS spectra. The deuterium incorporation values were then used to calculate the HDX kinetic rate constants of digested peptides. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) vesicles were incubated with lipid free apoA-I samples and the decrease in turbidity followed at 325 nm to compare the lipid clearance activity of the protein at different concentrations.

Results: At low concentration (0.07 mg/ml) lipid-free apoA-I is monomeric based on equilibrium PAGE, dynamic light scattering, and cross-linking studies. Nevertheless, the protein displayed bimodal HDX kinetics in multiple regions. Kinetic HDX studies further revealed that at higher concentration (0.7 mg/ml), a new HDX pattern appears for a small ten amino- acid apoA-I C-terminus domain (E_{223}-A_{232}). Lipid clearance kinetics studies showed that monomeric lipid free apoA-I interacts with DMPC vesicles at a faster rate than the multimeric protein.

Conclusion: The combination of unimodal and bimodal HDX kinetics indicates the presence of conformational dynamics in monomeric lipid-free apoA-I in solution. The emergence of new patterns of deuterium incorporation also suggests new intermolecular interactions and changes in secondary structure surface at higher apoA-I concentrations. Surprisingly, apoA-I self-association significantly slowed down the rate of apoA-I lipidation shown by the lipidation assay. Our studies collectively indicate that HDL genesis starts with lipidation of apoA-I monomers followed by apoA-I dimerization in an antiparallel fashion on the lipid surface.

B-117

Quantitative Characterization of Protein Content from HDL and LDL Size Fractions

B. A. Parks, Z. Kuklenyik, D. Schieltz, M. S. Gardner, J. Rees, L. McWilliams, Y. Williams, J. R. Barr. *Centers for Disease Control and Preventiron, Atlanta, GA*

Background:There is building evidence in recent years that measurement of LDL and HDL particle number concentration (LDL-P and HDL-P) in serum provides significant improvement in cardiovascular disease (CVD) risk assessment relative HDL and LDL cholesterol (HDL-C and LDL-C). The higher relative CVD risk associated with HDL-P and LDL-P is due to the particle size heterogeneity of both HDL and LDL. In spite of being potentially stronger risk factors, the more general use of clinical assays for quantification of particle number, particle size and other emerging lipoprotein associated protein/lipid biomarkers is impaired by lack of satisfactory inter-laboratory comparison and standardization. For this reason, we seek to develop a potentially gold standard method by preparatively separating lipoprotein sub-classes based on hydrodynamic size, coupled with quantitative protein/lipid measurements using isotope dilution mass spectrometry (IDMS).

Methods:A volume of 50 µL of human sera was separated by asymmetric field flow fractionation (AF4) into 20-40 fractions in a hydrodynamic diameter range of 5-45 nm in phosphate buffered saline. Size information was determined by dynamic light scattering measurements in each fraction after collection. An aliquot of each fraction was batch digested with trypsin. An additional aliquot was used for simultaneous basic hydrolysis of cholesteryl esters and triglycerides. Fast IDMS methods were developed for the high throughput quantitative analysis of all digested and hydrolyzed AF4 fractions and diluted serum aliquots, 3 min IDMS run for cholesterol and glycerol, and 8 min run for protein specific peptide cleavage products.

Results:Based on AF4 fractionation, size measurement, and IDMS analysis, highly selective concentration versus hydrodynamic size profiles were constructed for cholesterol, glycerol and apolipoproteins. Individual protein species profiles had a 2.5 nm half peak width on the hydrodynamic size scale allowing differentiation of 5 ApoA-I and >5 ApoB-100 containing subclasses using profile deconvolution, in the range 7-17 nm and in the 18-45 nm range respectively. The accuracy and precision of the concentration measurements of ApoA-I and ApoB-100 were evaluated based on currently used primary and secondary serum reference materials. Our IDMS method matched the assigned concentrations of ApoA-I and ApoB-100 with 95% accuracy and ~8% CV. This work has been expanded to the quantitative measurement of >10 proteins associated with ApoA-I and ApoB-100 containing sub-species based on size profile overlap in multiple serum samples with wide range of cholesterol and triglyceride levels. ApoA-II, ApoA-IV and ApoM were uniquely distributed in ApoA-I containing sub-fractions. ApoC-II/, ApoC-III and ApoE could be measured in both large size ApoA-I and small size ApoB-100 containing sub-fractions. A significant concentration of ApoE was in ~18 nm particle size fractions where it did not correlate with ApoA-I or ApoB-100 subclass concentration. The apolipoprotein profiles varied significantly between samples with low and high total cholesterol/triglyceride serum levels (low vs. high CVD risk samples).

Conclusions:We demonstrate an advanced lipoprotein particle sizing and sub-class particle number analysis approach which has the specificity, accuracy and precision to directly measure both size and composition of lipoproteins, in a manner that is traceable to universal calibrators.

B-118

A serum oxidized high-density lipoprotein marker and its association with metabolic syndrome in males

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Background: A high-density lipoprotein (HDL) particle, whose major protein is apolipoprotein A-I (apoA-I), plays relevant roles in cardioprotection with its antioxidant properties. The oxidative modification of apoA-I can be involved in dysfunctional HDL. Metabolic syndrome (MetS), a cardiovascular risk factor and an oxidative stress condition, is often accompanied by low HDL levels in the circulation. However, the cardiovascular burden of MetS is still not well understood with regard to dysfunctional HDL, and easy biomarkers for MetS-related HDL modifications are also needed. We developed a new assay to measure oxidized apoA-I, a suitable biomarker for oxidized HDL (oxHDL), since we found high oxHDL levels under several oxidative stress-related conditions. The aim of this study was to investigate the association between the oxHDL levels and the MetS status in males.

Methods: A total of 269 Japanese males (mean age, 61 years) were consecutively recruited from general health checkups. Any subjects who had been treated for cardiovascular diseases were excluded. Clinical data, including the waist circumference, blood pressure, and serum lipid and glucose levels, were obtained from the subjects in a fasting state. The serum oxHDL levels were quantified using a sandwich ELISA system, which utilizes monoclonal antibodies prepared by immunization with H2O2-oxidized human apoA-I. The presence of MetS was diagnosed by ≥ three of five metabolic criteria (including ≥ 85 cm of waist circumference for Japanese males).

Results: There were 77 subjects with MetS. The subjects with MetS showed a significantly lower level of mean HDL-cholesterol (1.27 mmol/L) than those without MetS (1.55 mmol/L, $p < 0.05$). The subjects with MetS showed a significantly higher level of the mean oxHDL/HDL-cholesterol ratio, an index of HDL oxidation, than those without (5.3 versus 4.3, respectively, $p < 0.05$). Additionally, a significant stepwise increase of the oxHDL/HDL-cholesterol ratio with an increase in the number of MetS criteria ($r = 0.2$, $p < 0.01$) was found.

Conclusion: The present findings suggest that MetS may oxidatively modify HDL particles, thereby leading to dysfunctional HDL in males. The oxHDL/HDL-cholesterol ratio may therefore be useful for assessing the cardiovascular burden in relation to the MetS status.

B-119

Standardized NMR Spectroscopy for Metabolic Profiling in Clinical Diagnostics and Life Science Research

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Background

NMR spectroscopy is a powerful method for metabolomic profiling of human body fluids such as serum and urine. In lipoprotein profiling NMR has proven its unique potential for structural analysis of the complex lipoprotein particles, giving access to lipoprotein particle numbers and sizes as well as lipid concentrations.

Despite its potential and distinct advantages compared to other diagnostic systems, advanced lipoprotein testing by NMR spectroscopy has so far only partly found its way into routine diagnostics. The lack of accessibility and standardization, the complexity of the system, as well as long-term spectrometer drifts and insufficient comparability of different devices are reasons for this situation.

Here we describe a robust and highly standardized measurement process which overcomes individual spectrometer variability and other contributors to variation to achieve consistently reliable results even on different devices. According to a high level of automation and standardization, NMR spectroscopy now becomes available for routine diagnostics.

Methods

Our method provides particle concentrations and subfractions, mean particle sizes, cholesterol concentrations in fractions and subfractions, standard lipid parameter (total cholesterol, triglycerides, LDL-C, HDL-C) as well as metabolic parameters such as glucose, lactate, alanine and other amino acids.

Lipoprotein analysis was conducted via deconvolution of the broad methyl group signal at about 0.90.8 ppm utilizing fourteen base functions. In this process, lipoprotein subclasses are reflected by this fixed number of predefined bell shaped base functions, each of which has a constant position and defined width. From the resulting fit parameters we compute the integrals attributable to each base function. NMR lipoprotein profiling is then carried out by finding an optimal linear combination of these base functions that deviates from the originally measured methyl NMR signal as little as possible. To assess the lipid concentrations, a conversion step is necessary to weight the achieved integrals of the base functions based on their contribution to the total signal fit.

Results

Based on our unique calibrator system, we end up with excellent precision, i.e. a mean total imprecision between 3 different systems of 3.7%, a repeatability of all within-runs < 4.5%, and a reproducibility between 3 different sites <5.1%. Thus, according to the high degree of standardization and automation, it is possible to acquire several hundred serum spectra per day on a single instrument in routine operation.

Conclusion

We have demonstrated that our automated NMR approach is capable of creating a standardized, stable and reproducible spectrum output, independent of spectrometer instabilities or differences, and thus is suitable for routine diagnostics. With respect to lipoprotein profiling our approach may be used for diagnostic tests to identify patients at risk for CVD, predict and monitor the course of the disease or monitor treatment efficacy. Furthermore, the approach can be used in Life Science Research for biomarker discovery, validation and development of new diagnostic tests

B-120

A comparison of lipoprotein associated phospholipase A2 and high sensitive C-reactive protein levels between diabetic and non-diabetic patients with coronary artery disease

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BACKGROUND : Lipoprotein-associated phospholipase A2 [LpPLA2] is known as an emerging marker of coronary artery disease (CAD). However its role and levels have not been documented clearly in diabetic patients with CAD in Indians. The aim of this study was to explore the association of LpplA2 levels between diabetic and non-diabetic patients with CAD and compare it with other established markers like hs-CRP.

METHODS:

Sixty individuals with angiographically proven CAD and 30 healthy individuals matched for age & sex were studied. CAD patients were divided into two groups based on presence (n=30) [Group I] and absence (n=30) [group II] of type 2 diabetes mellitus (DM). The serum levels of LpPLA2, hs-CRP were measured by ELISA. Angiographic clinical vessel scoring was also done for all the patients.

RESULTS:

Both groups of CAD with and without DM had significantly higher levels of LpPLA2 (Group I-408.48 +/- 38.96 ng/ml, Group II 272.88 +/- 34.21ng/ml respectively) and hsCRP (Group I-10.61 +/- 1.34 mg/l, Group II-5.75 +/- 2.59 mg/l respectively) when compared with healthy control subjects (LpPLA2 =200.82 +/- 20.97ng/ml & hsCRP=1.89 +/- 1.34mg/1) [p<0.001]. LpplA2 levels between the two CAD groups were highly significant($p<0.001$), levels being maximum for CAD with type 2 diabetes (Group I) which could be due an increase in its substrate sLDL and oxidised LDL in DM. Angiographic clinical vessel score of CAD severity was also higher in CAD with DM. LpPLA2 levels correlated strongly ($r=0.763, p<0.001$) with the angiographic clinical vessel score in diabetes patients with CAD while hsCRP has moderate correlation with the veesel score ($r=0.475$, $p<0.01$).

CONCLUSION:

LpPLA2 and hsCRP elevation is increased with patients of type 2 diabetes mellitus with CAD as compared to only CAD patients. Measurement of LpPLA2 may be considered as a marker for better prediction of cardiovascular risk in diabetes patients.

B-121

The LDLR variant c.1426C>T; p.P476S as a novel cause of Familial Hypercholesterolaemia

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Objective

The aim was to identify the cause of a raised serum cholesterol concentration of 8.8 mmol/L in a 26 year old male.

Methodology

On clinical examination there was no evidence of tendon xanthomas nor a family history of hypercholesterolaemia or premature death from vascular disease. The patient did not meet the Simon Broome criteria for Familial Hypercholesterolaemia (FH) and had a BMI of 31.5 kg/m^2. Lifestyle modification was suggested.

The following year a younger brother presented with a raised cholesterol concentration of 9.6 mmol/L. He was screened by fluorescent sequence analysis of the promoter and coding sequence (including intron-exon boundaries) of the LDLR gene, and for PCSK9 (exon 7) and ApoB (part of exon 26). MLPA analysis was also carried out for the LDLR gene. The variant c.1426C>T;p.P476S within exon 10 of the LDLR gene was identified. Although this mutation has been reported in one paper as a cause of FH, computer predictions are equivocal. The mutation was reported as possibly pathogenic and may be consistent with the diagnosis of FH.

Fluorescent DNA sequence analysis identified the same LDLR variant in the patient, their mother and a sister. The mother had a cholesterol concentration of 7.8 mmol/L and the sister had a cholesterol concentration of 9.1 mmol/L. The variant was absent in the father who had a cholesterol of 6.6 mmol/L. The mutation therefore does co-segregate with raised cholesterol concentrations within the family and the diagnosis of FH was given.

Outcome

LDL receptor mutations are the most common genetic defect and the prevalence of heterozygous FH in the UK population is estimated to be 1 in 500. The LDLR variant c.1426C>T;p.P476S is a novel mutation and the cause of the families raised cholesterol. Atorvastatin was prescribed to lower the cholesterol and due to the 50% risk of transmitting the variant to offspring the siblings have been referred for genetic counselling.

B-122

Large HDL-Cholesterol Concentrations Predict Long-term Outcomes in Patients with Acute Coronary Syndrome

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Background: HDL-cholesterol is an important independent predictor of atherosclerosis, however, it remains to be determined how HDL subfractions are associated with long-term cardiovascular events in patients with acute coronary syndrome (ACS).

Methods: Concentrations of cholesterol and apolipoproteins (Apo) in large (HDL2) and small (HDL3) HDL fractions separated by heparin-Mn and dextran sulfate precipitation methods were measured at the onset of ACS in 146 patients (119 men), aged 64.3 +/- 11.8 years. Cardiovascular events defined as death, ACS, stroke, heart failure requiring hospitalization and/or any revascularization were evaluated during 6-year follow-up periods.

Results: Not HDL-cholesterol, ApoA1, HDL3-cholesterol, or HDL3-apoA1 but lower levels of cholesterol and apoA1 in HDL2 were associated with higher incidence of cardiovascular events (p = 0.045 log-rank 4.037).

Conclusion: Measurement of HDL2 fractions is an important strategy to detect high-risk patients with ACS.

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Carotid artery IMT is more closely related to small dense low-density lipoprotein cholesterol concentrations than other lipid parameters

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BACKGROUND: Small dense low-density lipoprotein cholesterol (sdLDL-C) concentrations correlate more strongly with coronary heart disease than total LDL-C and large LDL particle concentrations. We investigated the association between carotid artery intima-media thickness (IMT) and sdLDL-C concentrations in Japanese subjects.

METHODS: Carotid artery IMT, blood pressure (BP), fasting plasma sdLDL-C, glucose metabolism, lipid, and C-reactive protein concentrations were measured in 97 native Japanese subjects. Carotid artery IMT was assessed by ultrasonography, and sdLDL-C concentrations were measured by a homogenous assay (Denka Seiken Co., Ltd.). Pearson's correlation coefficient analyses and multiple regression analyses were used to examine the relationships between carotid artery IMT values, sdLDL-C values, and other clinical variables.

RESULTS: After multiple regression analysis, including age, sex, body mass index, systolic BP, diastolic BP, fasting plasma glucose, HbA1c, estimated glomerular filtration rate (eGFR), total-cholesterol, high-density lipoprotein (HDL)-C, triglyceride, LDL-C, non-HDL-C, large buoyant LDL-C, and sdLDL-C, carotid artery IMT remained significantly associated with age, systolic BP, diastolic BP, and sdLDL-C, whereas sdLDL-C remained significantly associated with age, total-cholesterol, HDL-C, triglycerides, and carotid artery IMT.

CONCLUSION: Carotid artery IMT has a closer relationship with sdLDL-C concentrations than other lipid parameters in Japanese subjects. sdLDL-C may be a potentially useful risk marker when assessing carotid artery IMT in Japanese subjects.

B-124

Role of Serum Resistin and Adiponectin in Detection of NON-Alcoholic FATTY liver Disease

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Background : Non alcoholic fatty liver disease (NAFLD) is the commonest cause of abnormal liver function tests. Non-alcoholic steatohepatitis (NASH) is the extreme form of NAFLD . liver biopsy is the gold stone in diagnosis of NAFLD.

Methods : This study was conducted of 60 proved NAFLD patients (15 out of them had NASH) and another 30 healthy volunteers as a control group . Resistin and adiponectin were assayed by ELISA. Acoustic radiation force impulse elastography was evaluated in all subjects.

Results : Resistin level was significantly higher in NAFLD than control group (P=0.001) . At the cut off 10830 ng/ml , the area under ROC curve (AUROC) was 0.79 (raised to 0.86 in NASH subgroup) , with sensitivity = 85% and specificity = 20% . Serum adiponectin level was statistically significantly higher in the control group than in the patients (P=0.001). At the cut- off 2500 ng/ml , AUROC was 0.79, sensitivity = 93.3% and specificity =34.3%. Mean velocity of acoustic radiation force impulse was 1.46 ± 0.352 m/sec in patients and 1.29 ± 0.292 m/sec in control group with no significant difference (P=0.103).

Conclusion ; Results indicate that both resistin and adiponectin show good sensitivity but low specificity in detection of NAFLD. Addition of acoustic radiation force impulse may not significantly increase the sensitivity .

B-125

The AtherOx™ Assay detects oxLDL-β2GPI antigen complexes associated with atherosclerotic vascular disease

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Background: Low-density lipoprotein (LDL) is the principal form of cholesterol that accumulates in atherosclerotic lesions or plaques, but before accumulation occurs, it must be modified into oxidized LDL (oxLDL) by oxidative stress. Only oxidized LDL binds to β2 glycoprotein 1 (β2GPI) to form oxLDL-β2GPI complexes; initially as unstable complexes followed by the formation of stable complexes. While highly pathogenic free oxLDL can be detected in serum, complexed oxidized LDL is also pathogenic and can be detected more reliably in serum. OxLDL-β2GPI complexes act as pro-inflammatory chemotactic factors for T lymphocytes and macrophage uptake of complexes leading to the development of foam cells within atherosclerotic lesions. Thus, circulating oxLDL-β2GPI complexes are implicated as pro-atherogenic antigens which may represent a serologic risk factor for the development of thrombosis and atherosclerosis. Here we describe the analytical and clinical performance of the AtherOx™ ELISA for the detection of oxLDL-β2GPI antigen complex in serum.

Methods: The AtherOx™ test is performed as an indirect ELISA. Diluted serum samples, calibrator, and controls are incubated in microwells coated with a monoclonal antibody directed towards only complexed oxLDL-β2GPI. After washing, biotin conjugated detection monoclonal antibodies specific for human apoB100 are added, followed by horseradish peroxidase conjugated to Streptavidin. Following substrate development, sample values are determined by comparison of optical densities to a calibrator curve on a 0-5 units/mL scale. Pre-defined assay performance characteristics were compared across two lots including control recovery, determination of LOB/LOD/LOQ, Linearity, Precision, Prozone, and Interference Testing. OxLDL-β2GPI and free oxLDL levels were compared in human subjects with and without vascular disease.

Results: The Limit of Blank (LOB) of the assay is 0.025 units/mL. The LOD is 0.075 units/mL and the LOQ is 0.19 units/mL. The linear range was determined to be 0.2 to 7.5 U/mL. Control recoveries were within labeled ranges with CVs between 9 and 12%. Within run precision of three levels spanning the linear range of the assay was 6-11%. Total precision was 12-23%. The assay exhibits no prozone effect during assays of synthetic oxLDL or very high titer human serum. Twenty four interfering substances, drugs, uncomplexed oxLDL and β2GPI were tested in the AtherOx™ assay. Only high levels of hemoglobin interfered with value recovery of serum samples. The assay exhibits strong specificity for only complexed oxLDL- β2GPI. In clinical studies, patients with vascular disease showed no difference in free oxLDL levels (p=0.8), but significantly higher values (p<0.0001) of serum oxLDL- β2GPI than controls assayed with the AtherOx™ assay.

Conclusion: Based on the performance data, the AtherOx™ test kit can be manufactured consistently across multiple lots and meets performance criteria. The assay has robust precision, linearity, interference and LOB/LOD/LOQ profiles. A significant increase in AtherOx™ levels was detected in patients with atherosclerotic vascular disease. These data point to the utility of detecting complexed oxLDL-β2GPI as a biomarker for the development of thrombosis and atherosclerosis.

B-126

Correlation between subfractional high-density lipoprotein cholesterol levels and obesity traits: an electrophoretic method and a homogeneous assay method

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Background:

Subfractional high-density lipoprotein (HDL) analyses can be useful for assessing the cardiovascular risk relative to a simple measurement of total HDL. A homogeneous assay for HDL2- and HDL3-cholesterol (HDL2-C and HDL3-C) was recently developed with a good correlation with a standard ultracentrifugation method, and its clinical applications are being studied. Obesity is known to modulate HDL metabolism and often induces low HDL-C levels. This study was performed to see the correlation between the subfractional HDL-C ratio, as measured by the homogeneous method, and the obesity traits.

Methods:

The serum samples of 54 subjects were analyzed by the direct assay for HDL-C, as well as the homogeneous method (Denka Seiken Co. Ltd., Japan) and the electrophoretic method for subfractional HDL levels. The data obtained by the electrophoretic method were expressed as the cholesterol levels of respective subfractions (with multiplication by total HDL-C). The HDL2-C/HDL3-C ratio and the large (L)+intermediate (IM) HDL-C/small (S) HDL-C ratio are possible indices for the obesity traits.

Results:

The subjects' mean levels were as follows: body mass index (BMI) 24 kg/m2, HDL-C 67 mg/dL, HDL2-C 40 mg/dL, HDL3-C 23 mg/dL, HDL2-C/HDL-3-C ratio 1.8, L+IM HDL-C 59 mg/dL, S HDL-C 8 mg/dL and L+IM HDL-C/S HDL-C ratio 13. The correlation coefficients were as follows: HDL2-C/HDL3-C ratio and BMI -0.33 ($p = 0.02$), and L+IM HDL-C/S HDL-C ratio and BMI 0.07 ($p > 0.05$).

Conclusion:

The HDL2-C/HDL-3-C ratio by the homogeneous method was significantly correlated with the BMI, suggesting the subfractional HDL-C ratio by the method might be helpful for observing the obesity-related pathophysiology. Subfractional HDL analyses by the electrophoretic method may also provide different information from those by the homogeneous method, and further studies using both methods are warranted.

B-127

Accurate Assessment of LDL Cholesterol Reduction at Levels below 70mg/dL has Implications in the Estimation of Efficacy for New Drugs in Development

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Objective: The objective of this study was to compare low density lipoprotein cholesterol (LDL-C) measurements obtained by two calculated methods, the Friedewald and Hopkins formulas, and a direct homogeneous enzymatic assay to preparative ultracentrifugation (PUC).

Relevance: Calculated LDL-C by the Friedewald formula has been the foundation for assessing both clinical response to lipid modifying therapies and determination of efficacy in clinical trials. As new drugs in development achieve very low LDL-C levels, the validity of the Friedewald formula is questioned as it significantly underestimates LDL-C < 70 mg/dL, with increasing inaccuracy when used to estimate levels < 50 mg/dL. Recently, the Hopkins method for calculating LDL-C has been introduced; which is based on the Friedewald formula but uses a variable triglyceride (TG):very low-density lipoprotein cholesterol ratio. PUC is widely considered to be the "gold standard", reference method to measure LDL-C, but is labor-intensive and requires specialized instrumentation. Direct, homogenous enzymatic methods have been proposed as an alternative.

Methodology: LDL-C measurements for PUC, a direct homogenous enzymatic assay, and calculations by both Friedewald and Hopkins formulas were obtained from 1299 patients. Samples were analyzed in a CDC-NHLBI Part 3 lipid standardized central laboratory. The four methods were compared for LDL-C across TG cutpoints.

Results: See table. Overall, both direct and calculated methodologies were significantly different from PUC at LDL-C < 70 mg/dL with increasing bias at LDL-C ≤50 and 25 mg/dL. However, the direct assay varied less from PUC than the calculated methods.

Conclusion: At LDL-C levels < 70 mg/dL, LDL-C calculated by both the Friedewald and Hopkins formulas, and 'direct' LDL-C measurement, show significant differences when compared to LDL-C by PUC. Underestimation of LDL-C levels when post treatment LDL-C is very low has implications for the accurate assessment of LDL-C lowering by new therapeutic agents in development.

Summary Statistics of Calculated LDL-C and PUC LDL-C by PUC Categories

		PUC	Direct LDL		Friedewald		Hopkins	
PUC LDL-C Category (mg/dL)	N	Mean	Mean	% Diff,a	Mean	% Diff,b	Mean	% Diff,c
≤25	322	18.1	18.9	8.8	12.3	-32.9	14.6	-19.7
26-50	538	36.0	34.3	-4.3	28.5	-20.9	32.8	-9.3
51-70	87	59.5	57.0	-4.1	50.9	-14.3	58.1	-2.3
71-100	76	86.2	83.6	-2.7	80.2	-6.9	84.2	-2.2
101-200	258	138.0	132.9	-3.7	133.4	-3.4	135.8	-1.4
>200	18	267.5	235.6	-3.5	261.9	-2.4	261.4	-2.4
≤50	860	29.3	28.6	0.6	22.4	-25.4	26.0	-13.2
≤70	947	32.1	31.2	0.1	25.1	-24.4	28.9	-12.2
≤100	1023	36.1	35.0	-0.1	29.2	-23.1	33.0	-11.4

a, Percent difference = 100*(Direct LDL – BQ LDL-C)/BQ LDL-C; b, Percent difference = 100*(Calculated LDL-C by Friedewald – BQ LDL-C)/BQ LDL-C; c, Percent difference = 100*(Calculated LDL-C by Hopkin's – BQ LDL-C)/BQ LDL-C P-values are from a one sample t-test performed on percent difference.

B-128

An Immunoturbidimetric Assay for Measuring oxLDL-Beta-2-Glycoprotein

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Background: The pathophysiology of atherosclerosis is mediated by a myriad of factors including dyslipidemia, pro-inflammatory factors, and oxidative stress which leads to peroxidation of low density lipoproteins (LDL). These oxidized LDL molecules (oxLDL) are capable of binding to Beta-2 Glycoprotein (oxLDL-B2GPI), eventually resulting in covalently bonded complexes. As the chronic nature of atherosclerosis progresses, these complexes along with autoimmune anti-oxLDL-B2GPI-IgG can be taken up by macrophages (drawn by chemotactic factors into the vascular intima) via scavenger and Fc-gamma receptors. Ultimately these macrophages containing these complexes become Foam cells, a hallmark of the atherosclerotic plaque. Measurement of these oxLDL-B2GPI complexes in serum may be a useful marker for progression of atherosclerosis. Although immunological methods do exist to measure oxLDL, these are ELISA based assays and require over 2 hours to perform with intermittent manual intervention. Methods: Herein we report the first immunoturbidimetric-based assay for measuring oxLDL-Beta Glycoprotein complexes from serum. This assay can be run in 15 minutes on a standard clinical chemistry analyzer. Reagents for the assay include monoclonal antibodies specific for both the oxLDL and Beta-2 Glycoprotein components that were coupled to latex microparticles via standard coupling methods to generate the R2 component of the assay. This R2 reagent, in combination with a reaction enhancing R1 reagent and the oxLDL-B2GPI containing sample resulted in a specific agglutination reaction. Results: A preliminary precision study demonstrated a CV of less than 5% across 4 different calibration levels ranging between 4.5µg/mL to 0.9 µg/mL from 5 independent measurements using 3 different calibration curves. Initial results indicate linearity (n=7) from 5 to 0.15 µg/mL (CV>5%). Conclusion: The assay demonstrated robust initial precision and linearity in a performance range consistent with the predicate ELISA assay. This immunoturbimetric assay format will allow a higher throughput of samples and more rapid turnaround times.

B-129

Incidence of risk factors in patients with acute myocardial infarction at young age

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Background: The incidence of acute myocardial infarction (AMI) in people under age 45 years is rare, less than 5% of all cases. It is emphasized that presentation of AMI in this population is peculiar, whose etiopathogenic, anatomic and prognostic characteristics are singular, being different from older patients. The purpose of this study was describe social-demographic-metabolic outline and incidence of cardiovascular risk factors in patients (age below 45 years) with AMI diagnosis. **Methods:** This was a cross-sectional study conducted from November 2010 to January 2015 which included 103 young patients under age 45 years, both sex, with AMI diagnosis, established based on clinical and coronary angiography criteria. The laboratorial evaluation includes analysis of glycosylated hemoglobin, fasting blood glucose, triglycerides, total cholesterol and fractions. The cardiovascular risk factors evaluated were smoking, alcoholism, familiar history, dyslipidemia, hypertension, diabetes mellitus, sedentary behavior and metabolic syndrome. **Results:** A total of 103 patients were included. The mean age of participants at the time of study was 39,6 ± 5,7 years. Fifty-eight percent of the participants were men. Smoking was found in 57 % of cases. Alcoholism and sedentary behavior were presented in 7% e 81% of persons respectively. Diagnosis of hypertension and dysglycemia were evidenced in 42% and 69%, respectively. Metabolic syndrome was confirmed in 58% of patients. Forty-one percent of persons had positive familiar history for coronary arterial disease. The ST-segment elevation was identified in 59% of cases. The most commonly compromising coronary artery was descendent anterior (72%). The subjects showed unfavorable lipid profile characterized for low levels of HDL-cholesterol (91% of men with HDL-C ≤ 40mg/dL and 95% of women with HDL-C ≤ 50mg/dL). **Conclusion:** Acute coronary syndrome in people younger than 45 years is rare and appears to be associated with dysglycemia and unhealthy lifestyle: smoking, hypertension, sedentary behavior and low HDL-cholesterol.

B-130

Validation of a new equation for LDL-c estimation in a German population-based study cohort

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Background: A recently established simple equation by Cordova & Cordova [LDL-COR = 3/4 (Total Cholesterol - HDL-cholesterol)], provided an improved estimation of LDL-cholesterol and correlated higher with directly measured LDL-c compared to the Friedewald's Formula (LDL-FW) in a sample of 10,664 Brazilian individuals. It showed a better performance compared to other previously published equations using or not triglycerides (TG) values. However, validation of this new equation in a distinct population is mandatory.

Methods: We have evaluated the performance of this new equation in a German general population cohort (SHIP), also considering age, gender, use of lipid lowering therapy, and associated co-morbidities such as diabetes, hepatic, kidney and thyroid disease. In total, we used data from 4,075 individuals from SHIP (SHIP-0: 1997-2001) aged 20 to 79 years. We excluded subjects with TG>400 mg/dL or with an incomplete lipid profile. Data (in quartiles when appropriated) were analyzed by linear regression models (with 95% Confidence Interval) and also by fractional polynomials to account for possible non-linear associations.

Results: LDL-COR showed a better performance, with a lower standard deviation (28.83%), compared to the previously published equations (31.42% for LDL-FW). The differences between LDL-COR and LDL-FW increase with higher Total Cholesterol [-6.6 (-27.60 to 15.49); -9.9 (33.80 to 13.99); -12.67 (-38.27 to 12.92); -21.21 (50.38 to 7.96)] and HDL-c [-6.38 (-35.63 to 22.86); -11.73 (-38.89 to 15.43); -14.43 (-39.34 to 10.48); -15.93 (-40.76 to 8.90)] quartiles, and decrease with higher TG quartiles [-20.03 (-38.03 to -2.02); -17.76 (-37.23 to 1.70); -13.22 (-34.68 to 8.24); 1.87 (-25.03 to 28.78)]. Considering the co-morbidities and the other evaluated factors, all except fibrates, being TSH borderline (P=0.06), were associated with the difference between LDL-COR and LDL-FW (P<0.01).

Conclusion: It is possible to hypothesize that, if there is a change in the absolute difference between the two formulae in these conditions, the LDL-FW values are being mostly affected, and not the ones obtained by LDL-COR, since these co-morbidities are well known conditions to affect the values obtained by LDL-FW. A study evaluating the performance of the new formula using a reference method (ultra-centrifugation and beta-quantification) in a well-defined population is still necessary to validate its use. Support: DAAD, CAPES, CNPq, FURB.

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FABP 2 gene polymorphism and metabolic syndrome in Libyan Subjects

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Background: Metabolic syndrome (MetS) is a multifactorial disorder in which dyslipidemia plays an important role. Fatty acid-binding protein 2 (FABP 2) is responsible for transport of free fatty acids in the intestinal endothelium cells. *FABP2*-genetic variants might affect plasma lipid concentrations and intracellular lipid transport. The aim of this study was to investigate the association between FABP2 Ala54Thr genetic polymorphism and metabolic syndrome and some biochemicaland anthropological parameters in elderly subjects.

Materials and methods: This cross-sectional study included 150 men and 175 women of local Libyan subjects in Benghazi,Libya. Fasting serum concentration of glucose, lipid parameters, total proteins and C-reactive protein were determined by standardized methods. Presence (MetS(+)) or absence (MetS(-)) of MetS was determined according to criteria of the International Diabetes Federation. *FABP2* genetic polymorphism Ala54Thr (rs1799883) was genotyped with PCR-RFPL.

Results: The genotype frequencies for Ala/Ala, Ala/Thr and Thr/Thr genotype were 60, 36 and 6 in MetS(-), and 131, 70 and 13 in MetS(+), respectively, without statistical significance (P = 0.567). Ala/Ala genotype was a subgroup of non-carriers, while Ala/Thr and Thr/Thr genotypes were Thr54-carriers. Median triglyceride concentration was significantly lower in carriers then in non-carriers for whole MetS(+) group (P = 0.050); there were no significant difference between men with MetS (P = 0.144), but there was a difference between women with MetS (P = 0.020). T-test showed that mean HDL cholesterol concentrations in MetS(+) group for Thr54-carriers was significantly higher in whole group (P = 0.001), and for both genders (men P = 0.039; women P = 0.004) as compared to non-carriers.

Conclusions: *FABP2* genetic polymorphism is associated with lower triglyceride and higher HDL-cholesterol concentrations in Libyan subjects with MetS. This genetic variation might be a useful marker for understanding dyslipidemia in MetS.

B-132

Is lipoprotein lipase a monomer or dimmer in pre- and post-heparin plasma?

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Background:

The structure of lipoprotein lipase (LPL) has been long controversial because of the difference of enzymatic activity between pre-heparin and post-heparin plasma. Active form of LPL found in post-heparin plasma has been recognized as dimmer LPL, while inactive form of LPL found in pre-heparin plasma has been believed as monomer LPL. However,

we recently found that dimmer form of LPL prevalently exists in pre-heparin plasma. Using LPL-ELISA and LPL activity assay, we have investigated the structure and function of LPL in pre-heparin plasma. Also the relationship between circulating LPL and remnant lipoproteins (RLP) in plasma was investigated.

Methods:

Monoclonal antibodies against LPL (57A5 and 88B8) were raised against human recombinant LPL and established two sandwich ELISA systems. LPL mass and activity were determined in healthy volunteers (n=40) of pre-heparin and post-heparin

plasma with TG, HDL, RLP-C, RLP-TG, apoC1, apoC3, apoE and other plasma parameters.

Results:

Two LPL-ELISA systems were established. One assay used two different monoclonal antibodies against LPL (57A5 and 88B8) for the sandwich ELISA which can detect both monomer (inactive form) and dimer LPL (active form) (Assay 1). The other assay used the same monoclonal antibody (88B8) for the sandwich ELISA which can detect dimer

LPL only (Assay 2). The Assay 2 detected about 70 % of LPL concentration compared with Assay 1 in both pre- and post-heparin plasma using the same recombinant LPL as a calibrator. Both ELISAs were significantly correlated with activity assay in post-heparin plasma (r=0.6). Pre-heparin LPL mass was less than 20% of post-heparin LPL mass. In pre-heparin plasma, more than 80% of LPL mass was found in RLP but no activity was detected in it. LPL mass in RLP increased 2-folds with inactive form after heparin injection, while more than 5-fold increase of LPL mass in post-heparin plasma with significantly high LPL activity. Significant amount of apoC1 and apoC3 were detected in RLP, suggesting the inhibition of LPL activity.

Conclusion:

Sensitive and specific LPL-ELISA assays were developed to distinguish a monomer and dimmer form of LPL in RLP with and without heparin injection. More than 80% of LPL mass in pre-heparin plasma was found in remnant lipoproteins. LPL in RLP isolated from both pre-heparin and post-heparin plasma was found to be inactive, although those LPL was found to have mostly dimmer structure. ApoC1 or C3 in RLP may inhibit the LPL activity in spite of dimmer structure.

B-133

Does increased adiponectin influence lipid profile and inflammatory status in chronic kidney disease undergoing hemodialysis?

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Patients with chronic kidney disease present markedly elevated mortality from cardiovascular causes, even in patients undergoing hemodialysis (HD) substitutive treatment. Adiponectin (ADP) is an anti-inflammatory and insulin-sensitizing cytokine decreased in insulin resistance (IR) states. End-stage renal disease (ESRD) is associated with insulin resistance, nevertheless, ADP has been observed paradoxically elevated, probably due to an impairment in renal clearance rate of ADP. It is not clear if elevated ADP function in ESRD is conserved. Objective: To assess whether increased ADP in HD patients is associated with a more favorable metabolic and inflammatory profile.

We studied 120 HD patients. Fasting blood was drawn in the interdialysis day and serum total ADP was measured by ELISA method. According to the median of ADP (18 ug/ml) in the studied population, two groups were established (n=60 each): High ADP levels (ADP-HL) ≥18.0 ug/ml (range: 18.0 to 33.2) and low ADP levels (ADP-LL) <18.0 (4.3 to 17.9). Both groups did not differ in age (61±14 years-old vs 62±19; p=0.99), gender (F/M: 32/28vs 25/35; p=0.27), duration of HD treatment (12±3 years vs 14±5; p=0.86) and frequency of diabetes (diabetes/no-diabetes: 20/40 vs 24/36; p=0.18). Serum lipid profile, inflammatory and insulin resistance parameters were assessed.

The ADP-HL group showed lower triglyceride (TG) (1.32 ± 0.63 vs 1.81 ± 0.91 mmol/L, p <0.001) and higher HDL-cholesterol (1.14 ± 0.31 vs 1.01 ± 0.36 mmol/L, p = 0.03). Free fatty acids (p=0.87) and LDL-cholesterol (p=0.54) showed no differences between groups.

Insulin levels were lower in ADP-HL, median (range): 8.0 (2.0 to 32.9) IU/L vs 12.7 (2.0 to 44.9) p=0.008, without differences in fasting glucose concentration between groups (p=0.07). ADP-HL showed a HOMA-IR: 1.57 (0.30 to 10.70) and ADP-LL: 2.24 (0.31 to 9.65) p=0.003. As expected, ADP was positively associated with HDL-cholesterol (r=0.33, p<0.001) and negatively with TG (r=-0.27, p<0.001), insulin (r=-0.28, p=0.02) and HOMA-IR (r=-0.30, p=0.01).

Regarding the analysis of inflammatory markers, all patients with hs-CRP over 10 mg/L were excluded (n=8 in ADP-HL and n=10 in ADP-LL). No differences were observed between groups in hs-CRP (ADP-HL: 4.9±3.2 mg/L vs ADP-LL: 5.9±3.5 p=0.21) and interleukin-6 (ADP-HL: 11.5 (2.8-43.1) pg/mL vs ADP-LL: 10.0 (4.3-43.3) p=0.79. No significant correlations between ADP and inflammatory markers were found.

Conclusion: elevated ADP in HD patients would act favorably on lipid profile and insulin sensitization, but it may not exert its anti-inflammatory function possibly due to a deregulation of adiponectin signal pathways.

B-134

Elevated small dense LDL cholesterol in metabolic syndrome and diabetes patients with a fatty liver

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Background: Serum small dense LDL-cholesterol (sdLDL-C) levels were determined in healthy controls, type 2 diabetic patients (T2DM) and people suffering metabolic syndrome (MetS) with or without a fatty liver.

Methods: The plasma lipids and lipoproteins including sdLDL-C were determined in controls, MetS and T2DM patients (n = 5255). sdLDL-C levels were measured by a homogeneous assay (Denka Seiken Co., Ltd.). The cases with MetS and preliminary MetS (pre-MetS) as well as T2DM and preliminary T2DM (pre-DM) were selected based on the Japanese criteria. Fatty liver was diagnosed using the ultrasonography.

Results: The 75th percentile values for sdLDL-C were 27.5 mg/dl for men and 23.3 mg/dl for women and increased with age. Significantly increased sdLDL-C concentrations were found in the controls, pre-MetS, MetS and pre-T2DM and, T2DM cases with a fatty liver compared to the groups without a fatty liver

Conclusion: Fatty liver significantly increased serum sdLDL-C levels and the multiple regression analyses ascertained that fatty liver was an independent determinant for sdLDL-C levels in serum.

B-135

The usefulness of a new equation to estimate LDL cholesterol compared with the Friedewald formula and direct measurement for the assessment of cardiovascular risk according to current European guidelines

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Introduction: Cardiovascular risk (CVR) assessment is based on Total, HDL, LDL Cholesterol (TC, HDL, LDL) and Triglycerides (TG) concentrations. A new equation (NE) for estimating LDL has been proposed as an alternative to the Friedewald formula (FF). At our hospital we measure LDL (mLDL) with TG>250 mg/dL, due to possible inaccuracies using FF to estimate LDL.

Objective: Evaluate the usefulness of the new equation to estimate LDL cholesterol when compared to Friedewald formula for the assessment of cardiovascular risk in patients with moderate hypertriglyceridemia.

Patients and Methods: Results for TC, mLDL, HDL and TG (Advia 2400; Siemens HD) from patients with moderate hypertriglyceridemia (250-400 mg/dL) were gathered from laboratory information system over a ten months period, and NE and FF calculated. LDL intervals derived from 2012 European ESC/EAS Guideline were used. Concordance between mLDL and estimated LDL by both equations were calculated using the Cohen's Kappa index (k) with a 95% Confidence Interval (CI). Correct patient's classification according to CVR guidelines was studied.

Results: 7120 results from 5870 patients (60.9% males) were obtained; median age was 54 years (IQR 44-65). Medians and IQRs for concentrations in mg/dL are: TC 202 (174-230); mLDL 120 (95-147); HDL 39 (33-45); TG 289 (267-326).

Next table shows the concordance (Kappa index) and percentage of correct classification of both equations when compared to mLDL.

mLDL (mg/dL)	LDL NE	LDL FF
	Kappa index (CI 95%)	
	0.609 (0.595-0.623)	0.327 (0.313-0.340)
	% Correct clasification (CI 95%)	
<70 (n=667)	62.1 (58.3-65.8)	90.6 (88.3-92.8)
70-99 (n=1510)	72.8 (70.5-75.1)	42.0 (39.5-44.5)
100-154 (n=3558)	85.4 (84.2-86.6)	57.7 (56.0-59.4)
154-189 (n=998)	48.1 (44.9-51.3)	25.2 (22.4-27.9)
>189 (n=387)	57.4 (52.3-62.4)	41.1 (36.1-46.1)
Total (n=7120)	**73.9 (72.8-74.9)**	**52.0 (50.8-53.2)**

Conclusions: In patients with moderate hypertrygliceridemia there is a good concordance using the new equation for CVR assessment when compared to mLDL versus FF (fair agreement).

In this group, NE improves the correct classification of patient's CVR according to current European guidelines.

New equation can be an adequate alternative to assess the CVR in patients with moderate hypertriglyceridemia with an added advantage for saving of material resources.

B-136

Validation of a New Lipoprotein(a) Particle Number ELISA

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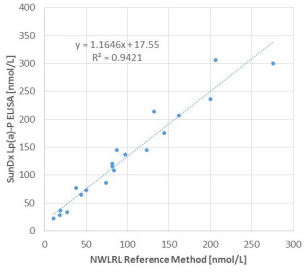
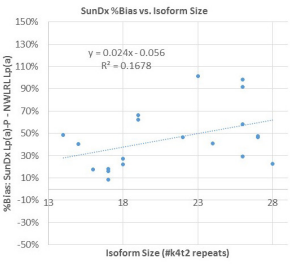
Background: Measurement of lipoprotein(a) particle number [Lp(a)-P] is important in CHD risk assessment. Unfortunately, turbidimetric assays for Lp(a) mass are biased due to apo(a) size isoforms. Therefore, we developed an ELISA for Lp(a)-P without isoform bias. Methods: Limit of the Blank (LOB) was determined using delipidated human serum assayed 50X over 5 days (LOB = mean ± 1.654 SD). Limit of Detection (LOD) was determined by measuring 2 serum samples with low Lp(a) ~50X over 5 days (LOD = LOB + 1.654 SD). LOQ was the lowest concentration with <20% CV. Precision was assessed by measuring 10 replicates of 5 serum pools over 5 plates. Linearity was assessed by intermixing samples with low and high Lp(a) pools. Accuracy and bias vs. size isoforms was assessed using 20 samples from Northwest Lipid and Diabetes Research Laboratory (Seattle, WA) with reference method-assigned concentrations and known kringle 4 type 2 repeats by linear regression analysis. Results: The LOB and LOD were 1 and 13 nmol/L, respectively. The LOQ was equal to the LOD. Imprecision at the extreme low and high ends of the analytical range were 12.1% and 11.4%, respectively. Imprecision in the midrange varied from 6.9% to 7.7%. Results were linear from ~2 to 500 nmol/L. Lp(a)-P was highly correlated to the reference method (r=0.971) with an average bias of 34 nmol/L, which was corrected by adjusting the master calibrator assigned values. There was no statistically significant association between bias and the number of kringle 4 type 2 repeats. Conclusion: The Sun Dx Lp(a)-P ELISA is sensitive, precise, and linear over a wider analytical range than most Lp(a) assays, and is strongly correlated to the reference method (r= 0.971). Importantly, the assay shows no bias due to apo(a) size isoforms.





B-137

Accuracy and Precision of Cholesterol, Triglycerides and HDL-Cholesterol Methods: A Retrospective Assessment of Quality

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Introduction: Accuracy-based proficiency testing programs utilize commutable samples and reference laboratory results to assess accuracy and precision of routine clinical methods. The Institute for Quality Management in Healthcare (IQMH) provides ISO 17043:2010 accredited Proficiency Testing (PT) programs for lipids. Ten years of data was evaluated to determine average bias and imprecision, and then compared to desirable performance targets based on biological variation.

Methods: Twenty-nine surveys distributed between June 2005 and December 2014 were included. PT samples consisted of single donor sera from healthy donors with no additives. Two challenges were distributed in each survey. Participants' results were assessed against the Centers for Disease Control and Prevention's certified methods. Robust statistics based on ISO 13528:2005 were used to calculate peer group means and standard deviations to eliminate outliers' effects.

Results: Cholesterol (CHOL) was assessed across all PT samples with 374 peer groups; average method bias: -0.23% (range: 19.4%-6.5%), average CV: 1.9% (range: 0.4%-11.3%). For triglycerides (TRIG), 362 peer groups; average method bias: -3.26% (range: -23.8%-12.2%); CV: 2.8% (range: 0.4%-14.8%). HDL-Cholesterol (HDL-C), 372 peer groups; average method bias: 0.9% (range: -34.6%-105.4 %); CV: 3.5% (range 0.7%-14.6%).

Ninety-five per cent of method bias estimates were within the desirable limits for CHOL; 91% and 69% for triglycerides and HDL-C. Ninety-five per cent of the peer group CVs were within the desirable CV limits for CHOL; 99% for TRIG and 66% for HDL-C.

Table 1. Average Method Bias and peer group CV values

	CHOL		TRIG		HDL-C	
	Bias (%)[b] (N)[c]	CV (%) (N)	Bias (%) (N)	CV (%) (N)	Bias (%) (N)	CV (%) (N)
Desirable Target[a]	4.1%	3.0%	9.6%	10%	5.6%	3.7%
Optimum Target[a]	2.1%	1.5%	4.8%	5.0%	2.8%	1.8%
Abbott	1.4 (40)	1.3 (40)	2.2 (38)	2.4 (38)	3.0 (38)	3.7 (38)
Beckman Coulter	-0.4 (80)	1.7 (80)	-6.3 (77)	2.8 (78)	-1.2 (80)	4.5 (80)
Ortho	-0.8 (80)	2.4 (80)	-2.0 (77)	2.5 (78)	-0.6 (80)	3.6 (80)
Roche	0.5 (34)	1.9 (34)	-2.1 (32)	2.8 (32)	1.3 (34)	2.7 (34)
Roche (BMC)	-0.7 (46)	1.7 (46)	-3.1 (45)	2.2 (46)	3.7 (46)	3.0 (46)
Siemens (Bayer)	-0.6 (36)	1.8 (36)	-3.5 (34)	1.8 (34)	0.0 (36)	2.5 (36)
Siemens (DB)	-0.1 (58)	2.3 (58)	-2.2 (56)	4.8 (56)	2.4 (58)	3.3 (58)

[a]Desirable and optimum targets refer to desirable and optimum specifications based on biological variation.
[b]Bias indicates the % difference between a peer group mean and reference laboratory result.
[c]N: number of peer groups. Analyte concentration ranges were cholesterol [117-364 mg/dL (3.0 -9.4mmol/L)], triglycerides [49 -1537 mg/dL (0.6 – 17.4 mmol/L)] and HDL-Cholesterol [22-115 mg/dL (0.6 -3.0 mmol/L)].

Conclusion: Overall biases for CHOL, TRIG and HDL-C and the imprecision for CHOL and TRIG were within the desirable limits. However there were peer groups bias and CV values exceeding these limits. This was more prominent in the HDL-C with only 69% of the HDL-C method bias and 66% of the CV estimates were within the desirable limits, indicating the lack of method standardization.

B-138

Hepatic triglyceride lipase is mainly distributed on apoE-rich HD in post-heparin plasma

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Background: We developed HPLC system with cation-exchange and heparin affinity columns to isolate and determine apoE-rich HDL in serum. As HTGL is known to metabolize HDL2 to HDL3, we have studied the relationship between HTGL activity and apoE-rich HDL, using our specific apoE-rich HDL isolation technique.Method: The HPLC system (Tosoh) consisted of AS-8020 sampler, five pumps (CCPS and CCPM-II) and UV-8020 detector and a SC-8020 system controller (Tosoh) was used for instrument regulation. Two columns were used tandemly to determine apoE-HDLc in plasma. First, a cation-exchange column (HiTrap SP HP, 1 mL, GE healthcare) was used for retaining non-HDL lipoproteins. Second, a heparin affinity column (HiTrap Heparin HP, 1 mL, GE healthcare) was used for retaining apoE-HDL. Three types of elution buffers for a step-wise gradient were mixed on line at a constant flow rate of 1.0 mL/min. To analyze HTG distributions in post-heparin plasma, 0.4-min fractions were collected and analyzed for HTGL activity by Imamura method (J Lipid Res 2008; 49: 1431). Remnant lipoprotein (RLP) fraction was isolated by immuno-separation method and used for the detection of HTGL and LPL activity and mass in RLP isolated by HPLC. Thirty units/kg of heparin was injected to healthy controls and post-heparin plasma was collected in 15 min. Tetrahydrolipstatin (lipase inhibitor) was added in test tube right after blood withdrawal. Results: Post-heparin plasma was applied to this HPLC system and fractionated into three groups, apoE-poor HDL, apoE-rich HDL and non-HDL, respectively. Also RLP fraction isolated from the post-heparin plasma by immunoaffinity gel was applied to this HPLC system. HTGL activity and mass was prevalently found in apoE-rich HDL. No LPL activity and mass was found in apoE-rich HDL. HTGL activity and mass was not found in apoE-poor HDL and non-HDL fraction. HTGL activity was completely inhibited by tetrahydrolipstatin in plasma and RLP.

Conclusion: This is the first report that apoE-rich HDL prevalently carries HTGL in post-heparin plasma. Although HTGL was found in RLP, HTGL did not bind remnant lipoproteins but bound apoE-rich HDL in RLP. Addition of tetrahydrolipstatin completely inhabited HTGL activity, similar with LPL activity.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Management

B-139

QC Failure Rates Assessed by Assay Specific Sigma Metrics in a Six Sigma Based QC Program

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Introduction: A Six Sigma Based Quality Control program was implemented in our laboratory two years ago to improve our ability to detect clinically important errors and decrease the number of false rejections. This type of QC program decreases laboratory costs in reagents, supplies and labor by minimizing unnecessary investigations due to false rejections of QC rules. A Six Sigma based QC program should result in reduced false rejection, while maximizing detection of the clinically significant errors. Since methods that are 5 Sigma or greater require fewer QC challenges per run and simpler "Westgard Rules" to monitor the method, it is important to select instruments with methods that deliver 5 Sigma performance or greater.

Objective: The goal of this study was to determine the rate of QC rules violations for a method depending on its Sigma metric. We also determined the number of QC points that were outside of 2 SD limits, but within the limits of the QC rule used to monitor the 5 and 6 Sigma methods.

Methods: QC values were reviewed for 70 different chemistry, TDM and immunoassay methods over a three month period. Methods were grouped by their Sigma Metric: 5 Sigma, 6 Sigma and 4 Sigma or less. For the 5 and 6 Sigma methods, the number of QC values outside 2 SD limits, but less than 3 SD (5 Sigma methods) or 3.5 SD (6 Sigma methods), were also tallied.

Results: Over 40,000 QC values for 70 chemistry, TDM and immunochemistry methods on two Abbott ARCHITECT c8000 and three Abbott ARCHITECT i2000SR were evaluated for QC rules failures. Six Sigma methods, using 1-3.5s Westgard Rule, had a QC failure rate of 0.9%, with 40% of the failures due to the wrong control being tested. Five Sigma methods, using 1-3s Westgard Rule, had a QC failure rate of 2.3%, with 23% of the failures due to the wrong control being tested. Sigma metric methods of 4 Sigma or less had a QC failure rate of 14.7%, requiring the use Multiple "Westgard Rules" to monitor the methods. For the 5 and 6 Sigma metric methods, 6.0% of the QC values were outside 2 SD limits but within 3 SD limits.

Conclusion: 5 and 6 Sigma methods had 85% fewer QC failures than the 4 Sigma or less methods when a Six Sigma Quality Control program was put in place. 5 and higher Sigma metric methods also require fewer QC challenges per run, resulting in lower costs in reagents, QC material and calibrators. Fewer QC failures mean less time required by staff to investigate the failures. By eliminating the need for the 1-2s rule for 5 and 6 Sigma metric methods, another 6% in QC failures were avoided. The combination of high quality methods with optimized QC design resulted in significant resource and labor savings, assuring quality while providing needed savings.

B-140

The need for a comprehensive and updated Medical Decision Levels database: TSH test as an example of this requirement

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Introduction: Medical Decision Levels (MDL) are used by laboratory professionals to verify analytical performances; set quality specifications; establish quality control plans and by IVD providers to validate analytical performance of their methods; select concentrations of their control materials. MDL presented in different guidelines and expert opinions, may not agree. Available databases collating MDL (Westgard and CLIA) provide a resource for potential users, but they are infrequently updated and not comprehensive. This could provide a significant issue for users of databases if the MDL do not reflect current guidance and opinion.

Aim: To investigate recommended MDL for Thyroid stimulating hormone (TSH) published in contemporary guidelines and to compare them with those held in current MDL databases.

Material and methods: TSH was chosen as a complex example for study. It has a high request rate; there are issues with standardization and has multiple MDL applying to many clinical situations.

We searched biomedical databases (MEDLINE, EMBASE, Cochrane, NICE) to identify relevant clinical practice guidelines related to thyroid diseases. MDL were collated taking into account diagnosis, follow-up or ongoing treatment.

Results: The Westgard and CLIA databases present a simplified strategy with two MDL: lower and upper limits (0.3 and 5.0 mU/L).

The table summarizes the MDL for TSH (mU/L) gathered from guidelines of 3 selected Thyroid Associations. These guidelines passed critical appraisal using Agree II (www.agreetrust.org).

Thyroid Clinical Condition	**Thyroid Associations**		
	European	**American**	**British**
Adults Reference Range	0.4-4.0	0.4-4.0	0.4-4.5
Hypothyroidism			
Adults	>4.0	>4.0	>4.5
Elderly (>70-80 years)	>7.0	>7.5	-
Subclinical (not need for treatment)	4.0-10.0	4.0-10.0	4.5-10.0
Subclinical (cutoff for treatment)	>10.0	>10.0	>10.0
Treatment objective	0.4-2.5	0.5-2.5	*
Hyperthyroidism			
Subclinical (consider for treatment)	-	0.1-0.5	0.1-0.4
(depends on clinical and/or freeT4)			
Subclinical (cutoff for treatment)	-	<0.1	<0.1
Others Clinical Conditions			
Pregnancy 1st trimester	0.1-2.5	0.1-2.5	0.4-2.0
Pregnancy 2nd trimester	0.2-3.0	0.2-3.0	**
Pregnancy 3rd trimester	0.3-3.5	0.3-3.0	**
Pregnancy with high risk of thyroid tumor	-	0.1-0.5	-
Pregnancy period considered tumor free	-	0.3-1.5	-
Thyroid surgery deferred until postpartum	-	0.1-1.5	-
Neonates clinical evaluation			>20.0
Thyroglobulin assay	-	-	>30.0
Objective treatment post thyroidectomy	-	-	<0.1
* Within Reference Range,	** Trimester-related reference ranges.		

Conclusions: For TSH, the existing MDL databases are not comprehensive. Many clinical scenarios and important clinical situations are not considered.

Current databases will not support user choice of the most appropriate MDL for their intended use or clinical requirements.

There is a need for a comprehensive database, similarly to the existing for Biological Variation but with granularity to support user choice. A systematic evidence based medicine initiative is required to assure its elaboration, harmonization and update.

B-141

HbA1c assay management: a commutability evaluation between cobas c501 and HPLC

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Background: Presently HbA1c is a powerful tool for both monitoring long-term glycemic control, and to diagnostic diabetes. Several methods are available to assay HbA1c, thus medical laboratories should use method certified by the NGSP. Our laboratory personnel have a prejudice regards to assay HbA1c by turbidimetric inhibition immunoassay (TINIA) and prefer to use high-performance liquid chromatography (HPLC). This study was aimed to assess the commutability between two methods certified by the NGSP - TINIA and HPLC - to assay HbA1c.

Methods: The protocol from CLSI EP14-A3 document was used to perform both the sample selection and statistical analyses. Briefly, twenty K2EDTA samples previously assayed by HPLC (G8 Tosoh) were selected to assess the commutability with Tina-quant Hemoglobin A1c Gen.3 in cobas c501 (Roche Diagnostics). The both analyzers (G8 and cobas c501) were previously calibrated against appropriate proprietary reference standard material traceable to both DCCT and IFCC, and verified using

third-party internal quality control. Moreover, a single lot of proprietary reagents was used, and all samples were assayed in triplicate. Appropriateness of data for linear regression analysis was checked regards CLSI EP09-A3 document, then performed both linear regression and difference plot analyses (Figure 1).

Results:

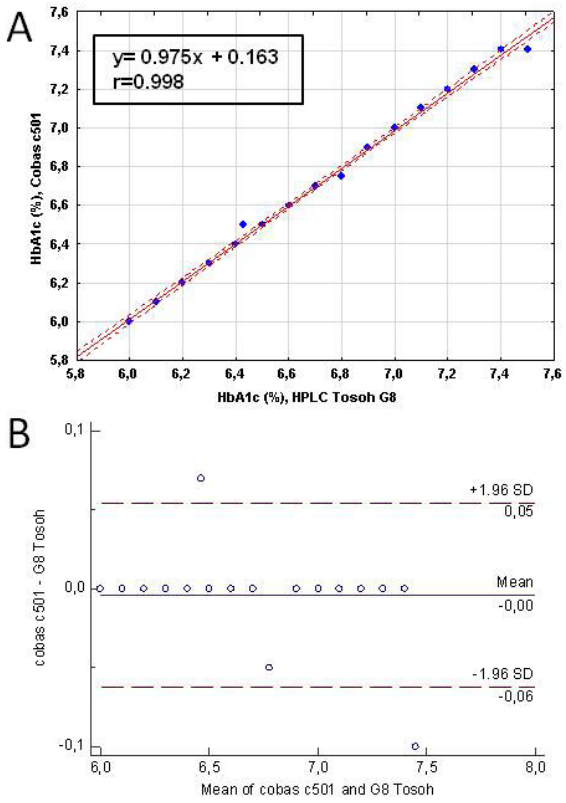

Figure 1. Comparison of HbA1c patient samples assayed by cobas c501 (Roche) and G8 (Tosoh).

A. Linear regression analyses. B. Difference plot.

Conclusion: Since the good correlation between TINIA in cobas c501 and HPLC in G8 for HbA1c assay (r=0.998), the commutability can be established by the equation:

HbA1c in cobas c501 = -0.142 + (1.022 * HbA1c in G8)

Consequently, our laboratory will can guarantee both analytical quality and patient safety using either TINIA or HPLC to assay HbA1c. Moreover, one possible advantage to assay HbA1c by TINIA onboard a robust analytical platform (i.e., cobas c501) connected to a preanalytical automation system, could be to reduce both turnaround time and human resource necessity.

B-142

Evaluation of test ordering patterns for serum Vitamin B_{12} and folate

B. M. Katzman, D. R. Block, R. Nett, W. Katrangi, N. A. Baumann. *Mayo Clinic, Rochester, MN*

Background: Serum Vitamin B_{12} (B_{12}) and folate testing are commonly ordered as part of the assessment of macrocytic anemia or when patients present with physical symptoms suggesting deficiency. Although B_{12} deficiency is more common than folate deficiency, deficits in both of these vitamins are predominantly associated with the elderly population. The aim of this study was to assess the frequency of serum B_{12} and folate test ordering in our institution and the prevalence of deficiency.

Methods: Serum B_{12} and folate test orders spanning one year (July 2013-July 2014) from Mayo Clinic in Rochester, MN were extracted from the laboratory information system. The prevalence of folate and overt B_{12} deficiency, defined as <4.0 mcg/L and <150 ng/L, respectively, was determined. Data were also analyzed to identify how often B_{12} and folate were ordered together, how often tests were ordered multiple times for the same patient, and the frequency of repeat test orders. The "appropriateness" of multiple test orders was evaluated based on the time interval between orders using literature reported intervals of 30 days when first test result indicated deficiency and 90 days when the first test result indicated no deficiency.

Results: A total of 18,126 B_{12} and 11,138 folate tests were ordered on 19,523 unique patients (9,115 male, 10,408 female; median age 61 y, age range 18-103 y). The prevalence of B_{12} and folate deficiency in the patient population was 2.5% (428 patients) and 0.8% (89 patients), respectively. 38% (8,057) of orders included both B_{12} and folate. Within the year studied, B_{12} or folate tests were ordered more than once on 1,104 (5.6%) patients. In 1029 patients with initial results indicating that B_{12} or folate were not deficient, 488 (47%) patients had the same test ordered more than once within 90 days of the original order. There were 111 patients with test results indicating B_{12} or folate deficiency, and 12 (11%) patients had the same test ordered again within 30 days.

Conclusion: Vitamin B_{12} and folate tests are frequently ordered together despite the rarity of folate deficiency in the patient population. The prevalence of B_{12} and/or folate deficiency in this study was comparable to previously published reports. The high frequency of test orders and interval between repeat test orders suggests that these tests may be over-utilized in patients without deficiency. These findings should encourage laboratories to investigate interventions that help guide appropriate utilization of Vitamin B_{12} and folate testing.

B-143

Indirect method for the estimation of Health Related Limits (reference intervals) for uric acid partitioned for decade using Cobas 8000 Roche in a large italian population aged 0-99 years

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Background: The development and the verification of reference intervals including early decades is very relevant and many authors investigated the subjects. Since the intervals are method, genre and age dependent, we applied a well known indirect method in a subregional laboratory which serves a population of 1,2 million in the Greater Romagna Area, in North Italy. The aim of the study was to establish a genre and decade Upper Reference Limit (URL) for uric acid.

Methods: We downloaded from our LIS (DnLab, Noemalife, Bologna, Italy) the uric acid results obtained between January 2011 and June 2014. All the assays have been carried out using the uricase and ascorbate oxidase method and Cobas 8000 analyzer (Roche, Mannheim, Germany). We assessed 169110 results in males: 9007 in decade 1 (M1); 11931 in decade 2 (M2); 21037 in decade 3 (M3); 20826 in decade 4 (M4); 25700 in decade 5 (M5); 22612 in decade 6 (M6); 23860 in decade 7 (M7); 25179 in decade 8 (M8); 8958 in decade 9 (M9) and 195369 results in females: 10132 in decade 1 (F1); 13033 in decade 2 (F2); 23074 in decade 3 (F3); 24987 in decade 4 (F4); 28561 in decade 5 (F5); 22767 in decade 6 (F6); 24539 in decade 7 (F7); 28442 in decade 8 (F8); 19834 in decade 9 (F9). Indirect Health Related Limits (HRLs) were estimated according to the method proposed by Kairisto et al (Graph ROC).

Results: The uric acid URL in males was respectively 7,0 mg/dL in M1-M4; 7,7 mg/dL in M5 and M6; 7,8 mg/dL in M7; 8,0 mg/dL in M8 and 8.2 mg/dL in M9; in females was respectively 5,2 mg/dL in F1; 5,5 mg/dL in F2; 5,7 mg/dL in F3, 5,8 mg/dL in F4; 6,0 mg/dL in F5; 6,1 mg/dL in F6; 6,7 mg/dL in F7; 7,0 mg/dL in F8; 7,6 mg/dL in F9.

Conclusion: The indirect method confirms its usefulness in assessing health related limits of uric acid that is affected by genre and age. The three most relevant points; are: 1) the genre difference is particularly relevant in the earlier decades and decreases after the decade 5 and is limited to 0.6 in decade 9; 2) the URL suggested by the manufacturer is proper in the earlier 4 decades but is underestimated in the decades 5-9; 3) the trends found in our study substantially confirm those found mostly in adults (decades 2-6) in Turkey, in Japan and other parts of Asia (Ilcol YO et al CCLM 2006, Ichihara K et al CCLM 2012, Yamamoto Y et al CCLM 2013; Ozarda Y et al CCLM 2014). The adoption of correctly tailored reference limits could further help in avoiding the high prevalence of inappropriate Xantine Oxidase inhibitors prescription that, for example, has been reported to reach in Italy about 90% of prescription of this class of drugs even if their potential for rare but life-threatening skin and systemic side effects is established (Pasina L et al Eur J Clin Pharmacol, 2014).

B-144

Summary and Analysis of Nine Years' Point-of-care Glucose Meter External Quality Assessment Programs in China

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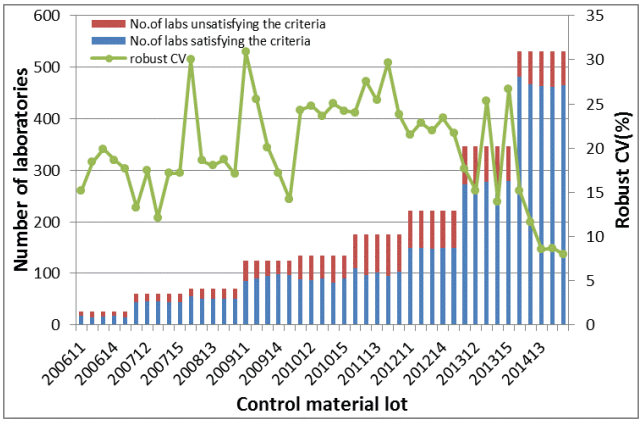
Background: With the increasing use of Point-of-Care(POC) glucose meters all over China, the accurate measurement of blood glucose with POC glucose meters seems more and more essential for correct treatment decisions for glycemic control. Despite the frequent use of POC glucose meter in hospital settings, its quality assurance is still challenging for many institutions.

Methods: External quality assessment(EQA) programs for POC glucose meter was organized by NCCL in china from 2006 to 2014, respectively. Five lots of control materials provided by Bio-Rad at different concentration were assigned to each participant laboratory in each year. The participants were asked to measure the concentration of each analyte and report their data through clinet-EQA reporting system V1.5. Percentage difference was calculated for each laboratory and each control material lot in each year. The measurement performance was then evaluated based on the minimum system accuracy performance criteria in ISO 15197. Robust coefficient of variation(CV) based on ISO 13528 for each control material lot was also calculated to evaluate the degree of variation among different laboratories in China.

Results: Number of laboratories satisfying and unsatisfying the accuracy performance criteria as well as the robust CV for each control material lot was shown in the picture below, respectively. The number of participant laboratories increased gradually from 26 to 531 while the pass rate increased gradually from 57.69%(15/26, lot 200612) to 90.77%(482/531, lot 201411) in nine years from 2006 to 2014. Although robust CV fluctuated largely among different lot, there was no obvious change trend among different years.

Conclusions: The POC glucose meter measurement performance in China has been improved gradually from 2006 to 2014. Laboratories should make persistent efforts to obtain better results in the future.



B-145

Implementing Lean Six Sigma Methods to Improve Workflow in the Clinical Flow Cytometry Laboratory

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Introduction:

Lean and Six Sigma process improvement methods have been utilized in the manufacturing industry for decades, and are now being applied to healthcare settings. In order to meet increased demand for Flow Cytometry evaluation for oncology and HIV patients in VT and NH while decreasing overtime; currently in excess of 20K annually for our laboratory section, now is the time to create capacity within Hematology and Flow to take on additional work without additional staffing.

An opportunity exists in the Flow Cytometry Lab to align the work load & staffing. Having the right person doing the right tasks with a goal to reduce the amount of technologist time spent on redundant and inappropriate tasks such as; manual ordering, paperwork, checking pending lists & bone marrow logs or physically checking the in basket for samples.

Materials and Methods:

The clinical Flow Cytometry workflow improvement project had multiple phases: documentation of the current state to establish baseline performance; workflow observations to determine areas for improvement; and review of work practices to determine/establish best practice for improvement. Process maps were created to document the current state of the workflow and a fishbone diagram was used to focus the group.

Laboratory or hospital information system solutions and technology were used wherever possible to simplify processes. Printers, worklists and pending lists were implemented during this phase. An additional goal was set to have the right duties assigned to the right role; i.e. technical staff performing technical tasks, clerical staff performing clerical tasks.

In the analysis phase, new process maps were created showing the reduction in steps with the improved workflow. Calculations were performed to determine the number of samples with automated/electronic orders versus those ordered by the flow cytometry technical staff.

Results:

At the start of this project the technical staff was ordering 80% of the immunophenotyping testing; they are currently ordering 6%. All workflows were improved, some as much as 80%. The laboratory has been able to implement a TAT monitor for immunophenotyping. The original goal set for this monitor was 24 hours; however the longest TAT since project completion was 20.50 hours.

Conclusions:

Within three months, the implementation of Lean workflow processes improved employee satisfaction, TAT, overall patient care and reduced waste in the clinical flow cytometry laboratory. Process improvement strategies, like Lean, should be embedded into all workflow analyses in a clinical laboratory.

B-146

Use and overuse of Immunofixation electrophoresis and Serum Free Light Chain assays

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Background: We examined the usage of serum protein electrophoresis (SPEP), serum immunofixation electrophoresis (SIFE) and serum free light chain assays (SFLCA) at a tertiary, medical school affiliated, 500 bed hospital. These tests are generally used as screening tests for diagnosis of monoclonal gammopathies. International Myeloma Workshop Consensus Panel 3 recommendation for diagnosis includes testing for SPEP, SIFE, urine protein electrophoresis, immunofixation electrophoresis, serum free light chain assay, bone marrow examination, cytogenetic studies and skeletal survey for diagnosis. Many of these tests are repeated to assess the response to treatment.

Methods: We assessed the utility of repeat SIFE and SFLCA to evaluate the status of patient on follow-up visits using the following criteria: if a monoclonal spike was not detectable on SPEP or was not quantifiable, SIFE and SFLCA were considered to be warranted. If monoclonal spike was detectable and quantifiable by densitometric scanning on SPEP, then SIFE and SFLCA were considered to be not warranted. If there was any doubt about the added value of SIFE and SFLCA, it was considered warranted. Serum immunoglobulins were also measured frequently, but we lacked objected criteria to judge the utility of this assay.

Results: Data from 184 patients yielded the findings shown in the table below.

Patients	SPEP	Peak	IFE	# Warranted	% Warranted	SFLC	# Warranted	% Warranted
184	1211	718	862	386	53.76	743	325	43.74

Using these criteria, nearly half of the SIFE and SFLCA were judged to be non-value added.

Conclusions: We recognize the value using these tests to establish a diagnosis of monoclonal gammopathy, but repeating of the tests does not add value. We are proposing that ordering option for these tests be limited to "SPEP for monoclonal gammopathy" and the pathologist signing out have the discretion to order additional tests as needed. This process has been shown to reduce the inappropriate utilization of SIFE by 53.7%. We believe that the same can be accomplished for SFLCA.

B-147

Implementation of six sigma as a quality control management tool

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Introduction: A priority for healthcare providers is to improve patient care with the overall objective of achieving patient satisfaction. Since laboratory results contribute significantly to patient diagnosis, the clinical laboratory plays an important role in improving patient care. The aim of this study was to see how Six Sigma implementation could improve our QC management.

Method: QC data for quantitative routine assays performed on the ADVIA 1800 Clinical Chemistry System (Siemens Healthcare Diagnostics, Tarrytown, NY) in our reference core laboratory were used for calculation of sigma metrics. Most of the selected quality goals were based on biological variation (BV) quality specifications; others were based on external quality assessment (EQA) peer capability and CLIA regulations. Biorad Unity software was used in calculation of sigma metrics and Westgard rules. Analyzer performance was compared to that of the market best performer as the standard.

Results: The ADVIA 1800 system's performance placed it among the world-class instruments. We were able to benchmark 62% of tests on-board at higher quality goals (at least the desirable BV quality goal). In addition, 89% of the tests under the study achieved sigma metrics greater than 4 ("fit for purpose"). Chol, Urea, Triglyceride, ALT, GGT, Fe and CK were some of the assays that were comparable to the industry best performers. Others with sigma metrics greater than 6 but not as good as the top performers included LDH, CREA, GLU, AST, PHOS, UA, HDL, AMY, DBIL, and TBIL. K and ALP had acceptable performance, with sigma metrics greater than 4 but less than 6. Others, with sigma metrics less than 4, included NA, CA, CL, TRF, ALB, and TP.

Conclusion: Our first experience with a Six Sigma quality approach was positive. Sigma metrics not only served as a quality indicator but also helped us to focus more on problematic assays. We were able to optimize error detection and minimize the false-rejection rate. As a result, patient care has benefitted from improved QC management and higher quality analyses.

B-148

Accreditation Inspection Case Study: How does ISO 15189 enhance CLIA regulations in laboratory compliance?

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The American Association of Laboratory Accreditation (A2LA) is the only international and national recognized laboratory accreditation organization that assesses clinical laboratories against both the CLIA'88 regulations and the ISO 15189:2012 Standard. In recent years, US laboratories have developed modest interest in incorporating ISO 15189 into their quality management system. However, many laboratories still rely solely on compliance to the CLIA regulations. CLIA laboratories that are not ISO 15189 accredited could potentially be missing opportunities to reduce systemic errors that cause decreases in efficiency and quality. In this study, we utilized long-term deficiency data derived from a subset of well-established CLIA labs that have also undergone an assessment against the ISO 15189 Standard. The study revealed that, of the findings cited during ISO 15189 assessments, approximately one-third were due to insufficiencies within the quality management system. When these findings are compared to the CLIA standard, a significant number of them would potentially go undetected during a laboratory assessment to the CLIA standard alone. Our findings establish the positive manner in which ISO 15189 enhances the CLIA regulations and why it is imperative that laboratories address quality system indicators that ultimately impact overall efficiency.

B-149

An Analysis of Specimen Rejection Rates in a Clinical Laboratory in a Medical Center

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Background

Specimen quality is crucial to laboratory reporting accuracy. Based on the previous researches, 50 to 70 percent of errors in the medical laboratory are from pre-analytical phase. Monitoring sample rejection rates can be an effective way to track pre-analytical errors, prevent avoidable errors, and provide better health care.The study aimed to determine the performance for the pre-analytical phase in an ISO 15189 accredited laboratory of a medical center.

Methods

The one-year period specimen rejection rates from emergency department, inpatient department, and outpatient services were evaluated. Moreover, types of error were investigated to discover the underlying causes of the problem.

Results

A retrospective review showed that 21,867 of the 1,188,120 specimens in 2014 were rejected, indicating an overall rejection rate of 1.8%.The rejection rates for the emergency department, inpatient services and outpatient services were 2.9% (6,629/232,589), 4.2% (14,572/345,403), 0.1% (666/610,128) respectively. The main reasons for rejection were hemolysis (44%) and inappropriate clotting samples (22%). According to the testing groups, chemistry ranked first (41.8%, n=9132), followed by hematology (22.8%, n=4977), coagulation (16.0%, n=3495) and gas analysis (5%, n=1096). Further analysis showed that the median turnaround time for the emergency department was 14 minutes, but 23 minutes for the redrawing.

Conclusion

Specimen rejection delayed the turnaround time, increased costs, and caused clinical problems. Hemolyzed and clotted samples were found to be the primary factors resulting in specimen rejection. Also, the rejection rates varied considerably in different departments. A rejection rate improvement project in the emergency department was conducted in 2004, leading to the decrease of the rejection rate from 4.4% to 1.1% that year. Therefore, the study recommended that the importance of specimen quality be addressed and continuous training programs be provided periodically.

B-150

Early comunication of non-critical, but clinically relevant, results to physicians by a medical team based outside the central lab.

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Background: Quality and safety accreditors place increased emphasis on the effectiveness with which critical laboratory results are reported to caregivers. CAP Q-Probes Study of 121 Institutions observed that, for all critical results combined, no rapid notification took place for only 10 (0.3%) of the 3545 critical results. On the other hand, notification of a wider group of clinically relevant results, although not considered critical, may benefit patient's health and be convenient to physicians in their daily practice, playing an important role in the relationship between prescribing professionals and the private lab.

Methods: Medical laboratory staff selected 57 tests to have a range of results notified to prescriber physicians, according to previous patient's results. After development and parameterization of LIS based tools and reports, lab medical staff, remotely based outside the central lab, daily accessed the LIS feature, in order to telephone to prescriber and inform the result. If the physician were not reached, patient was not contacted. The team goal was to successfully report 50% of detected results. The aim of the study is to access the dimension and efficiency of a new process of early notification of non-critical, but clinically relevant, results by a remote medical team, during year 2014.

Results: From January to December 2014, out of 63.4 million tests performed at the central lab, the LIS tool detected 1,943 (0.003%) test results parameterized for physician telephone notification. The medical team successfully reported 1,258 (64.8%) of them. We experienced instability of the LIS tool in the first months, and some of its features are still being continuously improved. The major cause of failure in reporting was insufficient prescriber data at LIS to allow proper contact.

Discussion: Although failure to notify caregivers of non-critical, but clinically relevant results may not represent an important patient safety vulnerability, it represents a lost opportunity in building a strong and reliable relationship between the private laboratories and the prescribers. We believe that having selected non-critical results notified by lab physicians brings a ense of higher standard of care for patients and prescribing physicians. Notifications done by a medical team, remotely based outside the central lab, and supported by LIS tools and reports, also allow lab staff to focus in critical results reporting and in lab routines, and may increase lab staff productivity.

B-151

Evaluation of the Analytical Performance of Creatinine in the Medical Decision Points

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Introduction: Given that commercial materials of internal control do not generally cover all the concentrations that represent the Medical Decision Points (MDPs) -where is relevant to evaluate the analytical performance of the tests-, this work shows a model of estimation of the analytical performance in the MDPs for creatinine in serum expressed like sigma metric.

Materials and Methods: A homogenous system, the analytical platform Architect ci8200 and Abbott reagents were used. The method used for the determination of creatinine was kinetic alkaline-picrate. The theoretical MDPs (0.60, 1.60, 6.00 mg/dL) were selected from the tables in Statland BE, Clinical Decision for Levels Laboratory Tests, Second Edition [Oradell NJ; Medical Economics Books, 1987]. In order to estimate the sigma metric in the MDPs, the ETa (Total Allowable Error) was selected from CLIA 88' (0.3 mg/dL and 15%), and it was calculated with the formula Sigma = [ETa (%) - Bias (%)] / CV (%). The bias that represents the systematic error of measurement was estimated as the absolute difference in percentage between the theoretical and estimated MDP, where the estimated MDP was obtained by interpolating the theoretical MDPs in the Deming linear regression obtained by charting the creatinine concentrations from 24 external quality control surveys which ranged from 0.56 to 11.30 mg/dL. The CV (%) which represents the random error was estimated in the MDPs through the equation obtained by charting different creatinine concentrations as a function of the coefficient of variation (precision profile). These data were collected by processing the internal quality control and pools of samples from patients with 11 different creatinine concentrations daily during a minimum of 30 days, together with the results obtained for the limit of quantification verification; the creatinine concentrations ranged from 0,05 to 6.44 mg/dL.

Results and Discussion: The slope of the Deming linear regression was 1.012 (CI 95%: 1.001 to 1.023) and the y-intercept -0.0110 (CI 95%: -0.0622 to 0.0402). The estimated MDPs obtained from the Deming regression were 0.60 mg/dL, 1.61 mg/dL and 6.06 mg/dL, the bias being of 0.00%, 0.63% and 1.00% respectively. The estimated CV (%) in the MDP of 0.60 mg/dL was of 2.84%, in 1.6 mg/dL of 1.71% and in 6.0 mg/dL of 0.86%. The sigma performance obtained in the MDP of 0.60 mg/dL was of 17.6, in 1.60 mg/dL was of 10.6 and in 6.00 mg/dL of 16.3.

Conclusions: The analytical performance calculated for creatinine in the MDPs by using the six sigma metric was highly satisfactory, which allows us to assure the quality of the results in the MDPs. The laboratory considers applying this complementary tool in the future to evaluate the analytical performance of those assays for which the concentrations of the internal quality control are far from the MDPs.

B-152

Statistical analysis and management of quality control data in clinical toxicology laboratory

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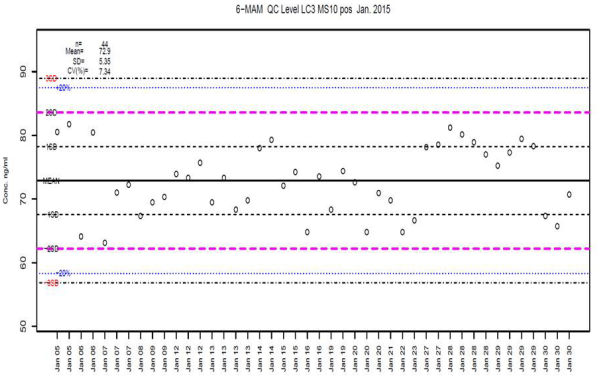
Background. Varieties of high-throughput liquid chromatography with tandem mass spectrometry (LCMSMS) techniques have been applied to clinical laboratory. These include multiplexing liquid chromatography systems, ultra-high chromatography flow rate, high throughput sample preparation and multiple reactions monitoring (MRM) that analyzes multiple target analytes within a single assay. Over the past decades, the amount of data generated each day from each mass spectrometry instrument has continually increased. Researchers and laboratory managers are constantly faced with the challenge of collecting, processing and storing large amounts of data on a daily basis as the result of an increase in samples processed each day and stringent regulatory requirements. A portion of these data are quality control (QC) sample data. We have used an effective method to manage and statistically analyze these data.

Method. The method used multiple scripts written with R statistical programming language to read QC data from multiple output files generated from mass spectrometry instruments and performed statistical analysis. The scripts sorted QC data based on the analyte name, QC level, test panel, and instrument. User-defined functions, control structure, reshaping data, and other data management techniques have been used. By executing scripting programs, multiple tables and plots were produced.

Results. Monthly QC results including mean, outlier, standard deviation, correlation variation (CV), bias and six sigma for each of QC levels were calculated and summarized in tables for each instrument. The format of these output tables could be in latex, pdf, htm or excel files. Each QC data point was also presented in Levey - Jennings plot. More than 20 tables and 2500 Levey-Jennings plots were produced monthly.

Conclusions. The method simplifies data management, saves time, and reduces costs. It significantly facilitates the process of periodical review and evaluation of QC data by laboratory managers and researchers.



B-153

Evaluation of events involved in the pre-analytical phase and the financial impact: a case of risk management study.

J. P. Padilha, M. Provasi, A. C. S. Ferreira, T. F. Barros. *Hermes Pardini Institution, Belo Horizonte, Brazil*

Background: The pre-analytical phase is a complex process that involves a series of steps, among them, first consider the request of the examination by the medical application, patient preparation and identification, collection of diagnostic specimens, transportation, preparation or screening. In addition to cost the patient's health, since about 70% of clinical decisions are informed in laboratory tests, there is a financial cost to the clinical laboratory generating impact on the overall revenue of the institution.

Objective: This study aims to quantify the cost of recollect caused by errors in the pre- analytical phase, of a private laboratory. To survey the costs involved industry-focused where it is performed sample collection of the patient in an outpatient clinical laboratory and verify the financial impact of this occurrences.

Methods: All data presented in this study were collected through computerized system of data collection (Sadig Análises®). Data on recollect one of a large private laboratory service unit were selected in the period of eleven months (Jan / 14 - Nov / 14). Based on these data was quantified the cost of labor added to the cost of material used to make the procedures for collection of diagnostic specimen. All financial data used were based on the Income Statement Report, of the same reporting period. The costs of process, analysis and sample transport were not included.

Results: In this scenario, were raised in 1442 pre-analytical occurrences, of which 24,48% of samples were contaminated to microbiology analysis, noting as the occurrency that was more frequent in the collection. Values obtained at the Income Statement Report were respectively of R$ 3,34 per test of personnel costs and R$ 0,48 per test; resulting of the total of R$ 3,82 of the total cost of recolletc of one exam . This total value was multiplied by total data of recollection requested, resulting in a financial impact of about R$ 5,508.40 per year.

Conclusion: We must consider the current quality internal processes that directly reflect the results of this study. Was performed about 754.000 examinations during the study period resulting in a percentage of pre-analytical cases of approximately 0,2 %, resulting, a low percentage, owing to intense work training, and optimization flows/processes focusing on progressive decrease in the number of recollect. Within this Optics, we can reflect that this cost when compared with a high percentage of pre-analytical occurrences, will rise significantly. As seen in studies, when we estimate the value of the pendencies, the cost can rises to 55,6 %, at least. So, we might consider that from the moment that raises the number of recollections cases, consequently the cost will raises persistently, therefore, the absence of a continuous improvement working with emphasis on recollect reduction has an important impact in the financial health of the private laboratory.

B-154

Verify the Analytical Measurement Range (AMR) in the clinical laboratory: a proposed tool

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Background: The linearity range evaluation defined by the manufacturers for analytical systems is a guarantee of the results' accuracy and reliability in the lower and higher values of the method range. Moreover, it is a requirement for many clinical laboratories quality accreditation programs. The development of a statistic protocol to aid Analytical Measurement Range (AMR) monitoring is a challenge for laboratories managers who need to guarantee workflow and quality with a low cost in the clinical laboratory. Objective: To present a new statistical tool and a protocol procedure for the monitoring of the AMR based on the CLIA '88 requirements and concepts review to verify the linearity for analytical systems. This tool named Easy Linearity Curve (ELC) provides a quick and effective data evaluation for clinical assay systems. Methods: The ELC was used as a monitoring tool for clinical chemistry and immune-hormone analytical systems in a clinical laboratory. The tool suggests the preparation of patients samples' pools selected from laboratory routine, including lower, zero, and higher concentrations within manufacturer's linearity range. In order to prepare them, samples storage and stability conditions were observed and five samples were processed in duplicate or triplicate during the laboratory routine. Then, ELC performed statistical analysis including the estimated bias, total error, coefficient of variation, linear regression, second and third degrees polynomials regressions. As the results were inserted at ELC, it generates statistical analysis, linear regression and scatter plots for each measured point. The criteria tools were based on CLSI guidelines and Dr. Westgard's established rules that consider appropriated results if they were within criteria of analytical quality specifications.**Results:** The method is considered linear if coefficient of regression is above 0.99 for the first degree polynomial model and if the coefficients for the cubic and quadratic terms are statistically equal to zero for the second and third degree polynomial model at 5% of the significance level. ELC monitored analytical systems according to Quality Assurance expectative when following tool's recommendations. Below an example of immune-hormones studies made in the laboratory: **Conclusion:** This tool establishes a self-inspection program and assures the efficiency and accuracy of procedures and results. The use of an AMR tool to evaluate assay linearity under four different statistical criteria provides additional safety and reliability to clinical laboratory results. After ELC implementation the laboratory could evaluate costs reduction, guaranteeing internal management of quality processes ensuring the accomplishment of accreditation programs and process standardization.

B-155

Enhanced performance of a Clinical Laboratory with the use of Auto-verification

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Background:

LEAN principles, focused on the reduction of unnecessary operational steps, were previously applied to the pre-analytical portion of the Clinical Laboratory. The improved performance was measured by the reduced time to release test results or turn-around time (TAT). In the spirit of continuous process improvement (CPI), the Clinical Laboratory strove to further reduce the laboratory's TAT for release of patient results. The Clinical Laboratory targeted the post-analytical, result-releasing process for the next step of improving TAT goal achievement, by the implementation of auto-verification.

Methods:

The TAT results for 36 routine Chemistry and Immunology assays were calculated from the operational timestamp data in the Laboratory Information System (LIS), spanning pre-analytical, analytical and post-analytical processes. Flanking data from one week before and one week after implementation of auto-verification were compared to determine the performance impact of auto-verification on the percentage of TAT goals achieved.

Results:

The TAT achievement goals of 49,310 tests before, and 48,273 tests after auto-verification implementation, were compared to evaluate the impact upon TAT. The following results were observed:

Laboratory Process	Before	After	Change
Total tests reviewed	49,310	48,273	
Pre-Analytical Process	21 min	22 min	4.76%
Analytical Process	43 min	39 min	9.30%
Post-Analytical Process	11 min	6 min	45.45%
Average TAT	75 min	68 min	9.33%
Achievement of TAT goals	**97.10%**	**98.90%**	

Auto-verification assisted the lab to realize an overall reduction of 7 mins in the average TAT, with the greatest improvement in the post-analytical processing time (45.45% reduction).

Conclusion:

The implementation of auto-verification in the post-analytical processes enabled the Clinical Laboratory to achieve performance improvement. The results demonstrated significant reduction of TAT in the post-analytical process, leading to an overall increased percentage achievement of TAT goals.

B-156

Celiac disease diagnosis. Are endomysial antibodies still necessary?

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Background:In the last 20 years the diagnosis of celiac disease (CD) has changed. The development of the tissue transglutaminase type 2 (tTG2) antibodies as the target antigen of the endomysial antibodies (EMA) and the subsequent development of anti-tTG2 immunoassays has by far contributed to this new scenario. Anti-tTG2 was the recommended first-line serologic screening tool for identifying individuals at risk for CD, replacing the EMA for this purpose. The longer and more expensive EMA measurement procedure, the operator dependence, subjective interpretation and variability between observers and centers, has also contributed. However the new European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines for the diagnosis of CD, states the need to confirm positives anti-tTG2 results with EMA measurement. Also that serological tests are sufficient for diagnosis (avoiding intestinal biopsy (IB)) in certain cases: children or adolescents with symptoms or signs suggestive of CD (including atypical symptoms) presenting a tenfold multiple of the upper limit of normal (equivalent to the optimal cut-off) of anti-tTG2 test (anti-tTG2>10x ULN), or if EMA and human leukocyte antigen (HLA) DQ2 or DQ8 are both positives.

The aim was to compare anti-tTG2, EMA and IB results in children presenting anti-tTG2>10x ULN, to find out if EMA measurement is necessary or if it is just a redundant test; also the concordance between serological tests and IB, when requested.

Methods:Our laboratory is located at a public University Hospital that serves a population of 234551 inhabitants. From January 1st 2012 to December 31th 2014 children suspected of having CD with anti-tTG2>10x ULN were prospectively included in the study. Patients having immune globulin A deficiency or gluten-free diet were excluded. EMA immunofluorescence methodology used sections of distal monkey esophagus and anti-tTG2, an enzyme-linked immunosorbent assay using human recombinant tissue transglutaminase (ELiA Celikey IgA kit Phadia AB, Uppsala, Sweden). Through a retrospective database search in our Laboratory Information System every children or adolescent (patients younger than 16 years old) with an anti-tTG2>10x ULN was searched. Every patient medical record was reviewed to find out IB result and final diagnosis.

Results:In the period of the study there were 66 anti-tTG IgA>10x ULN. EMA was requested in 22 cases and all results were positive. All of this 22 patients expressed HLA-DQ2 or HLA-DQ8. 12 patients were diagnosed as CD without a IB and in 10 IB was requested. Of them, 9 had a positive result and one did not confirm the diagnosis of CD. In that case faecal test indicated the presence of Giardia Lamblia.

Conclusion:Serial use of EMA and anti-tTG2 to CD diagnosis when anti-tTG2>10xULN could be redundant, and IB is frequently demanded despite ESPGHAN guidelines. Giardia Lamblia infection is a cause of false positive anti-tTG2 and EMA test. More studies are necessary to demonstrate the utility of the confirmation of the anti-tTG2>10xULN with EMA, and the serological tests sufficiency for CD diagnosis in certain cases.

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A Prospective Assessment and Physician Satisfaction Survey of Repeated Chemistry and Hematology Critical Results

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Background: Critical results alert healthcare providers of imminent danger that requires prompt intervention. Although not mandated at the national level, many clinical laboratories confirm critical results prior to their release; a practice that is not performed for non-critical results. Studies have examined the utility of confirming critical results but have not fully assessed the physicians' perspective regarding this practice. Our objectives were to corroborate that repeated critical results are unnecessary and to examine our physicians' opinions regarding the clinical laboratories' critical value procedures.

Methods: First, we prospectively evaluated selected critical results across five large hospitals (3 adult academic, 1 adult community, and 1 pediatric) during a 2-month period. The tests included hematocrit, hemoglobin, INR, platelet counts, PT, aPTT, WBC, ammonia, blood urea nitrogen, calcium, chloride, creatinine, cortisol, ethanol, glucose, lactate, magnesium, phosphorus, potassium, sodium, TSH, total bilirubin, total protein, troponin I, carbamazepine, digoxin, gentamicin, lithium, phenobarbital, theophylline, free phenytoin, and vancomycin. Blood specimens routinely submitted to the clinical laboratory were analyzed on the following instruments: hematology, DxH800 (Beckman Coulter, CA); chemistry, AU5822/AU680 (Beckman Coulter) or Dimension Vista 1500 (Siemens, DE); and immunoassay, DXI800 (Beckman Coulter). All specimens with critical results were reanalyzed to confirm the initial result per our laboratory protocol. The percent change was then compared with total allowable error criteria using the CAP and CLIA guidelines except for ammonia and troponin I, for which the American Association of Bioanalysis proficiency testing guidelines were applied. Subsequently, the clinical significance of repeated specimens via patient chart review and calculated paired t-tests were evaluated. Secondly, we developed and electronically distributed a survey (REDCap electronic data capture tools hosted at the University of Pittsburgh) to assess physician opinions concerning the current critical value list, procedures for confirming critical results prior to release in the EMR, and current and potential new methods to communicate critical results to healthcare providers.

Results: A total of 2,060 critical results were examined, and 34 exceeded the total allowable error. One result was clinically significant when repeated: ammonia (89 µmol/L versus 6 µmol/L). The discrepancy was attributed to a delay in re-analysis. Eighty-three percent of repeated analyte delta values were statistically significant (p value >0.05). There were 149 physicians with medical privileges across 11 hospitals representing anesthesia, emergency medicine, family medicine, internal medicine, surgery, and radiology that participated in the survey. Overwhelmingly, 92% of the physicians were satisfied with the current critical result list. Eighty-nine percent of physicians were in favor of the laboratory re-analyzing specimens before reporting the critical results. Interestingly, 72% preferred the current procedure in which the laboratory calls the results to healthcare providers, compared with 44% who welcomed the opportunity to have critical results communicated via HIPAA-compliant text message and email (percentages reflect physicians' selecting more than one preference).

Conclusion: Our data suggest that confirming critical results may not be warranted, but physicians prefer that the laboratory confirm and verbally communicate critical results. Communication of our findings with the physicians may impact their preference and collaborative efforts utilizing evidence-based laboratory medicine will be used as a model.

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Internal service culture and its influences on the quality of external customer service (investigation - SERVQUAL)

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Introduction: The quality of the production services in Diagnostic Medicine was always a present worry. Meanwhile, support activities (services offered within organizations to their own employees) have been relegated to a lower-level position, with unknown consequences.

Objective: The objective of the study was to evaluate the quality of service of 14 corporate processes and their possible impacts on the external client.

Method: It deals with a descriptive, quantitative investigation, whose data was given statistic treatment to analyze the results. The investigation consisted of 260 participants. The quality of service was measured for each of the five dimensions of the SERVQUAL scale and the identified satisfaction gap. The data collected was subjected to statistic calculations: medium, mode, and standard deviation. To ensure the consistency of the questionnaire, the linear correlation was calculated for both of the modules, Expectations and Perceptions, assuming a value of $r \geq 0.30$ for affirmative validation.

The quality of support services was calculated by the P-E (Perceptions - Expectations) difference, using the scores attributed to each affirmative module.

Results: The lowest average of service quality perceived was registered in three affirmatives related to Reliability: Services carried out by the promised deadline (-1.59); User informed about when the service will be carried out (-1.52); Services carried out correctly the first time (-1.47). **Conclusion:** The results indicate priority aspects to be improved (a) the communication with users regarding data for carrying out requested services; (b) the conclusion of the services by the promised deadline, and (c) the trust that the user has that their problem will be resolved. These critical points mostly reflect the importance that clients give to aspects related to reliability and show that they expect their support-related requests to be addressed correctly and reliably.

B-159

Is CV related to Productive Efficiency in the Clinical Laboratory Setting?

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Background: In the evaluation of renal function, estimating glomerular filtration rate (GFR) is the most used laboratory test. In our Laboratory we used Jaffe based test with low productive efficiency and proposed to change to aminohidrolase/oxidase method. We aimed to optimize production efficiency and to improve the accuracy of creatinine test, combining productive efficiency with the accumulated biannual inaccuracy of the results of the internal quality control.

Methods: The creatinine test was changed from colorimetric (Jaffe) to traceable enzymatic (Amidinohidrolase / Oxidase). We calculated the production efficiency and the accumulated biannual imprecision from 2013 to 2014. Productive efficiency is the ratio between the quantity of tests performed by presentation and the quantity of tests reported by the manufacturer. The biannual cumulative inaccuracy of the results of level 1 and 2 related to the internal control materials was calculated by the Pooled $CV = \sqrt{(\Sigma CVi2 / n)}$. We used chi-square test to evaluate if the Productive Efficiency of the test increased when occurred the decreasing Pooled CV of Level 1 and Level 2.

Resultados: The main problem observed was the presentation of each reagent bottle. Dedicated reagents optimized the onboard stability, precision and accuracy. An association between increased production efficiency and reduction of CV Pooled Level 1 ($p = 0.0014$) and level 2 ($p = 0.0240$) was observed.After mapping the daily demand and the activities we requested the manufacturer a new bottle size compatible with daily consumption, optimizing onboard stability and obtaining an effective reduction of 76,5% over budget.

Conclusions: Mapping the daily demand demonstrated that the use of a specific, precise and accurate test for creatinine facilitates obtaining higher productive efficiency and optimizes the financial resources of production.

Table 1 CV = Coefficient of variation EP = Productive efficiency

Metrics	1st Semester 2013	2nd Semester 2013	1st Semester2014	2nd Semester 2014
Pooled CV Level 1	7,06	5,81	1,78	2,12
Pooled CV Level 2	4,94	2,79	1,41	1,5
EP	45,82%	48,62%	95,72%	84,22%

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Follow up of Productive Efficiency on a Clinical Laboratory

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Background: Productive Efficiency occurs when a productive model explores the full potential of is installed capacity. We aimed to determinate the importance of periodic measurement of Productive Efficiency in production lines in a Clinical Laboratory.

Methods - We Adaptated the concept of Overall Equipment Effectiveness (OEE) on sample processing lines, followed by periodical measure of overall production efficiency of inputs directly connected to production of clinical laboratory tests. Productive input efficiency was calculated as:

EP (%) = Cr/Ci. Where EP (%) = productive input Efficiency; Cr = actual production capacity of the input; Ci = Ideal capacity of input output.

We stated the premise of 100% of maximum performance considering as high performance indicator the number of tests described by the manufacturer. The complementary of EP(%) (100% - EP(%), represent the global lost in the production line. This value was followed as an internal indicator of performance, focusing on investigation of causes of low efficiency (defined as 30%) such as loses or fails.

Results:After the 1st measurement with efficiency below designed by the manufacturer, were mapped all line for production processes, indicating the points of improvement and barriers in all production system.

Over the period and implementation of improvement actions we eliminated five barriers identified as impact points to low overall productive efficiency, reaching the "state of the art" in 4th quarterly measurement.

The importance of implementing the periodic measurement of Efficiency of Production Lines on a Clinical Laboratory context could be measured as maximization of resources in high-volume production. Applying periodic monitoring and promoting improvement actions in production processes, the efficiency indicator improved the budget in 23.13%. Implementation of production efficiency monitoring enables a simple way to evaluate the availability of tests described in the manufacturer specifications, in addition to monitor quality problems and other interference tests on the overall production system efficiency.

Global Production Efficiency Ratio measures in quarterly period.				
Period	1st Measurement	2nd Measurement	3rd Measurement	4th Measurement
	75,82%	82,59%	91,47%	98,95%

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Nonconforming Event Reporting: Essential Element of a Quality Management System

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Background: Errors in the clinical laboratory can lead to adverse clinical outcomes and significant financial and health system costs. Identifying these errors and preventing them is a key part of the quality assurance system of the laboratory. Analysis of nonconforming events, that is, events that are not according to rules or expectations, has been seen in other industries, such as aviation, as a way to prevent adverse events and help to develop a culture of safety. These events, even though they may not lead to significant errors in and of themselves, can be used as part of the continual improvement process. Ongoing monitoring of these events can show the effectiveness of the plan-do-check-act cycle. Capturing these events in an analyzable format can be challenging: the only system available that even approximated capturing these events was almost totally paper based and used a variety of forms and event criteria that varied across divisions.

Methods: A subcommittee was established to address capturing nonconforming events. A policy was drafted describing guidelines for accomplishing this within the laboratory. Three separate forms were created on the laboratory intranet Sharepoint site, incorporating the information previously captured on the paper logs throughout the lab and at the same time fulfilling the regulatory requirements. These included a Nonconforming Event Log, A Customer Complaint Log, and a Downtime/QC Issues Log. Workgroups at all levels, bench technologists through management, provided feedback on the forms to further refine their utilization in each division. It was decided that these forms were to be filled out by the person recognizing the nonconforming event, in real time, and not by a supervisor or manager. Criteria for reporting an event were made as uniform as possible across laboratory divisions and the relationship to the UHC event reporting system used by the hospital was established.

Results: The rollout of the new nonconforming event reporting system went live in February 2014.Staff were encouraged to document their nonconforming events in the appropriate Sharepoint log. Management was asked to support the transition and begin reviewing the information entered. There was some initial resistance to the electronic system and implementation was not totally uniform across laboratory divisions. Eventually, however, the number of events recorded increased to approximately 1300/month and totaled 15383 in one year. The increase in reported events which carried over into the hospital event system actually caused concern in the hospital outside the laboratory until the new program was explained.

Having the logs in Sharepoint provided a central database from which each divison could create custom reports to analyze these events. Reports can be created from numerous criteria to isolate specific information, such as all events of Critical Severity within a time period. Several divisions in the laboratory have developed specific programs to address recurrent events and prevent potential serious errors. There is a monthly meeting to discuss the nonconforming events and projects related to them across the laboratory.

Conclusion: A nonconforming event reporting system with support across the laboratory staff can be an effective and essential element of a quality management system.

B-162

Laboratory utilization of HbA1c, PSA and BMP in an academic medical center

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Objective: Testing with little or no diagnostic value is a significant source of unnecessary cost to the health care system. Using data available from an academic medical center we evaluated testing patterns for three diagnostic test scenarios to quantify test utilization patterns. The objective of the study is to quantify overutilization of Hemoglobin A1c (HbA1c), Prostate Specific Antigen (PSA), and Basic Metabolic Panels (BMP) to understand the effects of soft stops (warnings with information about indications) in improving utilization.

Methods: Using evidence-based guidelines, we determined test scenarios for which at least one test was largely unnecessary. The patient's medical history was reviewed to understand the indications of the test. The data was collected for one year (October 2013 to October 2014) and additional BMP data (October 2012 to March 2013) to compare the effect of soft stops on lab requisitions. An HbA1c ordered within 90 days is considered overutilization. The PSA ordered in patients less than 40 years, greater than 75 and ordered within 84 days is considered overutilization without any indications. The BMP ordered within 4 hours without any acute indication is considered overutilization. We also analyzed overutilization patterns in different departments, types of practitioners and time trends in utilization. We would like to extend this framework to other tests and regularly monitor the changes to improve overall laboratory utilization.

Findings: The HbA1c was over utilized in 15.16% cases (1112 tests) and the test was performed more than once on the same day in 94 patients. The top 5 departments that over utilized the test (more than 100 tests in last one year) were Neurosurgery/Neurology, Pediatric endocrinology, outpatient lab, Transplant division and gastroenterology divisions. Surprisingly, the top five attending physicians that over utilized HbA1c were from pediatric endocrinology, neurology, infectious diseases and emergency medicine. The PSA was over utilized in 20% (34 tests) tests. The BMP was over utilized only in 3.75% tests (5677). The comparison of before and after soft stop implementation for BMP order shows that overutilization has decreased from 7.01% (October 2012 to March 2013) to 3.77% (October 2013 to March 2014).

Conclusions: The amount of overutilization is significant in attending physicians, which may lead to continuation of these patterns in trainees. Education in divisions where overutilization is common may help improve the laboratory utilization. The data can also provide insight into financial impact on hospital and effect of warnings implemented in electronic health record systems.

B-163

Strategic Planning in the Clinical Laboratory: aligning greater participation of professionals, management excellence criteria and execution effectiveness

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Background:

A structured process of Strategic Planning is critical to the organization success, including clinical laboratories. This process, often have limited participation to directors and managers of companies and has huge challenges in the strategic actions implementation and results monitoring phases. The aim of this study was to propose, implement and validate a new model of strategic planning and execution applied to the clinical laboratory. In this model we propose a broad participation of laboratory personnel, at different levels of depth, alignment with excellence criteria (by Brazilian National Quality Foundation - FNQ, similar to Malcolm Baldrige Excellence Program), balanced structure of strategic objectives (Balanced Scorecard) and execution monitoring with a "Strategic Implementation Chart" for each different process, by the Consultive Board of laboratory.

Methods: The new model was validated in a medium clinical laboratory, in southern Brazil. The process began with a questionnaire distributed to all laboratory personnel. In this questionnaire each professional examined: mission, vision, organization values and comparative market informations (benchmarking). Strengths and weaknesses of the lab, and market opportunities and threats could be analyzed and marked in the questionnaire, according to different criteria of excellence (FNQ). From all this analysis, each team/process suggested strategic objectives balanced across different dimensions: Financial, Customer and Market, Innovation, Processes, Sustainability and People/Organizational Learning. These suggested strategic objectives were validated in one-day seminar, with all the leaders of the company. From this leadership consensus, strategic objectives were defined and, for each of these, were defined responsibility, and an action plan was approved. The strategic actions were shares to all organization professionals by a "Strategic Map" and monitored with the "Strategic Implementation Chart" and using key performance indicators, analyzed quarterly by the Consultive Board of the laboratory.

Results: Among the positive results obtained with the new model, the more important was a higher participation of the laboratory professionals in the strategy formulation process, which led to greater commitment to the proposed goals. The generation of ideas, which resulted in the strategic objectives defined, was more abundant and with greater depth and adaptation level to the current status of the organization and respective market challenges. The new strategy formulation model, aligned to excellence criteria and balanced strategic dimensions, facilitated the consensus of the strategic objectives and definition of responsibilities, with gains in the process of strategic actions execution control.

Conclusion: The new model of strategic formulation and execution proved to be fully adaptable to the clinical laboratory, aligning management excellence criteria and balanced performance dimensions. The biggest advantages were identified as being a broad participation and commitment of professionals, as well as more control in strategy implementation. The effectiveness of the strategic actions positively impacted the laboratory's customers, particularly related to improvements in processes and services, deployed with greater agility and safety.

B-164

Imprecision Investigation and Analysis of Carcino Embryonie Antigen in Clinical Laboratories in China

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Background: Carcino embryonie antigen(CEA) is a tumor biomarker widely used in the diagnosis, treatment and prognosis of a series of tumor. This study aims to investigate the imprecision of internal quality control of CEA so that we can have an overall knowledge of imprecision level of measurement in laboratories in China.

Methods: Internal quality control(IQC) information including cumulative coefficient of variation (CV) and measurement method of CEA were collected via an on-line questionnaire. To calculate the cumulative CV, laboratories had to collect all the results of controls from the first day on which the same lot of control was used to the last day until May 2014, outliers were removed for analysis. Then the percentages of laboratories meeting five imprecision criteria were calculated, which were specifications based on quality requirements (TEa) defined by National Center for Clinical Laboratories in China including 1/3TEa(8.3%) and 1/4TEa(6.3%) and the specifications based on biological variation including the desirable(6.4%), minimal(9.5%) and optimal(3.2%) allowable imprecision.

Results: There were 814 laboratories involved in the program. They were divided into 6 groups based on measurement methods as shown in table below. Pass rate varied largely with different allowable imprecisions. Electrochemiluminescence got the best result among all the measurement methods. The majority of participant laboratories can obtain a satisfied CV when 1/3TEa was applied. However, only a few laboratories can pass the imprecision requirement if optimal allowable imprecision was employed.

Measurement methods	Number of participant laboratories	Median of Cumulative CVs of Chinese Laboratories	Percentages of laboratories meeting quality requirements				
			1/3TEa	1/4TEa	Allowable imprecision specifications based on biological variation		
					Desirable Performance	Minimum performance	Optimum performance
All methods	814	5.97	79.85% (650/ 814)	54.91% (447/ 814)	56.63% (461/ 814)	86.86% (707/ 814)	10.93% (89/ 814)
Electrochemiluminescence	389	5.46	88.17% (343/ 389)	64.01% (249/ 389)	65.81% (256/ 389)	92.54% (360/ 389)	13.62% (53/ 389)
Acridine ester chemical Luminescence directly	205	6.63	67.80% (139/ 205)	45.37% (93/ 205)	47.80% (98/ 205)	77.56% (159/ 205)	7.80% (16/ 205)
Chemiluminescence Microparticle Immuno Assay	88	6.79	76.14% (67/ 88)	43.18% (38/ 88)	43.18% (38/ 88)	85.23% (75/ 88)	4.55% (4/ 88)
Chemiluminescent enzyme immunoassay	73	6.41	72.60% (53/ 73)	47.95% (35/ 73)	49.32% (36/ 73)	83.56% (61/ 73)	12.33% (9/ 73)
Microparticle enzyme immunoassay	22	6.37	81.82% (18/ 22)	50.00% (11/ 22)	50.00% (11/ 22)	90.91% (20/ 22)	9.09% (2/ 22)
Others	37	6.00	81.08% (30/ 37)	56.76% (21/ 37)	59.46% (22/ 37)	86.49% (32/ 37)	13.51% (5/ 37)

Conclusions: As only a few laboratories can pass the imprecision requirement if optimal allowable imprecision was employed, substantial effort is needed for CEA measurement in laboratories in China. Laboratories are recommended to adopt suitable measurement method with smaller CV, with which they can get more accurate results and provide high quality service.

B-165

Comparison of traditional and robust Youden plots in a PT program

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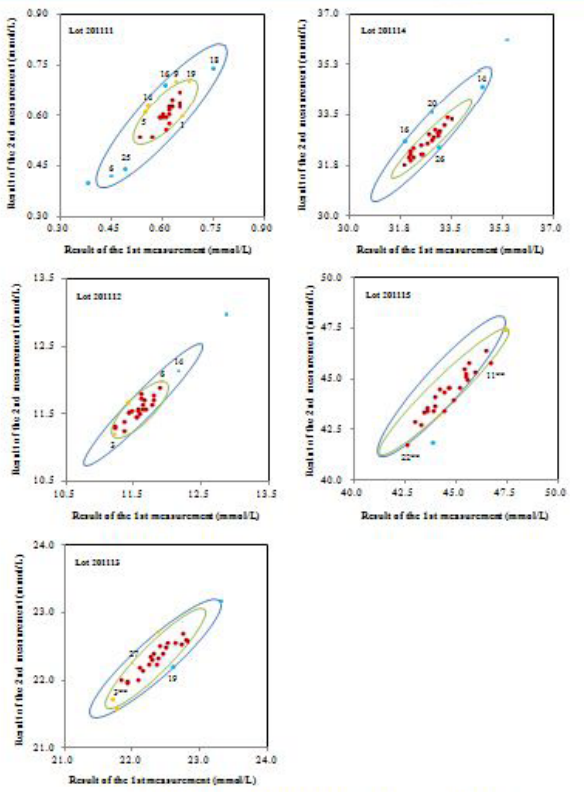
Background: Proficiency testing (PT) is the use of an inter-laboratory comparison to determine the performance of individual laboratories wishing to control the quality of their experimental results. Youden plot is a two-sample diagram for graphical illustration of inter-laboratory analytical results. The traditional Youden plot can be distorted by non-normally distributed data. In the PT program, the data usually are not normally distributed owing to outliers. The purpose of this study is to present an optimized Youden plot named robust Youden plot and to compare this plot with the traditional Youden plot.

Methods: The glucose concentrations of five serum samples (lots 201111 to 201115) were determined by 27 laboratories to provide data for the construction of traditional and robust Youden plots. The data were also tested for normality, using the Shapiro-Wilk test. To construct the traditional and robust Youden plots, non-robust and robust estimators were computed. To compare the plots, robust between-laboratory z-scores (ZB_i) and within-laboratory z-scores (ZW_i) were obtained. The expected results are: $|ZB_i|$ or $|ZW_i| \leq 2$, the corresponding points fall inside the ellipse; $2 < |ZB_i| < 3$ or $2 < |ZW_i| < 3$, the corresponding points fall on or near the ellipse; and $|ZB_i|$ or $|ZW_i| \geq 3$, the corresponding points fall outside the ellipse.

Result: The glucose data of lots 201111, 201112 and 201114 are non-normally distributed and lots 201113 and 201115 are normally distributed. The concordance rates of the expected and actual results for the traditional and robust Youden plots

were 66.7% and 100.0% (lot 201111), 88.9% and 100.0% (lot 201112), 88.9% and 96.3% (lot 201113), 85.2% and 100.0% (lot 201114), and 92.6% and 92.6% (lot 201115), respectively.

Conclusions: The traditional Youden plot can only illustrate normally distributed data; the robust Youden plot can explain data no matter what the data distributions are.



Traditional and robust Youden charts for glucose concentration

Blue line, traditional ellipse; green line, robust ellipse; red dots, $|z| \leq 2$; yellow dots, $2 < |z| < 3$; blue dots, $|z| \geq 3$. Points labeled lab code, lab code* and lab code** indicate that z-scores and the locations of their corresponding points on the traditional ellipse, robust ellipse, and both ellipses are inconsistent, respectively.

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Utilizing Lean Six Sigma to Improve Processes in Hematology

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Introduction: The Dartmouth Hitchcock hematology laboratory supports the established medical and research programs that make DH a world class medical and teaching facility. The hematology lab, through specimen analysis, actively supports the bone marrow and organ transplant program and trauma center. Seeking out and using the latest automation and fluorescent flow cell technology allows us to process over 800 CBCs per day. Hematology still relies heavily on manual methods for peripheral blood scans and differentials. These efforts support the needs of our inpatients, outpatients for the Norris Cotton Cancer Center, and outreach clients throughout NH and VT. An opportunity exists to utilize lean and six sigma process improvement methods to align the 24 hour staffing model in the Sysmex automated hematology work block, including the volume of peripheral blood differential slides and scans. **Materials and Methods:** The original stated goals in the project proposal were to improve staff satisfaction while maintaining turn-around times (TATs). Pre- and post-improvement staff satisfaction surveys were conducted. TATs were continuously monitored. The results of this survey were evaluated using various charts and affinity diagrams. Standard work processes were created and work was redistributed among the various work benches (a work bench is a collection of related tasks assigned to a person on a given day). These processes were then piloted and observed on all shifts. Job aids were created to facilitate the change. Pilots were designed to redistribute a small amount of the work in the early morning hours. The goal of the pilot was to shift some of the early morning diffs from a time with few staff to a time with more staff. The two inpatient heme/onc units (HSCU and 1WST) were selected as they generate the most manual microscope work; these samples are often the most time consuming due to decreased WBC counts or abnormal cells. Quick Wins: We were able to relocate our most frequently used printer to where the staff does most of their work. This printer is used for 2 hour TAT reports, pending lists and work lists. Additionally, we implemented automatic printing of requisitions for smear reviews, fluid reviews, hemoglobin electrophoresis and thrombosis screens upon arrival of the specimen in-lab. This relieved the tech of the task of manually printing each of these, which saves time and also serves as the visual trigger to perform the test. **Results:** The final survey results showed significant improvement in staff satisfaction. 100% employees responding to the survey were positive about the improvements made and there was no degradation in TATs. **Conclusions:** Lean and Six Sigma tools, although developed for manufacturing, are easily applied in the healthcare setting. Use of these tools improved employee satisfaction while maintaining TAT's and quality patient care.

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Retrospective Analysis of Patient Data for Reference Range Verification Following Implementation of New Chemistry Analyzers

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Background:

Our institution transitioned from chemistry analyzers based on dry slide technology (the OCD Vitros®) to one based on wet chemistry (Siemens ® Dimension Vista 1500). Reference ranges were verified for all assays on the new instruments before going live with at least 40 healthy individuals as recommended by the CLSI guideline (C28-A3). Following the implementation of the Vista instruments, clinicians began reporting an increase in the number of patients with hypoalbuminemia without clinical explanation, and questioned the appropriateness of the serum/plasma albumin reference range (3.9-5.1 g/dL for 1-54 years old). Therefore, we investigated the need for a potential change in albumin reference range using retrospective analysis of patient data from the electronic medical record (EMR).

Methods:

JMP software was used to analyze the histogram distributions of 169 and 185 patient albumin results over a period of one month before and after transitioning to the Siemens® Dimension Vista 1500 analyzer, respectively.

Results:

For albumin measurements, the OCD Vitros used bromocresol green dye-binding method whereas the Siemens® Vista uses bromocresol purple (BCP). While the BCP method is known to be negatively biased compared to the BCG method due to its greater specificity for albumin, our initial method comparison using 46 samples showed only a minimal negative bias of 0.13 g/dL for the Vista method. Given this minimal constant bias in the initial method comparison, the reference range of 3.9 - 5.1 g/dL for ages 1-54 years old was not changed with implementation of the Vista analyzer. However, after receiving feedback from clinicians on increased rates of hypoalbuminemia, we compared the distributions of patient albumin results before and after implementation of the new analyzer which revealed a larger negative bias. Specifically, the albumin results before go-live had a median of 4.1 g/dL (with the 2.5th to 97.5th percentile range of 2.6-4.8 g/dL) whereas the albumin results after go-live had a median of 3.8 g/dL with the 2.5th to 97.5th percentile range of 2.2 -4.6 g/dL. The difference in the medians of the distributions indicated a larger negative bias of 0.3 g/dL for the Vista method, larger than the negative bias of 0.13 g/dL seen on the initial method comparison. This was consistent with the observations made by clinicians that the reference range for albumin of 3.9-5.1 g/dL was inappropriately high, leading to overdiagnosis of hypoalbuminemia in healthy patients. Therefore we lowered the reference range of albumin to the Siemens® recommended reference range of 3.4 - 5.0 g/dL.

Conclusions:

While performing method comparison with 40 samples is recommended per CLSI guidelines, analysis of larger volumes of patient data post-implementation may unmask discrepancies between methods that are not apparent from comparisons using 40 samples. In this case, clinician input triggered a re-evaluation of the albumin reference range, which identified a larger negative bias than was apparent during instrument validation. Therefore, post-implementation data mining and retrospective analysis is a useful tool in lab management for re-evaluation of reference ranges.

B-168

RDC20: What has changed in the Brazilian legislation concerning the transport of biological material?

J. P. Padilha, N. Lima, A. C. S. Ferreira, G. Lima. *Hermes Pardini Institution, Belo Horizonte, Brazil*

Background: The samples to be representative, its integrity should be maintained during the pre-analytical phase, including transportation and handling of the material. In order to set health standards for the transport of biological material of human origin, was published RDC No. 20 on 04.10.2014. The resolution determines the standardization of processes and management of errors, with record of non-compliance in order to ensure the quality of the results of the analysis of these materials.

Objective: Review proposed by the resolution, and indicate what is needed for the health service to suit, in the routine of a large private laboratory.

Methods: Analysis of current legislation and checking each item in the processes adopted in the routine of a private laboratory.

Results: As analysis has been verified as a very important factor, the right direction of the sender's responsibilities, carrier and the recipient, which should be defined and documented. In addition, all the people engaged should receive proper training. It is up to the sender to ensure compliance with the requirements for packaging material, which must be done according to their biological risk category, including packaging, which should be considered the specificity of biological material and the purpose of transport, preserving its integrity and stability as well as the safety of personnel involved in the process. And yet maintain document validation and standardization of the process. The transporter must ensure the necessary infrastructure to process, porting document enabling cargo traceability, and check the packing conditions upon receipt. To outsource the transport, the provider must be licensed and trained. The recipient must ensure that the opening of packaging occurs in an appropriate and safe manner site, according to the specifics of each material.

Conclusion: Observed the importance of the standard from the Brazilian regulatory institutions, the evaluation of processes for compliance is essential, we identified several conducts that already is consistent with the regulator text in our service, and new procedures were implemented. Such as use of bags (packaging) for transport by identifying and seal, housed in a standardized process, in writing, like the standard operating procedure of transport; and other necessary documents and validations necessary. In addition to the traceability of cargo and sample system, the RDC 20 clarifies the procedures necessary to ensure the quality and stability of samples to be analyzed for diagnostic purposes, and it's in the Guide of ANVISA for Transport of Human Biological Material for Clinical Diagnostic Purposes, this manual was published in 2014, and will be updated soon. It is essential to adequacy of all health services, as well as ensuring sample integrity for accuracy of results, non-compliance constitutes a health violation. Those involved in the handling of samples must obey the rules of biosafety and worker health. Also emphasizing the need for ongoing training to all involved.

B-169

Westgard Sigma Verification Program, as a Tool to Improve Laboratory Performance

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Background:

Quantification of improvements in laboratory performance is essential for continuous quality improvement (CQI) programs. The use of Sigma metrics to quantify changes of analytical performances, however verification of performance changes are not addressed by internal or external quality control programs. The Westgard Sigma Verification Program represents an independent external assessment to validate improved Sigma performance to supports CQI goals.

Methods:

The performance of analytical process is still the most important part of laboratory services, then the analytical performances were calculated by Sigma metrics using Sigma metric equation of "Sigma = [(TEa-%Bias)/%CV]".

Results:

The Sigma values of 28 chemistry assays during Q3 and Q4 2014 were calculated as part of the laboratory implementation of the Westgard Sigma Verification Program. We found that at the beginning of the program during Q3, there were 21 assays (75.0%) with performance equal or better than 6-Sigma, 3 assays (10.7%) with performance between 5- to 6-Sigma, 2 assays (7.1%) with performance between 4- to 5-Sigma, 1 assay (3.6%) with performance between 3- to 4-Sigma, and 1 assay (3.6%) below 3-Sigmal. After the second round of the program during Q4, there were 23 assays (82.1%) with performance at or above 6-Sigma, 2 assays (7.1%) with performance between 5- to 6-Sigma, 1 assay (3.6%) with performance between 4- to 5-Sigma, 1 assay (3.6%) with performance between 3- to 4-Sigma, and only 1 assay (3.6%) with performance below 3-Sigma.

Conclusion: We found that participation in the Westgard Sigma Verification Program aided our laboratory in understanding the analytical performance of our assays as well as the choice of quality requirement for each assay. This further allowed us to focus our continuous quality improvement (CQI) activities to target the most problematic assays. We also identified which assays were significantly impacted by the choice of allowable total error (TEa), particularly when comparing performance between laboratories. We plan to implement multi-stage SQC designs to ensure equivalent analytical quality throughout all patient testing cycles.

B-170

Strategies to increase General Practitioners awareness of clinical decision making laboratory results: The vitamin B12 experience.

M. Salinas[1], E. Flores[1], M. Lopez-Garrigos[1], M. Leiva-Salinas[2], J. Lugo[1], M. Ahumada[2], C. Leiva-Salinas[3]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]Universidad Miguel Hernandez, San Juan, Spain, [3]University of Virginia, Charlottesville, VA*

Background: It is considered that laboratory workers should also think about the steps that occur outside the laboratory and thus prevent errors related to the interface between laboratory and clinician.

However, post-post-analytical phase, when the test result is received, interpreted and acted upon, is less studied. In fact, 25 to 46% of laboratory errors are referred to be generated for delayed or missed reaction to laboratory reporting, incorrect interpretation, inadequate follow-up plan or failure to order the appropriate consultation.

Vitamin B12 deficiency should always be immediately treated, because may lead to DNA damage, cognitive decline or dementia and because the lack of toxicity of vitamin supplementation. However in our setting a percentage of primary care patients presenting vitamin B12 results below 100 pg/ml did not receive vitamin B12 supplements.

The aim was to find out if General Practitioners (GPs) awareness of vitamin B12 deficits results has improved after strategies design and implementation.

Methods: The laboratory is located at a Public University Hospital that serves a population of 234551 inhabitants and receives samples from inpatients, outpatients and primary care patients. Laboratory requests are made electronically from the primary care patient's electronic medical record (PEMR) by the GPs and the reports are automatically sent from the laboratory information system (LIS) to the PEMR.

Through a retrospective database search in our LIS (from 1st January 2007 to 31th May 2014) primary care patients with a result of vitamin B12 lower that 100 pg/ml were studied. Every PEMR was reviewed to find out if patients had visited their GP, if the result was communicated (laboratory report available in PEMR) and received by the doctor, and if the result was reviewed (patient treated with vitamin B12 supplements before one year period).

Through 2 meetings, laboratory personnel and GPs agreed the criteria to consider that low vitamin B12 results were "interpreted correctly and taken the consequently action": when the patient received intramuscular treatment prescription before one month after phlebotomy.

On 1st November, 2014 to 31th January (post intervention period), a strategy was designed in consensus with GPs to improve clinician awareness of vitamin B12 results lower than 100 pg/ml: firstly automatically (via LIS) is recommended, through a comment on the laboratory report, to treat the patient. Secondly a laboratory report is printed on garish colour paper and lastly in PEMR is signaled the convenience for a patient appointment.

In pre and post intervention periods was compared the rate of vitamin B12 results communicated, received and interpreted correctly and taken the consequently action.

Results: 100% of the 197 low vitamin B12 results of the 197 patients in the pre intervention period were communicated and received. 85% were reviewed and 65 % interpreted correctly and taken the consequently action. In the post intervention period there were 12 patients. 100% of the results were communicated and received, and 92% were interpreted correctly and taken the consequently action.

Conclusion: From the Laboratory and in consensus with clinicians, strategies can be designed to increase GPs awareness of clinical value laboratory results.

B-171

Laboratory Information System utilization as a previous step of guidelines implementation: HbA1c as a tool to Diabetes diagnosis.

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Background: American Diabetes Association recommends requesting HbA1c to every person older than 45, when it was not measured the previous 3 years, as a tool to diagnose type 2 diabetes mellitus (DM).

The prevalence of DM in Valencia Community among subjects older than 18 years is 13.3%; however 6.25% is unknown. By applying recommendations the detection of the disease could improve and hence its prognosis. However, previous to guidelines implementation it is necessary to find out the current pattern of HbA1c requesting to be able to calculate and predict the cost if recommendations were followed. The aim was to find out how many additional HbA1c would have been measured if the test would have been processed in every primary care patient above 45 years with a lab test requested, no HbA1c measured the previous 3 years, not demanded by General Practitioner (GP) and sample availability in the current request.

Methods: The laboratory is located at a Public University Hospital that serves a population of 234551 inhabitants. It receives samples from inpatients, outpatients and primary care patients. Laboratory information system (LIS) (OMEGA 3000, Roche Diagnostic®, Barcelona, Spain) manages laboratory requests of every inhabitant independently of attendance as an inpatient, emergency patient, outpatient or primary care patient.

The strategy consisted in a "quality test", that was automatically registered by the LIS in the request of every primary care patient above 45 years and no HbA1c measured the previous 3 years. It was also studied in which cases HbA1c was not requested by GP, when a Cell Blood Count (CBC) was also demanded (sample availability to measure HbA1c) and the cost if HbA1c would have been measured in CBC specimens as a strategy to detect DM patients.

Results: In one year period, 91219 primary care patients' requests were received. 61955 were older than 45 years. 25242 had not a HbA1c requested the previous 3 years. In 7894 HbA1 was requested by GPs. However in 17348 (68.7%) HbA1c was not requested by the GP in the current request. In 13085 patients, in whom a CBC was requested, there was sample availability for HbA1c measurement. The cost if the sample would have been processed would be 18004 dollars.

Conclusion: Guidelines implementation for DM diagnosis by GPs is insufficient in our population. From the Laboratory and through LIS, it is possible to gather information to be evaluated in communication with all the figures involved in medical process to decide the convenience of strategies design and implementation in order to improve clinical decision making.

B-172

Occupational Safety Management in Clinical Laboratory

A. L. ARAUJO, N. ALMEIDA, L. F. A. NERY, S. COSTA. *Sabin Laboratory, Brasilia, Brazil*

A Clinical Laboratory in Brasilia, Brazil, has an occupational safety management system in place since 2003 and, among the indicators monitored and followed up, one of them is the work-related accidents. These accidents are classified as typical (when they occur in the laboratory and are inherent to the activities of the professionals involved), needle-stick injuries (during the collection of biological material) and transport accident (Transport home-work, work-home). Methods: when accidents occur, they are reported immediately to the health sector and company security, which communicate this accident to the governmental organ.The worker is sent for medical care where an investigation around the cause of the accident is made to take such actions as prevention and control and retraining. Results: In 2014 there were 23 occupational accidents, 7 typical, 8 needle-stick injuries and 8 transport accident. In 1.796 million customers served, there were 12.8 accidents per million. Conclusion: According to the security and control measures established, training and routine inspections, and compared to national benchmarking, the series of work-related accidents is small, concluding that the rules and occupational safety management practices have been satisfactory.

B-173

Risk-Based Quality Grades - An Alternative to Sigma Metrics

Z. C. Brooks[1], K. A. Przekop[2]. *[1]AWEsome Numbers Inc., Worthington, ON, Canada, [2]Rutgers, The State University of New Jersey, School of Health Related Professions, Newark, NJ*

Objectives

1.to introduce the Risk-Based Quality Grade© (R-B-Q Grades©) as a metric to:

a.combine the current risk of the analytical process producing a medically-unreliable result with

b.the potential risk of the quality control process failing to detect a clinically significant error

2.to compare the R-B-Q Grade to sigma as a metric to manage patient risk

Methodology:

1.We examined analytical process quality and quality control practices from three distinct groups separated by dates and QC practice, with a total of 46 laboratories, 274 methods, and 612 QC samples for a total of seven analytes.

2.For each Q.C. sample, we gathered: 1. Measured mean; 2. Measured SD; 3. Peer mean; 4. TEa limit, 5. Q.C. Chart assigned mean; 6. Assigned SD; and 7. Q.C. rule(s)

3.We used Quality OptimiZer™ software to:

a.measure the Current Risk Level as the percent of results that currently fail TEa limits and may be medically-unreliable;

b.simulate a shift that would cause 2.3% of results to fail TEa limits;

c.recommended a mathematically-optimized 5-part Q.C. process;

d.measure the Potential Risk Level as the probability that a patient tested after the simulated error will be exposed to unacceptable risk

e.compare the effectiveness of current and recommended QC processes to detect this significant shift

f.grade patient risk based on current and potential risk

g.measure Margin for Error (to calculate sigma)

4.We compared the interpretation of acceptability of quality and probable action based on R-B-Q Grades and sigma

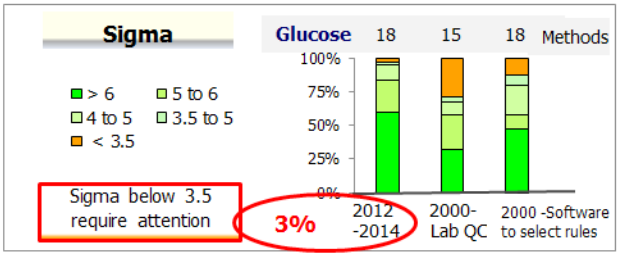
Results

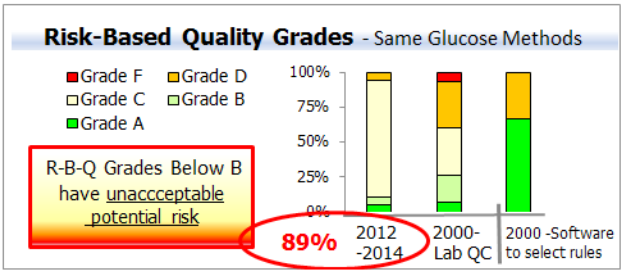
Sigma metrics would indicate that 3% of methods require attention.

R-B-Q Grades would indicate that 89% of methods have unacceptable potential risk

Conclusions

The Risk-Based Quality Grade© should be further investigated as a metric to manage patient risk





B-174

Issue and Complaint Management in a Large Integrated Laboratory System Using a Web-based Electronic Tool

E. R. Giugliano, H. Poczter, A. Atwaru. *North Shore LIJ Laboratories, Lake Success, NY*

The North Shore LIJ Health System Laboratories is a large health system based laboratory comprised of a central core lab, 12 hospital based labs performing approximately 16 million billable tests/year servicing the New York area including Long Island, Brooklyn, Queens, Manhattan and Staten Island. The Core Laboratory performs approximately 8 million of those billable tests. Currently there are approximately 1400 clients, including outreach, faculty practice, hospitals, nursing homes and in 2014, The New York City Health and Hospitals Corporation Laboratories has partnered with the NSLIJ Health System Laboratories. Since 2013, our client base has been growing rapidly, by approximately 8% per year, with a large increase in test volume (20%) and complexity. Concomitantly, there has been an increase in the number and complexity of client complaints and issues. Due to the number of new EMR interfaces, there have been many more LIS related issues. There was an associated increase in accessioning related errors, such as the ordering of incorrect tests and missed orders, as well as an increase in client generated issues such as incorrect specimen collection, unavailable specimen pick-up, amongst others. Our standard process of receiving complaints through our call center, logging cases into a front-end complaint management software system and resolving issues by assigning cases to management responsible for the lab section where the error occurred, has become less than optimum in providing excellent client satisfaction. To address this situation and provide clients requiring immediate information about corrective and preventive actions (CAPA), we have supplemented this process with the implementation of a real-time web-based solution to enable clients to view the progress of our complaint resolution on-line. This change also involves daily oversight by our Quality Management (QM) department of any new issues and complaints which may arise. QM expedites issue resolution by identifying process related issues , by ensuring immediate follow-up, and by implementing appropriate CAPA. On a monthly basis, the QM department then evaluates and trends occurrences and performs additional in depth analysis to make existing processes more robust. The incorporation of an electronic web-based tool allows for not only better enhanced real-time communication between the laboratory and its clients, but also engages the laboratory team in expediting complaint resolution. This new technology will allow for more effective communication, documentation and issue resolution for our prospective client complaints.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Molecular Pathology/Probes

B-175

Study of Patatin-Like Phospholipase-3/Adiponutrin I148M Polymorphism and Biochemical Markers in Nonalcoholic Fatty Liver Disease

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Background: Nonalcoholic fatty liver disease (NAFLD) is the most common disease associated with abnormal liver function test. Its diagnosis requires the demonstration of fat in liver by either imaging study or liver biopsy. Some gene polymorphisms have been reported to be associated with NAFLD in a number of studies. We hypothesize that the single-nucleotide polymorphism rs738409 C>G at position 148 in the patatin-like phospholipase-3 gene (PNPLA3) will increase the susceptibility of NAFLD. This study aims to examine the association of PNPLA3 polymorphism with the prevalence and severity of NAFLD among Kuwaiti lipid clinic patients and to study the association of NAFLD with different markers of liver damage including alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total bilirubin, and tissue inhibitor of metalloproteinases-1 (TIMP-1). Method: 137 Kuwaiti lipid clinic patients (99 cases and 38 controls) were enrolled. The main inclusion criterion was attendance at the lipid clinic, and the main exclusion criteria were evidence of other causes of liver disease. Anthropometric parameters, general biochemistry profile, full lipid profile, TIMP-1, PNPLA3 gene and abdominal ultrasound were analyzed. Statistical analysis were performed including linear and binary logistic regression analysis which was used to ascertain the association of variables with the NAFLD and metabolic syndrome as dependent variables with and without adjustment for the confounding effects of age and waist circumference. Mean values were compared by Kruskal-Wallis one-way ANOVA test. Spearman correlation coefficient was used to describe the association between TIMP-1 and other variables of interest. A p-value < 0.05 was considered as statistically significant. Results: The I148M variant of PNPLA3 gene and markers of liver damage including ALT, ALP, GGT, total bilirubin, and TIMP-1 were not associated with NAFLD in Kuwaiti lipid clinic patients. NAFLD patients were older ($p < 0.001$), had significantly increased body mass index (BMI) ($p = 0.002$), waist circumference (WC) ($p = 0.004$), hip circumference (HC) ($p = 0.007$), waist-hip ratio ($p = 0.017$), fasting blood glucose ($p < 0.001$) and triglyceride (TG) ($p = 0.002$) and lower high density lipoprotein-cholesterol (HDL-C) ($p = 0.046$) in comparison to control group. Diabetes mellitus showed significant association with NAFLD (odds ratio [OR], 4.66; 95% confidence interval [CI], 1.68-12.97; $p = 0.002$). Moderate-to-severe ultrasound-defined hepatic steatosis was significantly associated with older age ($p = 0.034$), increased BMI ($p = 0.045$), WC ($p = 0.044$), HC ($p = 0.046$), TG ($p = 0.002$), ALT ($p = 0.026$), and low HDL-C ($p = 0.002$). Severity of steatosis showed significant association with metabolic syndrome ([OR], 2.77; 95%[CI], 1.35-5.71; $p = 0.005$). Patients carrying the CG and GG genotypes had significantly higher levels of total bilirubin ($p = 0.043$) compared to those carrying the CC genotype. TIMP-1 showed direct correlation with ALP ($r = 0.23$) and inverse correlation with total cholesterol ($r = -0.24$) and low density lipoprotein-cholesterol ($r = -0.19$). Conclusion: Unlike reports from other populations, the I148M variant of PNPLA3 gene and biochemical markers of liver damage including ALT, ALP, GGT, total bilirubin, and TIMP-1 are not associated with NAFLD in Kuwaiti lipid clinic patients.

B-176

Rapid Screening for Targeted Genetic Variants via High-Resolution Melting Curve Analysis

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Background: Appropriate dosing of pharmaceuticals is critical to prevent sub-therapeutic efficacy or toxicity. Genetic variability in drug-metabolizing enzymes, including members of the cytochrome P450 (CYP450) superfamily, may significantly alter drug pharmacokinetics. CYP450 family members, including CYP2C9, CYP2C19, and CYP2D6, are responsible for the metabolism of >75% of commonly used therapeutic agents. Thus, targeted genetic screening for single nucleotide polymorphisms (SNPs) associated with aberrant enzyme function may considerably improve therapeutic regimens. In this study, we describe the validation of a method for the rapid screening for non-wild-type genotypes for targeted genetic polymorphisms in the CYP2C9, CYP2C19, and CYP2D6 enzymes via high-resolution melting curve (HRM) analysis. The method can rapidly identify individuals with non-wild-type sequences who should be further investigated for aberrant cytochrome P450 enzymatic activity.

Methods: DNA containing wild-type sequences of targeted SNPs for *CYP2C9*, *CYP2C19,* and *CYP2D6* were acquired from Coriell Institute. Sequence-specific primers were designed to amplify regions flanking targeted SNPs for *CYP2C9 (*2,*3*), *CYP2C19 (*2*, **3*, **17*), and *CYP2*D6 *(*2*, **10*) and acquired from Integrated DNA Technologies. Polymerase chain reaction (PCR) parameters were optimized and performed under the same conditions for all SNPs: 5 min hold at 95°C followed by 5 s at 58°C and 7 s at 72°C for 40 cycles. Post-amplification, HRM analysis was performed over a temperature gradient of 65°C to 95°C with a 0.1°C step function. Development and validation studies were conducted on the Rotor-Gene Q (QIAGEN) thermocycler. Melting temperatures for each control were defined by the Rotor-Gene software as the midpoint of the melt phase, and data analyses were performed in Microsoft Excel. The multiplexed assay was validated through the assessment of primer specificity, intra- (n=5) and inter-assay (n=20) precision of melting temperatures, dynamic range analysis of DNA input concentrations, and concordance with Sanger sequencing. Validation studies were performed on wild-type, heterozygote and homozygote DNA templates. The entire assay run time for PCR amplification and HRM analysis is <2 h.

Results: Sequence-specific primers successfully amplified DNA amplicons of interest for targeted *CYP2C9*, *CYP2C19*, and *CYP2D6* regions, as determined by DNA gel analysis and classical Sanger chain-termination sequencing. Identical PCR amplification and HRM parameters were optimized and implemented for the screening of all aforementioned polymorphisms. Intra-assay precision of melting temperatures for all SNPs was ≤ 0.09%, while inter-assay precision of melting temperatures ranged from 0.04% to 0.21%. Further, upon normalization to wild-type sequences, melting temperatures of heterozygotes differed from wild-type melting temperatures by 0.12% to 0.33% for *CYP2C9*, 0.21 to 0.41% for *CYP2C19* and 0.13 to 0.15% for *CYP2D6* ($p < 0.05$). HRM screening for all SNPs displayed thermostability over a wide range of input DNA concentrations (10-200 ng/µl). Slopes of resulting melting temperatures versus input DNA concentrations did not exceed 0.0012 °C/(ng/µl), indicating that results were constant over all DNA concentrations.

Conclusion: The presented assay provides a high-throughput method for the rapid screening for genetic variants in targeted regions of the *CYP2C9, CYP2C19, and CYP2D6* genes. This method can be used to screen samples prior to targeted, probe-based confirmatory testing.

B-177

The comparative Cq method can be used in the quantitative assessment of JAK2 V617F mutation by allele-specific qPCR in whole blood: no requirement for standard curves.

G. B. Barra, T. H. Santa Rita, L. F. R. Velasco, L. F. Abdalla, S. S. S. Costa. *Laboratório Sabin de Análises Clínicas, Brasília, Brazil*

Background:

The diagnostic value of JAK2 V617F mutation in myeloproliferative neoplasms is well established. The most widely used detection method involves allele-specific (AS) qPCR. This method also allows for quantification of JAK2 Wild-type (WT) and mutant (MUT) alleles percentages in the sample, generally, by using standard curves. However, the unique difference between WT and MUT AS-qPCR is the first nucleotide of the AS primers. It means that both reactions should have similar efficiencies and the ΔΔCq method could be applied for relative quantification of JAK2 alleles. The aim of the present study was to prove this hypothesis.

Methods:

This study enrolled whole blood samples (EDTA) from 27 healthy volunteers and 117 patients with known JAK2 V617F status (50 positive and 67 negative). Genomic DNA was extracted by using easyMAG (Biomerieux) and was quantified by a qPCR. The JAK2 WT and MUT were assessed by separated AS-qPCR reactions. The RNAse P was co-amplified in both reactions to function as a normalizer gene. The percentage of JAK2 MUT allele was calculated by the ΔΔCq method using JAK2 WT allele as comparator sample. The WT and MUT AS-qPCR efficiencies were evaluated by serial dilution of DNA samples with different JAK2 MUT levels. The assay linearity was determined by testing selected samples (JAK2 MUT from 0.5 to 99.69%). The lower limit of detection (LOD) was determined by probit regression analysis (JAK2 MUT 1:2 dilutions from 1.2 to 0.01%). For assay precision, the one-per-day run method (CLSI EP5-A2) and samples with 93%, 54% and 2,5% of JAK2 MUT were used. The accuracy was evaluated comparing the agreement between ΔΔCq method and ipsogen JAK2 MutaQuant kit (Qiagen).

Results:

The JAK2 WT and MUT AS-qPCR reactions showed similar efficiencies in all tested concentrations. The assay presented a linear response from 1 to 99.96% of JAK2 MUT allele. The LOD for the assay was 0.2% (95%IC 0.15-0.52%). The within run, between-run and total CV% were 0.24, 0.26 and 0.4% for the 93% of JAK2 MUT sample, 1.53, 1.14 and 1.9% for the 54% of JAK2 MUT sample and 6.21, 9.22 and 11.11% for the 2.5% of JAK2 MUT sample, respectively. The agreement with ipsogen JAK2 MutaQuant kit was high (R^2=0.997). The JAK2 MUT signal was observed in 50 out of 50 positive samples, in 0 out 67 negative samples and in 0 out of 27 healthy volunteers.

Conclusion:

The proposed ΔΔCq method along with AS-qPCR can be used for JAK2 V617F mutation detection and quantification in whole blood without the use of standard curves.

B-178

Differentially expressed miRNAs are potential biomarkers for poor response to dual antiplatelet therapy in patients with coronary arterial disease

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Background: The variability of response to antiplatelet therapy depends on genetic background and environmental influence. Gene expression is regulated by pre- and post-transcriptional factors, including non-coding RNAs such as miRNAs. MiRNA profile has been proposed as important circulating biomarker for disease and therapeutic control. This study may contribute to prognosis of inadequate response to dual antiplatelet therapy in patients with coronary arterial disease (CAD). **Objective**: To investigate potential biomarkers of poor response to dual antiplatelet therapy by miRNome analysis in peripheral blood cells of patients with CAD. **Methods**: One-hundred-eleven CAD patients were selected, aged 30-70 years, under 100 mg aspirin and 300 mg clopidogrel loading dose 24 h prior to angioplasty. Platelet reactivity was measured by electrical impedance using ASPI and ADP antiaggregation tests. Patients in the low (T1) and high (T3) tertiles of aggregation units were considered responders and poor-responders to dual antiplatelet therapy, respectively. Six patients from each T1 and T3 groups were selected and total miRNA was extracted from whole blood. The mirnome was analyzed by NGS. Two libraries from T1 and T3 miRNA pooled samples were loaded on separated single-read flow cells and sequenced. The trimmed sequences were counted on mirDeep2 software and mirnome profiles of both responders and poor responders were compared using Deseq2 Biocondutor pakcage. Targeting and pathway analysis of the differentially expressed miRNAs was performed with the Ingenuity® suite, using filters for cardiovascular and inflammatory disease. **Results**: Poor-responders presented augmented expression of 12 miRNAs while 4 miRNAs had lower expression compared to responders ($p<0.05$). Ingenuity analysis revealed that these miRNAs have relevant targets, which are involved in platelet aggregation, platelet activation and clopidogrel metabolism, such as thrombin, Rac-1, ITGA2, ITGB3 and paraoxonase-1. **Conclusion**: The results are suggestive that differential expression of miRNAs in peripheral blood cells may be associated with variable response to antiplatelet therapy in CAD patients, and mirnome could be used to early prognosis of inadequate response to aspirin and clopidrogrel and possibly prevent restenosis after angioplasty.

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Mutation Analysis by Next Generation Sequencing (NGS) in de-novo Acute Myeloid Leukemia (AML)

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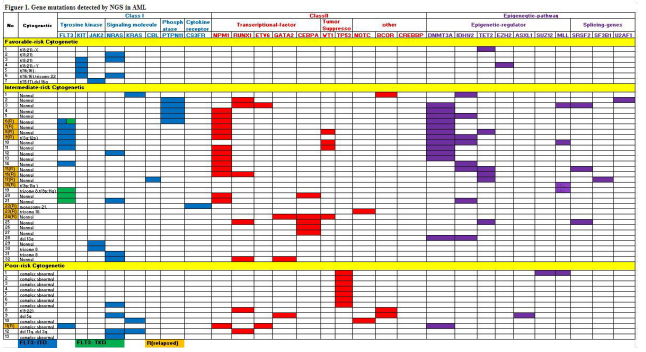
Background: Somatic mutations have been implicated in the pathogenesis of acute myeloid leukemia (AML). The classification and prognosis of AML increasingly depend on molecular and cytogenetic analysis. Mutation screening in AML is an integral part of risk stratification to guide therapy and monitor disease response/relapse. Next Generation Sequencing (NGS) can simultaneously detect multiple mutations in leukemia. We report mutations in AML using targeted NGS.

Methods: The study included 52 de novo AML from the pathology department/Knight diagnostic laboratory/Oregon Health and Science University (OHSU) (2013-2014). The targeted-NGS-panel covered 42 genes relevant to hematopoietic malignancies. Sequencing required 20ng of DNA from patients using Ion-Torrent-PGM. Bioinformatics-analysis was performed by Torrent-Suite-v.3.2 -pipeline (Life-Technologies, CA). Open-source programs and lab-developed algorithms were used for annotation and amino acid prediction.

Results: As shown in Figure 1, 100% of AML cases showed at least one mutation by NGS. In the intermediate-risk-cytogenetic-group, the most common mutations include transcriptional factors (NPM1, RUNX1, CEBPA) and epigenetic-regulators (DNMT3A, IDH, TET2, MLL) at the rate of 66% (21/32) for both. Mutations in splicing-genes (U2AF1, SRSF2, SF3B1) were less frequent (16%, 5/32). Multiple mutations (>2) were often seen (75%, 24/32); nearly all FLT3-ITD (100%, 7/7) or NPM1 (93%, 13/14) mutations occurred with epigenetic-regulators (DNMT3A, IDH and TET2). In the favorable-risk-cytogenetic-group, Kit (57%, 4/7) and Ras (43%, 3/7) mutations were common. In the poor-risk-cytogenetic-group, P53 (54%, 7/13) and Ras (38%, 5/13) mutations are frequent. Mutations with both DNMT3A and FLT3-ITD showed the high relapse rate (71%, 5/7).

Conclusion: AML is a biologically heterogeneous leukemia. Numerous recurrent mutations have been found and are used for risk stratification, targeted therapy and monitoring therapeutic response. This study demonstrates that targeted NGS mutation analysis is applicable in the clinical setting and provides a better understanding of leukemogenesis.



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Association between CYP2C19 genetic polymorphisms and platelet responses in clopidogrel-treated patients with coronary artery disease

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BACKGROUND:

Elevated platelet activity will increase the risk of major adverse cardiovascular events (MACE) after percutaneous coronary intervention (PCI). Clopidogrel was used to inhibit platelet activity in patients after PCI. CYP2C19 loss-of-function (LOF) alleles were reported to be associated with the low response of clopidogrel.

PURPOSE:

This study was carried out to analyze the gene frequencies and contributions of CYP2C19 polymorphisms to the variable clopidogrel responses in Chinese CAD patients undergoing PCI.

MATERIALS AND METHODS:

Clot strength and ADP-induced platelet inhibition were assessed by thromboelastography (TEG) in 2793 patients undergoing PCI with loading dose of 300 mg clopidogrel and a maintenance dose of 75 mg once daily. The genetic polymorphisms of CYP2C19 were analyzed by DNA microarray hybridization.

RESULTS:

With respect to the *1, *2 and *3 LOF CYP2C19 alleles, patients were classified into three phenotypes with different clopidegrel metabolism: extensive metabolizer (EM)(40.53%), intermediate metabolizer (IM)(45.22%), and poor metabolizer (PM)(14.2%). Among the enrolled patients, 63.14%, 31.33% and 5.53%, respectively, were the carriers of *1, *2 and *3 alleles. There was a significant difference in clot strength (MAADP) and ADP-induced inhibition between PM and either EM (MAADP: 35.89±16.80mm vs 31.52±16.08mm, P=4.2×10-6; ADP-induced inhibition: 46.34±30.96% vs 55.61±29.58%, P=1.5×10-7) or IM group (35.89±16.80mm vs 33.92±16.23mm, P=0.036; ADP-induced inhibition: 46.34±30.96% vs 50.41±30.51%, P=0.019). And also significant difference in clot strength and ADP-induced inhibition were shown between EM and IM groups (MAADP: 31.52±16.08mm vs 33.92±16.23mm, P=3.1×10-4; ADP-induced inhibition: 55.61±29.58% vs 50.41±30.51%, P=2.6×10-5). Nevertheless, there was no difference in clot strength (MAA, MAAA, MACK) and AA-induced inhibition between these three metabolism groups. Attentively, 7% (79/1132) of the EM patients had ADP-induced inhibition≦10%, while 13.1% (52/398) of PM patients had ADP-induced inhibition≧90%.

CONCLUSION:

The distribution of CYP2C19 genotypes in Chinese is different from Caucasians and Blacks. The genetic polymorphisms of CYP2C19 significantly affect the effectiveness of platelet inhibition by clopidogrel.

Effectiveness of platelet inhibition by clopidogrel with three different metabolisms

Parameter	EM (N=1132)	IM (N=1263)	PM (N=398)	P-Value (All)	P-Value (EM VS IM)	P-Value (EM VS PM)	P-Value (IM VS PM)
ADP-induced inhibition(%)	55.61 ± 29.58	50.41 ± 30.51	46.34 ± 30.96	<0.01	<0.01	<0.01	0.019
MAADP(mm)	31.52 ± 16.08	33.92 ± 16.23	35.89 ± 16.80	<0.01	<0.01	<0.01	0.036
AA-induced inhibition(%)	86.80 ± 25.36	85.78 ± 25.23	85.87 ± 23.84	>0.05	>0.05	>0.05	>0.05
MAAA(mm)	15.84 ± 13.56	16.19 ± 13.61	15.92 ± 12.84	>0.05	>0.05	>0.05	>0.05
MAA(mm)	9.99 ± 4.85	9.88 ± 4.81	9.72 ± 5.55	>0.05	>0.05	>0.05	>0.05
MACK(mm)	58.68 ± 6.79	58.41 ± 6.73	58.11 ± 6.85	>0.05	>0.05	>0.05	>0.05

All data represent as arithmetic mean ± standard deviationP-Value with EM IM and PM by LSD Test.

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Promoter hypermethylation of tumour suppressor genes (BRCA1 and RASSF1) in serum DNA of epithelial ovarian cancer patients

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Background:: Novel strategies for early detection of Epithelial Ovarian Cancer (EOC) are urgently needed because it is the most lethal of all gynaecological cancers and the third leading cancer amongst Indian women. Silencing of tumor suppressor genes by DNA methylation is the most frequent epigenetic alteration that may initiate and drive many human cancers including EOC. Promoter methylation is a common and relatively early event in epithelial ovarian cancer.

AIM: To determine the frequency of epigenetic alterations of tumor suppressor genes BRCA1 and RASSF1A in serum DNA of EOC patients and correlate with clinicopathological parameters, to explore the possibility of identifying potential minimally invasive, clinically useful diagnostic biomarkers.

Methods:

Thirty newly diagnosed, untreated, histopathologically confirmed EOC patients were selected for the study and DNA was extracted from their serum and corresponding cancer tisuue samples, followed by sodium bisulfite modification of DNA. Promoter methylation of BRCA1 and RASSF1A genes was detected using methylation-specific PCR (MSP). Control group 30 normal healthy individuals) were also examined to determine the specificity of hypermethylation in serum DNA.

Results:

Frequency of hypermethylation for BRCA1 gene in serum DNA of ovarian cancer patients was 70% and for RASSF1A gene 43.3% in a cohort of 30 histopathologically confirmed cases of EOC .None of the genes were found to be methylated in serum DNA of healthy controls. (p value < 0.0001) .There was no significant difference of clinicopathoogical parameters including age, menopausal status and stage between two groups but methylation of BRCA1 was significantly associated with the grade of the tumor.

Frequency of methylation of genes BRCA1 and RASSF1A in paired tissue samples with frequencies 73.33% and 53.33% respectively. positively correlated with serum DNA Methylation.

On combination of both genes in a single panel sensitivity and specificity increased markedly.

Conclusion: Our results suggest that aberrant epigenetic signatures of BRCA1 and RASSF1A genes is significantly associated with epithelial ovarian cancer in cell free DNA .They may be useful clinically as more sensitive, specific noninvasive biomarker for early diagnosis of EOC using MS-PCR.

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Microdeletion/microduplication of Y choromosome may cause Swyer syndrome

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The 46, XY Swyer syndrome is a rare form of sex reversal in no menarche female characterized by a 46, XY karyotype. Here, we first reported a family in which the propositus' (her phenotype is female) karyotype was 46,X,del(Y)(q11.2) as the G-banded chromosome analysis showed. However, the other family members had the normal phenotype and karyotype. The propositus had a female appearance and voice, with little subcutaneous fat, no beard and laryngeal prominence. At the same time, the propositus also exhibited female external genitalia, with hypogenetic labia majora and minora, sparse pubic hair and a visible vaginal orifice. The results of B-mode ultrasound showed a promordial uterus, without the left ovarian, and right adnexa with 28×24 mm cystic separation. The propositus had a higher follicle-stimulating hormone (FSH) and luteinizing hormone (LH) level, lower free testosterone and testosterone level, as well as normal estradiol and prolactin level.

In most cases, sex determining region Y (SRY) gene deletion, translocation, rearrangement and point mutation contribute to female sex reversal syndrome. In the study, molecular analysis revealed that the AZFa, AZFb and AZFc region were normal, and the SRY gene was present. Here, the sequence of the SRY gene showed that the propositus and her normal younger brother had a same mutation (C-T) compared to their father. High-resolution single nucleotide polymorphisms array (SNP-A) was used to further define the chromosome microdeletion breakpoints and the results showed that beside the microdeletion, a microduplication was also present in Y chromosome. The microdeletion region from Yq11.223-qter (24,985,375-28,799,654)×0 and the microduplication region from Yp11.31-q11.223 (2,650424-24,636,055)×2 were found by SNP-A. Fluorescence quantitative polymerase chain reaction (QF-PCR) and fluorescence in situ hybridization (FISH) with probes of Y chromosome centromere further confirmed there was the microduplication in Y chromosome. All these results showed microdeletion and/or microduplication in Y chromosome may cause that Y chromosome is inactivate, and then may lead to the reversal of masculinised phenotype as Turner syndrome.

Kaywords: Swyer syndrome; microdeletion; microduplication; karyotype; sex determining region Y (SRY)

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Dengue virus serotypes distribution in a high-endemic country

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Background:

Dengue is an escalating health problem, predominantly in tropical areas where dengue viruses (DEN) can be transmitted. Four antigenically related but genetically distinct dengue viruses named DENV1, DENV2, DENV3 and DENV4 cause the disease.

Since the late 1980's the incidence of dengue disease have increased in Brazil, a high endemic country where the majority of the cities are infested by *Aedes aegypti*. During the last 13 years, Brazil had four epidemics, with a total of about 4 million cases; in 2013 there were almost 1.5 million cases, which mark the highest of cases in this period.

Objective:

To evaluate the dengue virus serotype distribution from positive tests performed in a national base laboratory through the period from Jan 2012 to December 2014.

Methods:

Records from the laboratory were used to identify all positive tests obtained during the period of the study from all states of Brazil. The methodology for the tests were by reverse Transcription and Real-Time PCR (SYBR) followed by typing using Direct Sequencing.

Results:

Throughout the study period, the serotype dengue analysis was performed for 261 patients; 58 (22.2%) tested positive. DENV4 was the most frequent (33, 57%), followed by DENV1 (19, 33%), DENV2 (4, 7%), and DENV3 (2, 3%). The distribution varied according the year: DENV4 predominated in 2012 (57%) and 2013 (77%), whereas DENV1 predominate in 2014 (79%). The serotype distribution was similar between male and female, but changed according the age group.

Conclusion:

Our data are in concordance with the serotype surveillance data reported in Brazil. The co-circulation of multiple DENV serotypes and high dengue disease endemicity may be responsible for the increased distribution and severity of dengue disease in Brazil. Continuous monitoring of serotypes circulations is important to health public politics to control the dengue; the magnitude of this risk of epidemics also depends on the immunity levels established in each age group for each serotype and specifically the serotype that is more intensive circulating at each time.

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Enrichment of genomic DNA amount in serum by transport and storage at ambient temperature makes it an alternative matrix for molecular assays: high-yield of DNA, automated DNA extraction-friendly and direct use as template in qPCR.

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Background:

EDTA-whole blood is the matrix of choice for genomic DNA (gDNA) isolation for molecular testing. However, its automated DNA extraction has additional steps compared to serum/plasma and the presence of hemoglobin and EDTA impair its direct use as template for qPCR amplification. Curiously, the blood clot continually releases gDNA to serum ex-vivo. Thus, the aim of this study was to evaluate if this phenomenon can be explored in order to enrich the gDNA amount in serum making it an alternative matrix that surpass the whole blood drawbacks.

Methods:

Blood samples from fifteen healthy volunteers were drawn in Vacuette serum clot activator tubes (Greiner Bio-one). Five tubes were collected from each participant. The tubes were stored for 0h, 24h, 48h, 96h and 192h at room temperature before serum separation. The gDNA present in the serum was extracted by Easymag (Biomerieux). The extracted DNA or the crude serums were used as template for qPCR amplification of 65bp, 202bp, 440bp and 688bp amplicons. Next, blood samples from other fifteen volunteers were collected in the same above described tubes; they were shipped from Brasília (Federal District, Brazil) to Manaus (located in the Amazon rainforest) and then returned at ambient temperature. The samples came back after five days. The same above described qPCR was performed in these samples. Genotyping of hemochromatosis C282Y and H63D were also performed (amplicons of 102bp and 101bp, respectively). After that, crude serum (separated after five days at room temperature) from 21 consecutive patients from JAK2 V617F routine was used as template for qPCR. The results were compared to the standard assay performed on DNA extracted from EDTA-whole blood.

Results:

The median quantity of gDNA extracted from serum were 1.25, 2.03, 3.08, 10.1, 32.1µg/mL based on the 65bp PCR amplicon; 0.22, 0.41, 0.60, 2.6 and 9.4µg/mL based on the 100bp amplicon; 0.1, 0.25, 0.33, 1.44, 4.6µg/mL based on the 202bp amplicon and 0.001, 0.01, 0.02, 0.03, 1.8µg/mL based on the 688bp amplicon for 0h, 24h, 48h, 96h and 192h, respectively ($p<0.0001$ for 96h and 192h versus 0h, for all instances). Using the crude serum as template, the 65bp and 100bp amplicons were detected in all instances; the 202bp amplicon was detected only after 96h and the 688bp amplicon was not detected. In the samples shipped to Manaus, the median quantitative cycles for gDNA extracted from serum were 22.36, 23.61, 23.67 and 29.97 and for crude serum were 26.34, 29.34, 32.54 and not detected for amplicons of 65bp, 100bp, 202bp and 688pb, respectively. Furthermore, serum's gDNA and crude serum showed full concordance for hemochromatosis C282Y and H63D genotyping. Finally, crude serum and DNA extracted from whole blood showed complete agreement for JAK2 V617F mutation detection (17 negative and 4 positive).

Conclusions:

There is a significant increase of gDNA quantity in serum after 96h at room temperature that makes this specimen a source of high amount of gDNA compatible with rapid automated DNA extraction, with transport/storage at ambient temperature and with its direct use as template for amplification of small sizes' amplicons, except the DNA extraction.

B-185

Primary drug resistance among HIV-1 recently infected patients in Brazil

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Background: According to the Brazilian Sexually Transmitted Diseases (STD) Program, the incidence of HIV is about 20 cases per 100.000 habitants. Genotyping test provides information about HIV-1 resistance to protease (PR) and transcriptase reverse (NRTI and NNRTI) class drugs. The goal of the government is to provide free and effective treatment to HIV patients but a new issue has arisen, the propagation of HIV-1 drug resistance strains among non-symptomatic individuals. This event called primary resistance can bring clinical and public health complications and may undermine HIV treatment with currently available drugs

Objective: To determine the prevalence of primary HIV-1 drug resistance in naive patients in a large private laboratory in Brazil.

Methods:We analyzed data from 782 plasma and blood samples genotyped for HIV-1 resistance mutations at the Molecular Diagnostics Laboratory (DASA) from the period of July 2014 to January 2015. Information about previous and current HIV treatment was taken from a patient's questionnaire completed prior to sample collection. Viral RNA was isolated using QIAamp RNA Viral Mini kit (Qiagen, Hilden, Germany). We amplified the protease gene and a fragment of reverse transcriptase from HIV-1 and sequenced in 3730 DNA Analyzer platform (Life Technologies, Foster City, CA). The calibrated population resistance tool (available through the Stanford University HIV Drug Resistance Database http://hivdb.stanford.edu) was used to identify the HIV subtypes and drug-resistances.

Results: From 782 samples, 145 (18%) naïve patients showed primary drug resistance or accessory mutations related to drug resistance, 32 patients (4%) presented primary drug resistance. From these 32 patients, 6 presented primary resistance to PR inhibitors and 25 primary resistance to NRTI and NNRTI´s class drugs. One patient presented primary resistance to all three classes of drugs.

The frequency of mutations mostly found in naïve patients were: K103N/S (NNRTI) 66.6%, M184I/V (NRTI) 15.1%, followed by D30N (PR) 12.1% and G48V / L90M (PR) 6%.

Conclusion: There are few studies about HIV-1 drug resistance in Brazil. The K103N is the most frequent mutation and confers resistance to Efavirenz, the first-line treatment in Brazil. The frequency of primary drug resistance was 4%, which is in accordance with five large studies in Brazil with rates from 2.2 to 8.1%.

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Increased Sensitivity of JAK2V617F Mutation Detection by COLD-PCR-based Sanger Sequencing

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Background: The point mutation of Janus kinase 2 V617F (JAK2V617F) has become a valuable marker for diagnosis of Philadelphia chromosome-negative chronic myeloproliferative neoplasms (MPNs) and an excellent target for therapeutic drug development. Although Sanger sequencing represents the criterion standard for characterization of the mutation, it is typically able to detect DNA variants present at moderate-to-high abundance, approximately 20% or greater. Co-amplification at lower denaturation temperature (COLD)-PCR has been reported as a powerful method to selectively amplify minority alleles from mixtures of wild type and mutation-containing sequences. In this study, we evaluated the efficiency of the combination

of COLD-PCR and Sanger sequencing to detect JAK2V617F, in comparison with the conventional PCR-based Sanger sequencing.

Methods: Genomic DNA purified from cell line HEL with JAK2V617F mutation was diluted to 1%, 3%, 5%, 10%, 20% and 50% of JAK2V617F mutation DNA in wild-type DNA from cell line K562. The DNA mixtures were amplified by fast COLD-PCR and conventional PCR, followed by Sanger sequencing on an ABI 3730XL DNA analyzer. Meanwhile, HEL cells was diluted to 1%, 3%, 5%, 10%, 20% and 50% of mutant cells in human healthy wild type whole blood cells, then genomic DNA was extracted with QIAamp DNA Blood Mini Kit from the cell mixtures. Then, the extracted DNA were amplified by the same COLD-PCR and conventional PCR protocols and sequenced. Furthermore, 114 JAK2V617F-positive DNA samples from patients were used to verify the established COLD-PCR-based Sanger sequencing method.

Results: The JAK2V617F mutation was detected in 20% mutant DNA mixture using conventional PCR and Sanger sequencing analysis, while not detected in less than 20%. However the mutation was clearly detected following fast COLD-PCR and Sanger sequencing even in 3% mutant DNA mixture. One the other hand, the JAK2V617F mutation was still detected in 20% mutant cells in wild type cells in whole blood matrix, not detected in less than 20%. By contrast, the mutation was clearly visible in 3% mutant cells in wild type blood cells using COLD-PCR based Sanger sequencing in a good accordance with the sensitivity observed in mutant DNA mixtures. Moreover, the JAK2V617F mutation was detected in 102 (89.37%) DNA samples from patients, not detected in 12 (10.63%) samples using conventional PCR based Sanger sequencing, while with COLD-PCR based Sanger sequencing analysis, the mutation was successfully detected in all 114 (100%) DNA samples from patients.

Conclusion: Compared to conventional PCR, COLD-PCR could greatly improve the sensitivity of Sanger sequencing method for JAK2V617F detection. COLD-PCR is powerful for mutation enrichment and mutation screening using clinical specimens with low-level mutations.

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Genotyping of the G6PD (Glucose-6-Phosphate Dehydrogenase) Gene: Applications in Pharmacogenomic Testing.

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Intro/Background:

G6PD deficiency is the most common human enzyme defect, estimated to be present in 400 million people worldwide. The *G6PD* gene is located on the X-chromosome. G6PD deficiency is inherited in an X-linked recessive manner therefore males are more commonly affected than females, but due to the high prevalence of G6PD deficiency, homozygous and compound heterozygous females are not uncommon.

G6PD is an important pharmacogene. With the exception of rare chronic nonspherocytic hemolytic anemia (CNSHA) related G6PD deficiency; individuals with G6PD deficiency are typically asymptomatic until an oxidative stress challenge occurs. Oxidative stresses, including those caused by many medications, can induce acute hemolytic anemia (AHA) in individuals who are G6PD deficient. AHA in G6PD deficient individuals can vary in severity, ranging from mild to life-threatening. The FDA recommends that patients at higher risk of G6PD deficiency, such as those with African or Mediterranean ancestry, be tested for G6PD deficiency before initiation of rasburicase therapy for hyperuricemia. *G6PD* genotyping is not widely available although enzyme testing is available. Our lab aims to make *G6PD* genotyping available to complement and, in some cases, replace G6PD enzyme testing.

Material/Methods:

A comprehensive genotyping of the *G6PD* gene was performed on six samples that had low G6PD levels (<0.3-6.4U/g Hb) by enzymatic assay, seven normals and a heterozygous deficient control. DNA was extracted from whole blood or saliva for analysis. A tagged-primer amplification method of all 13 exons, the intron-exon junctions, and 500 base pairs of the 3' untranslated region (UTR) followed by an enzymatic purification before performing bi-directional fluorescent dye-terminator sequencing, covering all previously described G6PD deficient variations. The sequencing was analyzed to determine variations using Mutation Surveyor™v.4.0.9 software.

Results:

In the sample set of fourteen, ten different single base variations were detected in the region of interest. Five of the variations detected result in amino acid changes, all classified as deleterious variations. All six samples selected for low G6PD levels had deleterious mutations found by sequencing. Four previously described WHO class III variations: A- (202)/Ferrara I, Mahidol, Seattle/Lodi/Modena/Ferrara II/Athens-like/Mexico, and Gaohe were seen in our initial deficient population. The lowest of the G6PD enzyme levels were associated with hemizygous variant males, while the heterozygous variant females exhibited deficient G6PD enzymes levels but not as severely suppressed. No deleterious variations were found in the normal samples sequenced.

Conclusions:

Comprehensive *G6PD* genotyping will allow clinicians to accurately characterize the G6PD status of an individual, impacting pharmacogenomics decision making.. This testing allows clinicians to employ pharmacogenomics to identify those at risk for an adverse drug reaction due to G6PD deficiency prior to prescribing drugs known to elicit an AHA reaction in G6PD deficient individuals. This testing also has applications to identify the genetic cause of G6PD deficiency, infants at increased risk of neonatal hyperbilirubinemia, determine G6PD status in individuals with inconclusive/abnormal phenotyping results, carrier status in women, and classify patients experiencing CNSHA.

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Micro-RNA Signatures In The Vitreous And Plasma Of Patients Suffering From Exudative AMD

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Purpose: Age-related macular degeneration (AMD) is the leading cause of blindness affecting people over the age of 50. The exudative form, known as neovascular AMD (NV AMD), is characterized by choroidal neo-vascularization (CNV) that consists of abnormal leaky choroidal vessel growth into the retina. Recently, a number of studies have demonstrated specific micro-RNA (miRNA) signatures in plasma or urine for several diseases such as cancers and kidney disease. MiRNAs are small non-coding RNA of 22-24 nucleotides length which can circulate in human body fluids. To date, there is a lack of information on miRNA signatures associated with NV AMD. The aim of this study is to detect miRNA profiles in the vitreous and plasma of patients with NV AMD.

Methods: All patients were previously diagnosed with AMD (with CNV) and were followed and operated by a single vitreoretinal surgeon (F.A. Rezende) before receiving anti-VEGF treatment (AVASTIN). Control patients underwent surgical treatment for nonvascular pathology (epiretinal membrane or macular hole) by the same surgeon. In addition, for plasma miRNA detection, blood was collected the same day prior to the surgery. First, a screening of miRNAs in vitreous samples (3 controls and 4 AMD) was perfomed by microarray (including 384 miRNAs). Next, we validated miRNA profiles in both vitreous and plasma with individual TaqMan qPCRs with a higher number of patients (n=15) in each group.

Results: Microarray analysis identified significantly higher levels of miR-548a (2-fold) and miR-146a (4-fold) and lower levels of miR-16, miR-152 and miR-106b in human vitreous from patients with NV AMD when compared to controls. Individual qPCRs validated vitreous expression patterns of miR-146a, miR-106b and miR-152. In addition, analysis of plasma miRNAs by TaqMan miRNA assay identified a decrease in the level of miR-152 and miR-106b. In contrast, plasma levels of miR-146a were not significantly induced in the AMD group as they were for vitreous samples. Importantly, no correlation was drawn for miRNA profiles and patient age.

Conclusion: To our knowledge, our study is the first to characterize miRNA profiles in both vitreous and plasma from a cohort of patients suffering from NV AMD. Identification of a miRNA signature in circulation is conceptually promising for future development of a novel class of biomarkers for NV AMD. Moreover, elucidating miRNA profiles may provide insight on gene regulation during disease progression and potentially provide novel therapeutic avenues.

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MELPA: a Novel Technology for High-throughput, Multiplex Genotyping Directly from Dried Blood Spot without DNA Extraction, with an Application in the Screening of Multiple G6PD Gene Variants at Risk for Drug-induced Hemolysis

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Background: Hundreds of thousands of single nucleotide polymorphisms (SNPs) have been found to be associated with disease susceptibility, drug response and complex phenotypes. Clinical population screening of significant SNP markers calls for multiplexed genotyping of SNPs on a large scale. Current SNP genotyping tools (e.g, TaqMan and microarray-based assays), despite many advantages, invariably require DNA extraction, which remains a key throughput-limiting step for population screening, and is technically challenging for small-volume precious specimens or long-term stored archived collections. In addition, multiplex-PCR amplification employed by these genotyping methods suffers from complex primer design and/or amplification bias. Here, we describe a novel high-throughput genotyping approach, Multiplex Extension and Ligation-based Probe Amplification (MELPA), which has multiplex SNP genotyping capability, eliminates DNA extraction, achieves uniform PCR amplification using a single pair of universal primers, and is suitable for archival Dried Blood Spot (DBS) samples.

Methods: Instead of nucleic acid extraction, MELPA lysed samples and captured the target DNA directly to 96-well plate by sandwich hybridization using multiple oligo probes with universal tail sequences. After enzymatic extension and ligation of the probes, a single-stranded template for each target SNP site was formed, and all templates were PCR-amplified using universal primers targeting the tail sequences. Multiplexed genotyping by single-base primer extensions were analyzed with a MALDI-TOF mass spectrometry platform (MassARRAY by Sequenom). We tested the feasibility of the new assay for whole blood and DBS samples, and evaluated the accuracy by comparing MELPA with both a commercial multiplex SNP assay (iPLEX) and DNA sequencing, for the detection of 23 G6PD gene variants known to be at risk for primaquine-induced hemolysis in antimalarial therapy. Finally, we employed MELPA in a G6PD variant preemptive screening of DBS samples from malaria-endemic areas.

Results: MELPA can be successfully applied on fresh or archival blood and DBS samples, with call rates >97%. G6PD genotyping by MELPA on 2 randomly chosen archival blood samples gave results more accurate than the iPLEX assay, and were 100% concordant with DNA sequencing. We conducted MELPA genotyping of 106 archival blood samples of *P. vivax* malaria patients taking primaquine and found 10 G6PD-deficient variants from 9 cases, including one hemizygous male patient with G6PD *Mahidol* mutation who had hemolysis. A preemptive G6PD genotyping of 438 DBS samples from a malaria-endemic area in Yunnan Province was conducted using MELPA, and 3 G6PD variants were identified. A sample-pooling strategy can further increase the sample throughput of the MELPA assay; elevated wild type background introduced by sample pooling can be reduced by adding specific wild-type allele blockers.

Conclusion: MELPA represents an efficient and cost-effective approach to multiplex SNP genotyping at population level. Preemptive genotyping of G6PD variants in high-risk population in malaria endemic areas presents a good paradigm for high volume epidemiological surveillance, and will be valuable for assessing drug-induced hemolytic risk for the development of optimal malaria therapeutic strategy for an endemic area.

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Whole-exome sequencing as a powerful diagnostic tool in clinical laboratories

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One of the major concerns of medical sciences is finding the causal genes underlying human diseases. In the last decade, great efforts were applied in the development of new molecular technologies, such as next generation sequencing and oligonucleotide arrays, aiming to elucidate the genetic basis of disorders. In this sense, with the advent of whole-exome sequencing (WES), identification of disease-causing genetic variations is progressing at rapid pace, improving the disease management by either available treatments or genetic counseling and risk assessment of patients andrelatives. In Hermes Pardini Institute, one of the largest clinical and diagnosis laboratory from Brazil, began offering this molecular test in 2014 and since then has enabled the genetic diagnose of many diseases. Here we report an overview of the WES use in different clinical situations by our lab group. Coding exons from 20 Brazilian patients were captured by Illumina Nextera technology and sequenced in the Illumina HiSeq 2500 platform. Reads were aligned to the human genome reference (hg19/GRCh37) and variants were identified through bioinformatics analysis. WES identified probably disease-causing mutations in nine cases from different disorders: Encephalopathy (2 cases), Cognitive Delay with Epilepsy, GLUT1 Deficiency Syndrome 1 (GLUT1DS1), Epilepsy, Spinal Muscular Atrophy type 1 (SMA1), Leigh Syndrome (LS), Tay-Sachs Disease (TSD) and Cat Eye Syndrome (CES). From these, WES revealed 9 previously undescribed mutations (4 missense mutations, 2 deletions, 1 insertion, 1 splice donor site mutation and 1 frameshift with stop gained mutation) and 2 previously described mutations (1 missense mutation and 1 splice donor site mutation). From these 11mutations, 4 were classified as damaging mutations, 5 as probably damaging and 2 were variants of unknown clinical significance in genes related to the clinical phenotype, and thus, need to be further investigated. WES failedto identify disease-causing mutations in 11 patients and this could be due to many factors, such as polygenic diseases, structural variants, epigenetics changes, insufficient coverageof few exons in exome sequencing and intronic or mitochondrial mutations. In conclusion, WES is an efficient, sensitive, specific and cost-effective method for disorders difficult to diagnose and has important implications for genetic counselling and clinical services.

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Association of GCK gene 6 new SNP loci polymorphisms with Han Chinese patients with Type 2 Diabetes

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Background: The incidence of type 2 diabetes (T2D) continues to increase in China, and genetic causes may account for predisposition to insulin resistance and diabetes. In order to investigate the relationship between these SNPs and T2D, six single-nucleotide polymorphisms (SNPs) of the glucokinase(GCK) genein southern Han Chinese were chosen and evaluated.

Methods: 499 T2D patients and 499 healthy control, which were qualified in this study, were selected. Six SNPs (include rs12702070, rs2971672, rs2268569, rs2268573, rs2300587 and rs1476891) from GCK gene were detected by improved multiple ligase detection reaction (iMLDR), and then the multivariate logistic regression analysis and haplotype association analysis were employed to investigate the association between these SNPs and T2D.

Results: The SNP rs2971672 genotype C/A frequency was 45.9% in T2D patients which is significantly lower than control group (51.9%) (odds ratio (*OR*) =1.57; 95% confidence interval(*CI*) =1.17-2.12; $P<0.01$), C allele showed 0.4% in the T2D group, which is still lower than control group(0.45%) (*OR*=1.25; 95%*CI*=1.04-1.52; $P<0.05$). The SNP rs12702070 genotype C/T frequency was also significantly more prevalent among the T2D patients (9.4%) than healthy control (14.8%) (*OR*=1.88; 95%*CI*=1.25-2.84; $P<0.05$), C allele showed 0.06% in the T2D group, lower than control group(0.08%) *(OR*=1.51; 95%*CI*=1.06-2.14; $P<0.05$). In addition, five SNPs of GCK (rs2300587, rs2971672, rs12702070, rs1476891 and rs2268569) formed 2 linkage disequilibrium blocks, which means that there are gene-gene interactions among these five SNPs.

Conclusion: Gene variants are associated with diabetes mellitus, SNP rs2971672 and rs12702070 of GCK could increase risk of T2D in southern Han Chinese.

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Performance characteristics and comparison of two real-time PCR systems for hepatitis B virus DNA quantification.

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Background: Detection and accurate quantification of hepatitis B virus (HBV) DNA are essential tools for the diagnosis and management of chronic HBV infected patients undergoing antiviral treatment. The use of real-time PCR assays for HBV

DNA quantification is strongly recommended. With several HBV DNA real time PCR quantification assays available, it is important to use the most efficient and effective testing system for accurate virological monitoring. In this study, we evaluated the performance characteristics and comparability of two HBV real time PCR quantification systems: Artus HBV real-time PCR assay on Step one Applied Biosystems analyzer with manual viral nucleic acid extraction using QIAamp DNA blood kit, and COBASAmpliPrep/COBAS TaqMan HBV Test, v2.0 with automated viral nucleic acid extraction.

Methods: We evaluated precision, bias and linearity (Upper and lower detection limits). Precision data had been collected using pooled serum of HBV positive patients; this pooled serum was very well mixed, divided into aliquots and stored at -20 ºC then analyzed in triplicates in different PCR runs. Bias was calculated from proficiency testing results (UK-NEQAS) as the difference between the measured and the intended results for the provided samples. Linearity was determined using serial dilutions of a high viral load sample, regression line analysis was done between the expected and the measured values to verify the analytical measurement range of each assay (Upper and lower detection limits). Method comparison was done between both assays and correlation coefficient was calculated.

Results: Results for precision study revealed an overall variance of 0.0410 log IU/ml (CV: 0.89 %) for Artus HBV real-time PCR assay and 0.078 log IU/ml (CV: 2.24%) for COBASAmpliPrep/COBAS TaqMan HBV Test, v2.0 . Average bias estimation using 4 proficiency testing samples (UK-NEQAS) showed an average bias of 5.36 % for Artus HBV real-time PCR and 5.64 % for COBASAmpliPrep/COBAS TaqMan HBV Test, v2.0 . The analytical measurement range for Artus HBV real-time PCR was found to be from 2.0 X 10^0 to 2.9X 10^8 IU/ml (Regression line analysis; slope:1, r^2:1) while that of COBASAmpliPrep/COBAS TaqMan HBV Test, v2.0 was found to be 2.0 X 10^1 - 2.4 X 10^7 IU/ml (Regression line analysis ; slope:0.99, r^2: 1). Method comparison between paired quantitative results of both assays showed strong correlation with r^2 0.99

Conclusion: The performance characteristics and the strong correlation between results of both Artus HBV real-time PCR with manual nucleic acid extraction using QIAamp DNA blood kit and COBASAmpliPrep/COBAS TaqMan HBV Test v2.0 with automated nucleic acid extraction assays; suggest that both assays can be used for management and therapeutic monitoring of chronic HBV infection.

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15q11.2 microdeletion syndrome

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Background: Comparative genomic hybridization to arrays (array-CGH) analyzes the human genome to detect gains and losses of genetic material associated with microdeletion and microduplication syndromes. Array-CGH detects the presence of microdeletions and microduplications that would be undetectable by conventional cytogenetic techniques.

Case report: Child two years old with neurological dysfunction that has delayed motor development. The constitutional karyotype and genetic study Prader-Willi were negatives. Chromosome analysis was performed by array-CGH, Human G3 used CGH Microarray SurePrint 400K (Agilent®), with an average spacing between probes of 5.3 Kb and 4.6 Kb for RefSeq genes. The reading was performed using microarray Microarray Scanner G2565CA (Agilent®) at a resolution of 3µm and analysis software results Cytogenomics v 2.0.6.0 (Agilent®). The results show a genomic male pattern with the formula: arr (1-22) x2 (XY) X1, (ISCN 2009). The heterozygous microdeletion on chromosome 15 was detected between breakpoints BP1 and BP2 (15q11.2) with genomic coordinates chr15: 18692865-20308073, including deletion of GOLGA6L6, GOLGA8C, BCL8, LOC646214, CXADRP2, POTEB, NF1P1, LOC727924, OR4M2, OR4N4, OR4N3P, LOC646396, GOLGA8DP, GOLGA6L1 genes.

Discussion: Recent studies suggest that this area is a genomic region of susceptibility to neurological dysfunction, including developmental delays, autistic features, behavioral disturbances, attention deficit hyperactivity disorder, and mild dysmorphic features, leading to a new 15q11.2 microdeletion syndrome and might be associated with the clinical history of this patient. Also, the 15q11.2 microdeletion syndrome has been associated with proximal esophageal atresia, distal tracheoesophageal fistula, congenital cataracts idiopathic, generalized epilepsy, schizophrenia, and Alzheimer's.

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Genotyping the PKLR gene (Pyruvate Kinase, Liver, Red Blood Cell) for Non-spherocytic Hemolytic Anemia

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Background: The *PKLR* gene encodes for two isoforms of the enzyme pyruvate Kinase (PK), a glycolytic enzyme that transfers phosphate groups from phosphoenolpyruvate (PEP) to ADP creating pyruvate and ATP. The two isoforms have different exon 1 sequences that cause tissue-specific expression, L (liver) and R (red blood cell). The second leading cause of Non-spherocytic Hemolytic anemia is a deficiency of the enzyme pyruvate kinase. Patients have a wide range of phenotypic expression of hemolysis ranging from minor effects to life-threatening neonatal situations requiring blood transfusions. Anemic patients that have inconclusive findings by enzyme assays could benefit from the characterization of the *PKLR* gene to aid in the diagnosis of PK deficiency. Carrier testing is also possible.

Methods: Genotyping of the *PKLR* gene was performed on samples that had low PK (pyruvate kinase) levels by enzymatic assays. The DNA was extracted from whole blood then genotyped using two different methods (*PKLR* Full gene and Deletion assay). The first methodology employed tagged-primer amplification of all 11 exons for isoform R and included exon 1 for isoform L, followed by an enzymatic purification before performing bi-directional fluorescent dye-terminator sequencing. The sequencing was analyzed to determine variations using Mutation Surveyor™v.4.0.9 software for visual inspection. The second methodology utilizes fragment detection of a single amplicon (~10kb) covering intron 2 through the 3-primed untranslated region (UTR) for the presence of a large deletion. The amplified fragment was then sized using the Agilent 2100 Bioanalyzer and visually verified to be within the acceptable range.

Results: The tagged sequencing assay detected the causative mutation in patients sequenced exhibiting a low PK level. The large deletion detection assay identified patients with a deletion. One pair of samples had one individual with a heterozygous large deletion and a family member with a homozygous large deletion. The two method approach was very beneficial in these two samples. Neither sample had a mutation detected by the tagged sequencing assay however the large deletion was detected by the fragment assay. Combining the results from both assays gave greater understanding of the patient's genotype.

Conclusion: The *PKLR* full gene and large deletion detection assay utilized two different methodologies thereby giving a clearer picture of the cause of anemia in patients with PK deficiency. Most causative mutations were found in the sequencing portion of the assay however for the patients with a large deletion present, this was detected only by the fragment detection assay. The combination of the two assays together created a more accurate genotype for the patients.

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Promoter polymorphism in Wnt signaling pathway is associated with tuberculosis risk in Chinese subjects

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Objective: Compelling studies have implicated that Wnt signaling pathway plays an important role in the development and progression of tuberculosis, however, the exact mechanism remains unknown. For the first time, we took a pathway based candidate gene approach in a Chinese Han population to investigate the association of promoter polymorphism the Wnt signaling pathway with tuberculosis risk from the host genetic perspective.

Methods: Fifteen single nucleotide polymorphisms (SNPs) from promoter regions of five key genes in the Wnt signaling pathway (Wif1, Dkk1, Wnt3a, Wnt5a, Ctnnbl) were selected via searching the International HapMap Project and dbSNP database. The genotype analyses were conducted by the Massarray method for 912 unrelated Chinese subjects. Common inflammatory markers C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and hematocrit (Hct) were also detected using the automatic analyzer IMMAGE® 800 and Test® 1. Gene mRNA expressions were measured on a subset of 561 subjects whose buffy coat fractions were available. Logistic regression analyses were conducted to test the associations of the promoter polymorphisms with tuberculosis risk and inflammatory markers.

Results: The study group comprised of 450 tuberculosis patients (60.67% males and 39.33% females, age [mean ± SD] = 42.11 ± 17.18 years) and 462 control subjects (58.23% males and 41.77% females, age [mean ± SD] = 43.11 ± 12.01 years), and

no significant differences in age and sex were observed in cases and controls (p = 0.539, 0.784, respectively). Genotype distributions of all SNPs were in agreement with Hardy-Weinberg equilibrium (p all > 0.05). We found that three SNPs of Ctnnb1 gene (rs3864004, rs9859392, rs9870255) were associated with decreased tuberculosis risk, from the perspective of allele, genotype distribution and dominant model (p all < 0.05). Ctnnb1 haplotype [GCA] was associated with a decreased tuberculosis risk (OR = 0.65, p = 0.008) and haplotype [CGG] showed a higher risk with an odds ratio of 1.53(p = 0.008). The mRNA expressions of Ctnnb1 in tuberculosis cases were significantly higher than those in healthy controls (15.21 ± 3.10 vs. 5.28 ± 1.09, p < 0.001). The dominant models of rs3864004, rs9859392, rs9870255 were associated with a decreased mRNA expression trend (p all = 0.025). Subsequent analysis indicated the polymorphism of Ctnnb1 didn't connect with the expression levels of CRP, ESR or Hct (p all > 0.05).

Conclusion: Our study indicates that Ctnnb1 promoter polymorphism is associated with tuberculosis susceptibility and may also affect its mRNA expression levels, predicting some SNPs in Wnt signaling pathway as genetic markers for determining the susceptibility to tuberculosis infection. Further epidemiological and functional studies in larger populations are warranted to verify our results.

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Frequency of microdeletions of the Y chromosome in a routine laboratory in Brazil

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Background: Y chromosome microdeletions are the second most frequent genetic cause of genetic infertility in men following Klinefelter syndrome. Molecular studies have shown that mcrodeletions at Yq11 may represent the etiology factor in as many as 10 to 20% cases of idiopathic azoospermia or severe oligoszoospermia. Most of the deletions occur de novo and fall in 3 non overlapping regions, designated Azoospermia Factor Regions (AZF): AZFa, AZFb and AZFc, of which the distally located AZFc is the most frequently deleted. The molecular diagnosis of these deletions became an important diagnostic test to investigate spermatogenic failure.

Objective: The aim of this study was to evaluate the frequency of Y microdeletions in the routine of a private laboratory in Brazil.

Methods: From January to December 2014, 491 blood and sperm samples were isolated at the QIAsymphony Platform (QIAsymphony DNA mini kit - QIAgen). The amplification strategy consisted in a multiplex polymerase chain reaction (Multiplex PCR - QIAgen) targeting six regions (AZFa: sY84, sY86, AZFb: sY127, sY134 and AZFc: sY254, sY255). The multiplex-PCR products were detected at QIAxcel Systems (DNA Screening Kit - QIAgen). All steps were processed and validated at Molecular Diagnostics Laboratory (DASA). Information about infertility problems was taken from patient's questionnaire filled prior to sample collection.

Results: We detected 15 (3%) patients that presented microdeletions from a total of 491 samples. From these positive for microdeletion patients, 4 patients showed deletions at the AZFb (sY127, sY134) region , 8 showed deletions at the AZFc (sY254, sY255) region and 1 presented all regions deleted (AZFa, AZFb and AZFc).

Conclusion: Diagnosis of a microdeletion of the Y chromosome permits the cause of the patient's azoospermia/oligozoospermia to be established and to formulate a prognosis. Deletions in the AZFc region on Y chromosome are the most common cause of spermatogenic failure, which is in accordance with the results of this study.

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A New Universal Multiplex Digital PCR Method with Improved Precision for the Quantification of Donor Derived Graft cfDNA Traces.

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Background: Graft-derived cell-free DNA (GcfDNA) quantification is gaining high interest as biomarker for graft integrity after transplantation. Using high throughput sequencing (HTS)1 or digital PCR (dPCR)2, the quantification of informative SNPs in total cell-free plasma DNA (cfDNA) became feasible. Molecule counting as employed by both methods enables the detection of small changes in GcfDNA levels with higher precision than qPCR. However, both methods include a preamplification using adapters ligated to the DNA, which introduces non-systematic amplification variability. Here we present a preamplification-free, multiplexed dual dPCR assay approach yielding a low overall imprecision.

Methods: Informative SNP assays were selected for each patient as described2. Four-plex dPCRs were performed in separate reactions for graft and recipient. Molecule concentrations were calculated by Poisson statistics using molecule counts of four combined graft-specific dPCRs with 5-7µL sample volume and one recipient-specific reaction (1µL sample). Simulations were computed to assess the achievable total precision of dPCR quantifications with and without preamplification. These results were experimentally validated by direct comparison of both methods in 11 cfDNA pools of mixed normal and 19 transplant patient samples containing 0.5%-30% GcfDNA. Twenty-four different four-plex dPCRs were performed in 207 HTx patient samples.

Results: The ligation efficiency is the limiting step in preamplification and ranges from 40% to 85%. In silico simulations, considering this ligation variability and the low DNA amount, indicated that direct multiplex quantification is superior to dPCR with preamplification, despite 40-fold higher positive events in the latter. Consistently, the relative standard deviation (CV) in 11 cfDNA pools was 2.1-fold higher after preamplification. The CVs of the multiplex method in 19 LTx patient samples (mean:11%,SD:6%) were significantly lower than those obtained with preamplification (mean:28%,SD:15%, p=0.009). In 207 HTx patient samples with a median total cfDNA concentration of 63,210cp/mL plasma (range: 2,415-2,102,861) the median GcfDNA was 0.57% (range: 0.02-12.1%) resulted in a median CV of 7.6% (range: 1.0-38.3). A CV of <30% was achieved in 98.5% of all patient samples and 90% yielded <20% CV; the GcfDNA in the latter group ranged from 0.03-12.1% and 21-43,530cp/mL plasma.

Conclusion: The reliable quantification of rare DNA moieties is technically challenging, especially in cfDNA, with the scarce total DNA amount. The use of dPCR or HTS eliminates a potential calibration bias by direct molecule counting. Due to the hypergeometric contribution to the total measurement error, the variability in preamplification is deteriorating the precision, especially if the number of targets is very low, as in GcfDNA in HTx. Since this is a random error, it cannot be eliminated and therefore, avoiding preamplification resulted in lower imprecision. The advantage of direct cfDNA molecule counting is the ability to assess the total error by the Poisson counting error. With the testing of four independent SNPs in one dPCR and the summation of five multiplex dPCRs into one final result the number of counted molecules is sufficiently high to quantify GcfDNA levels in HTx patients with high precision at lower costs compared to HTS based methods.

1)DeVlaminck et al. Sci.Trans.Med(2014);6:241ra77

2)Beck et al. Clin.Chem(2013);59:1732-41

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Early Detection of Rejection after Heart Transplantation by a Universal Digital PCR Method.

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Background: Acute allograft rejection (AR) is a major complication after heart transplantation (HTx). Current standard for early detection of rejection is percutaneous-transvenous endomyocardial biopsy (EMB), a procedure, which is burdening, associated with risk for serious complications and false negative results, which can occur due to the patchy nature of AR. The quantification of Graft derived cell-free DNA (GcfDNA) is reported as biomarker for graft integrity, which can early detect AR in solid organ transplantation, only requiring a simple blood draw. Aim of the study was to investigate the diagnostic use of GcfDNA during the first year after HTx.

Methods:30 Patients (23m,7f) undergoing HTx were included with an age range of 26 to 69 years, of which 26(81%) survived the first year. 16 samples were drawn per patient during the first year post Tx. Immunosuppression was based on CsA or Tacrolimus with Mycophenolate and 6 patients received an mTor inhibitor. EMB-proven AR occurred in 13 Patients, where two had a Grade 2 AR after more than 120 days post HTx. GcfDNA was measured with a modified published digital PCR method, based on an universal probe set. The data were calculated as percentage of graft cfDNA per total cfDNA and as absolute values in copies/mL (cp/mL) of plasma.

Results: Immediately after engraftment, the GcfDNA was high with a median of 4.3% (IQR:1.9-5.2) and 3,478cp/mL (IQR:1,733-8,172), and decreased with an approx. half-life of 3.3 days (%) and 4.5 days (cp/mL). In uncomplicated courses the levels after 2 weeks were below a threshold of 0.6% and 160cp/mL respectively (95thpercentile).

In the initial 2 weeks distinct increases in individual courses are better suited to indicate a rejection, since the GcfDNA and total cfDNA levels show high inter-individual variability during the post Tx decay period.

In two cases of late rejections, both at one year with samples at the time of EMB-proven AR levels of 3% (694cp/mL) and 11% GcfDNA (512cp/mL) were detected. In three other cases of EMB-proven AR the GcfDNA increased from a) 0.15% to 2.88%, where the first significant change was seen after 6 months and the rejection was diagnosed after 12 months. b) in one earlier EMB-proven AR (2 months) GcfDNA increased beginning at 1 month to 3% and was falling to 0.6% after successful treatment. And c) the third case had a subtle steady increase from 0.2 to 0.7% beginning 2 months before diagnosis of AR at 6 months; GcfDNA reverted to 0.2% after treatment.

Conclusion:A modified dPCR method without pre-amplification was used to quantify GcfDNA. The measurements can be done within one working day at reasonable costs. Compared to earlier observations in liver recipients, the post reperfusion phase is characterized by a longer recovery time of the graft as assessed by the GcfDNA half-life. During this phase increases of GcfDNA are subtle, but distinguishable during AR. After that period, rejections can be early distinguished by increasing and elevated GcfDNA levels, which in the later phase is characterized by early and sustained increasing levels up to 6 months before diagnosis of AR.

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Unusual mosaicism 45,X/47,XXX attenuates Turner's phenotype

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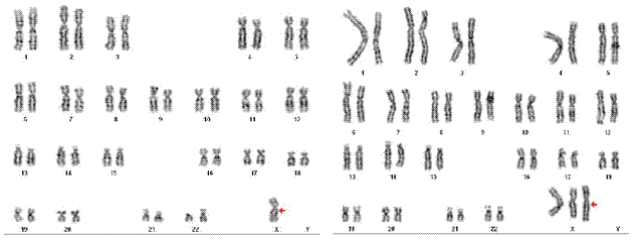
Introduction: Turner is syndrome characterized by ovarian failure, primary amenorrhea and short stature, it comprises a partial or complete absence of a sex chromosome and it's estimated that 1of 2.000 female newborns are affected. The ovarian failure generally occurs before puberty and leads to infertility. The triple X Syndrome has an incidence of 1 in 1.200 live born female and the phenotype varies greatly from women with normal intelligence and minimal dysmorphism to patients with severe mental disability. **Objective:** To describe an unusual karyotype finding with a mosaic for Turner Syndrome and Triple X Syndrome in a patient with Turner phenotype but without gonadal dysgenesis. **Method:** A 400-band Karyotype was performed in peripheral blood lymphocyte and 30 metaphases were analyzed in a 13 years old female patient to investigate short stature. The patient presented a suggestive phenotype for Turner Syndrome with 153 cm of height but with normal development of secondary sexual characteristics, menarche and a normal pelvic ultrasound. **Results:** The thirty metaphases analyzed 63% presented a monosomy of the sex chromosome X and 37 % a complete trisomy of the sex chromosome X: mos 45,X [19]/47,XXX [11] (Figure1). **Conclusion:** Mosaicism 45, X / 47, XXX is a very sporadic cause of ovarian dysgenesis. Many cases have been characterized by a variable Turner phenotype, including a 33 year old women with previous normal ultrasound, regular cycles and normal secondary sexual characteristic; at age 33 her ultrasound revealed evidence of streak-like ovaries, weight loss and breast regression. We might infer that this specific mosaic may attenuate the Turner phenotype enabling these women to have a menarche and normal pubertal development but the regression of the phenotype can occur in adulthood requiring constant monitoring and possibly a new karyotype to evaluate if the percentage of mosaicism remains the same.

45,X [19]/47,XXX [11]



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Mitochondrial DNA rare variants are genetic risk factors for type 2 diabetes for Chinese

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Abstract:**Background** The mitochondrial DNA variants and mitochondrial haplogroups have been suggested as risk factors for T2DM. However, the role of common variants, low frequency variants, rare variants, and singleton variants of mitochondrial genome with the risk of T2DM still have not been fully understood. Moreover, the interaction between haplogroups and variants in the mitochondrial DNA is also still clear. **Methods** In our studies, a total of 282 blood samples of type 2 diabetic patients and 396 blood samples of healthy controls were enrolled for analyzing from 2010 to 2013 in Zhejiang province, China.The entire mitochondrial genome for both patients and controls were sequenced. **Results** We demonstrated that the subhaplogroup M8a, included in mitochondrial haplogroup M, was associated with an increased risk of T2DM. While subhaplogroup N9a included in haplogroup N, was also associated with increased risk of diabetes. A total of 115 common variants (MAF≥5%), 288 low frequency variants, 599 rare variants (MAF<1%), and 835 singletons variants were identified across the entire mtDNA for the 2 major haplogroups M and N.For the common variants, our studies indicated that common variants in *CytB* were associated with decreased risk of diabetes only for haplogroup M. But the low frequency variants of mtDNA in both haplogroup M and N were not associated with risk of T2DM. In addition, the rare variants in *ND5* and *CytB* genes might contribute to the elevation risk of diabetes in haplogroup M. In contrast, the rare variants in *ND6* and *16S* genes were associated with increased risk of diabetes in haplogroup N. **Conclusion** Take those results together, our studies may provide the novel genetic evidences of rare variants in mtDNA are crucial for increasing the risk of T2DM in Chinese.

B-201

Performance Evaluation of New Solid Sample Types and Extraction Protocols on the Fully-Automated Tissue Preparation Solution

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BACKGROUND: Formalin-fixed, paraffin-embedded (FFPE) tissue samples are standard materials used by molecular pathologists, reference labs, research environments, and biobanks. An automated solution to extract high-quality nucleic acids from this challenging sample type is crucial to ensure accurate assay results and patient treatment. The IVD Tissue Preparation Solution (Siemens Healthcare Diagnostics, Tarrytown, NY), consisting of the Tissue Preparation System (TPS) and VERSANT® Tissue Preparation Reagents (TPR) Kit, is the only fully-automated method for deparaffinization and extraction of nucleic acids from FFPE samples with the proven flexibility to process a variety of sample type inputs as well. The new software delivers functionalities with expandable test definitions and an enhanced graphic user interface experience. The new software will include the existing *in-vitro* diagnostics (IVD) protocols (DNA, RNA, and SPLIT) as well as the new DNAext protocol. The new DNAext protocol leverages the validated workflow of DNA protocol while optimizing its extraction parameters to meet the increasing demand of novel molecular applications. The goal of this study was to evaluate the TPS protocols' extraction performance using the new software with FFPE samples and other solid sample types. METHODS: Four unique FFPE tissue samples were purified using the fully-automated IVD DNA protocol and the new DNAext protocol. DNA eluate yields from three sample replicates were compared between both extraction protocols. In addition, four replicates each of fresh frozen (FF) bone marrow tissue*, cultured cell lines*, and peripheral blood mononuclear cells (PBMC)* were extracted with the Tissue Preparation Solution. Paired DNA and RNA eluates (n = 4) from each sample were generated using the IVD SPLIT protocol. Nucleic acids extracted using the TPS protocols were qualified with in-house DNA and RNA surrogate gene real-time PCR/RT-PCR assays. The DNA and RNA eluates were also qualified using the industry-standard QUANT-IT PICOGREEN dsDNA and QUANT-IT RIBOGREEN RNA ASSAY KIT. RESULTS: When comparing the two DNA extraction protocols, the DNAext protocol, with increased lysis conditions, showed enhanced recovery of DNA from FFPE tissue samples. Furthermore, the SPLIT protocol extracted both DNA and RNA from a single sample of FFPE tissue, bone marrow, cell cultures, and PBMC. The extracted nucleic acids met all in-house acceptance criteria, demonstrating being both amplifiable and free of any inhibitory contaminants. In addition, commercially available qualification assays demonstrated that the DNA yield and integrity are suitable for molecular applications such as PCR and sequencing. CONCLUSION: The Tissue Preparation Solution is capable of extracting high-quality DNA and

RNA from FFPE tissue, FF tissue, bone marrow, cell cultures, and PBMC. The new TPS software with the DNAext protocol successfully extracted FFPE tissue DNA at higher concentrations than the DNA protocol. The Tissue Preparation Solution offers flexibility for processing multiple solid sample types using one instrument and universal IVD reagents. The newly developed Tissue Preparation Software version 2.1 provides workflow advantages by generating DNA and/or RNA eluates within a single extraction.

*Not a validated sample type.

B-202

Multiplex Ligation-Dependent Probe Amplification (MLPA) as a diagnostic tool for detection of Williams-Beuren syndrome (WBS).

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Williams-Beuren syndrome (WBS) is caused by a hemizygous contiguous gene microdeletion of the critical region on chromosome 7 at a position 7q11.23, which contains approximately 28 genes. Patients with WBS have specific dysmorphic features and are characterized by growth deficiency, mild cognitive delay with relative strength in expressive language, overfriendliness, hypercalcaemia and a supravalvular aortic stenosis (SVAS). The WBS is estimated to occur at a frequency of approximately 1 in 7500 live births with no ethnic or sex bias. Familial cases have been reported with apparent autosomal dominant inheritance. The most common deletion including the ELN gene is found in approximately 90-95% of the clinically typical WBS patients but in a lower percentage of atypical cases. The commonly deleted or duplicated chromosomal region has a size of approximately 1.5 to 1.8 Mb and is flanked by two highly homologous DNA sequences. However, smaller deletions involving only the ELN gene or the ELN and LIMK1 genes have also been described in SVAS and atypical WBS patients. Multiplex Ligation-Dependent Probe Amplification (MLPA, MRC-Holland) has been introduced into DNA diagnostic laboratories for the detection of molecular genetic alterations that are of diagnostic and prognostic significance. The MLPA kit for WBS, when compared to other techniques, is capable of detecting smaller and atypical deletions and duplications in the WBS critical region. Twenty patients with diagnosis hypothesis of WBS were tested using a commercial MLPA kit P029 specific for WBS and thirteen with kit P064 designed for mental retardation. The analysis was performed using the GeneMarker v2.6.2 software. Furthermore, 7 patients tested with Kit P029, were also evaluated by Short Tandem Repeat technique (STR) using 3 markers (HEI135, LINK1, D7S5613). Results obtained with MLPA kit P029 were concordant with MLPA kit P064 in all cases tested (n=13). The microdeletion was present in 3 patients and absent in 10. Using the STR technique, the results were concordant with MLPA kit P029 in 4 patients. However, we found 3 discrepant results comparing the microsatellite markes and MLPA method. The STR technique was not informative in these 3 cases since the absence of amplification of one allele does not discriminate the hemizygous from the homozygous condition that often leads to diagnostic difficulties the WBS. The most important advantages of the MLPA are its relative simplicity, low cost, rapid turnaround (2 days), ease of multiplexing to permit high confidence in the results, high accuracy of copy number estimation, and the potential for combination of copy number analysis with other applications. In conclusion, MLPA consists in a highly informative and easily manageable tool for the diagnosis confirmation of WBS and a faster and more economical method carried out in a single assay for the detection of deletions in WBS.

B-203

Detection of microdeletion syndromes by MLPA: A case of validation

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BACKGROUND: Chromosomal rearrangements that result in deletions and duplications of the part of the chromosome are among the main causes of congenital abnormalities. About 15-25% of the changes detected are microdeletions or microduplications, such as Velocardiofacial (del 22q11.2), Angelman and Prader-Willi (del 15q11) and Wolf-Hirschhorn(del 4p16) syndromes. The remaining cases are caused by other subtelomeric changes, balanced translocations, inversions, insertions, or mosaicism. Alteration of gene dosage due to gains or deletions of large genomic regions causes many genetic disorders that are frequently associated with intellectual disability (ID),multiple congenital anomalies (MCA), autistic spectrum disorders (ASD) and other phenotypic findings. Intellectual disability is characterized as impaired cognitive function and deficits in two or more adaptive behaviors and affects 1-3% of all children and cause a great impact on the lives of patients and their families. Understanding of the ID etiology is essential for guidance and genetic counseling for families as well as for the establishment of preventive measures.The Resolution of conventional karyotype isn't appropriate to detect small (less than 5-10Mb) genetic alterations under the microscope. However, techniques of molecular cytogenetics, having higher resolution, such as FISH (Fluorescent in situ Hybridization), MLPA (ligation-dependent probe amplification multiplex) and genomic microarray screening reach double the detection rate of chromosomal abnormalities.Our work aimed to verify the performance of the MLPA P064 kit in the laboratory's routine by comparing the results obtained in our verification and the results from 22 samples studied by MLPA at the Institute of Education and Research of Santa Casa Belo Horizonte - IEPSCBH.

METHODS: The MLPA p064 reaction was carried out following the manufacturer's instructions with little modifications. The Kappa statistic was used to calculate the concordance between the tests.

RESULTS: Among 22 samples, 2 individuals were positive to two different syndromes. A third individual had a positive result for one of the syndromes detected by the kit but he doesn't was considered a discordance because the different versions of kit used to do the test. Result's accordance obtained from these 22 samples using Kappa statistics was perfect 1,0 (0.582 to 1.0 CI 95%). There was no statistically relevant difference ($p < 0.001$) among compared results.

CONCLUSIONS: We concluded that the kit MLPA P064 could be used in the laboratory's routine for multiplemicrodeletionsyndromes simultaneously.

B-204

CX3CR1 mRNA expression is related to weight gain in obese individuals from Brazil

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Background: Obesity is cosmopolitan endemic disease caused by an imbalance between energy intake and expenditure. Pro-inflammatory status has been suggested to be involved in the adipogenesis, and similar to atherogenesis, may increase the susceptibility to cardiovascular disease. Polymorphisms in genes encoding proteins involved in the inflammatory process may contribute to the risk for obesity. Objective: To investigate whether polymorphisms e mRNA expression in inflammatory-related genes are associated with obesity in a Brazilian population. Methods: One-hundred-ninety-nine individuals attended at two Medical Centers of the Sao Paulo city, Brazil. were enrolled in this study. Anthropometrics, fat mass and clinical variables were recorded and blood samples were taken for DNA and RNA extraction and analysis of metabolic and inflammatory markers and adipokines. Individuals were grouped as lean (n=40, BMI: 18.5-24.9 kg/m2), overweight (n=55, BMI: 25.0-29.9 kg/m2) and obese (n=104, BMI: > 30kg/m2). CD40 (rs1883832) C>T, CX3CR1 (rs3732379) C>T, CX3CR1 (rs3732378) G>A, E-selectina (rs5368) C>T, ICAM-1 (rs1799969) G>A, ICAM-1 (rs281432) C>G, LIGHT (rs344560) G>A, LIGHT (rs2291668) C>T, RAGE (rs2070600) G>A, RAGE (rs2236493) C>T e VCAM (rs3176878) C>T gene polymorphisms and mRNA expression in peripheral blood leukocytes were analyzed by pyrosequencing and qRT-PCR, respectively. PAI-1, IL-6, TNF-α, resistin, adiponectin, and leptin were determined by and Luminex xMAP technology, and CRP-hs by imuno-nephelometry. Results: Serum CRP-hs, PAI-1, IL-6 and TNF-α were higher in obese than in lean group ($p<0.001$). The studied polymorphisms were not associated with obesity, and anthropometric and laboratory variables. Univariate logistic regression analysis showed that weight gain (increased BMI) was directly related to CX3CR1 mRNA expression (OR: 4.24, 95%CI: 1.27-14.08, p=0.018). Conclusions: These results are suggestive that mRNA expression of CX3CR1 increases with weight gain, reflecting the inflammatory status of obese individuals and, therefore, may be a useful biomarker.

B-205

Frequency of thrombophilic mutations associated with karyotype in women in laboratory investigation for infertility.

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Introduction: Infertility female factors can occur in 5 to 25% of cases, male causes 30 to 40% of cases and 10 to 15% cause can not be determined. Furthermore, infertility can be influenced a woman's age, frequency of sexual intercourse and body weight, therefore different diagnostic and therapeutic approaches may apply. The failure of embryo implantation is considered a relevant cause of failure in *in* vitro fertilization procedures. The blood disorders leading to hypercoagulability, in other words, thrombophilia may compromise the process of embryo implantation. Therefore, the interest in improving the embryo implantation rates led to the study of angiogenesis at the implantation site and, consequently of diseases related to alterations in blood coagulation system. The relationship between the thrombophilic factors and infertility should be considered as a possibile cause of spontaneous early miscarriage, caused by alteration in hemostasis of thrombophilic origin, at the implantation site. This abnormality affects vascular Trophoblastic invasion and placental vasculature.

Different genetic factors may contribute to infertility, but chromosomes number and structure stabilty is the main one. It is estimated that approximately 0,65%, i.e. one out of 153 live births, have chromosomal abnormalities, in cases of miscarriage, this number reaches reaches approximately 48,8%.

Chromosomal heteromorphisms are structural variants common in the population, without apparent phenotypic effect. It has been reported the increased incidence of chromosomal heteromorphisms in cases of infertility, recurrent miscarriages and other changes.

Method: We aimed to evaluate the prevalence of factors of hereditary and acquired thrombophilia, and its associaton with karyotype abnormalities, in females reffered to a large reference laboratory. We retrospectively surveyed LIS database, from January to December 2014, for concomitant results of thrombophilic alterations (Factor V Leiden; Antithrombin III; gene mutations on C677T, A1298C and MTHFR, and in prothrombin gene) and G-band Karyotype in females aged between 18 and 50 years-old.

Result: Eighty six women fitted the inclusion criteria, 59% [51/86] presented some type of alteration on coagulation related factors studied. Twenty seven (53%) of those with thrombophilic abnormalities presented some chromosomal heteromorphisms, found in chromosomes 1, 9, 15 and 21. And also, one woman (1.96%) presented translocation on chromosome 46,XX,t(11;17)(p11.2;p13), associated with alteration of antithrombin.

Conclusion: The heteromorphisms have been observed with increased frequency in infertile couples, with history of miscarriages, parents of children with chromosomal abnormalities, and chromosomally abnormal live births. The inefficient placental blood flow caused by thrombophilic events or vascular insufficiency can lead to deleterious effects on the development of pregnancy. Therefore the association chromosomal and thrombophilia or both disorders may contribute aditionally to female infertility. This study showed that more than half of women reffered to a large laboratory for infertility investigation presented both conditions together. This association may increase the severity of infertility and, therefore, it is recommended to evaluate Karyotype and thrombophilia, in order to better understand the causes and manage female infertility.

B-206

Screening of alterations in copy number variation (CNV) in subtelomeric regions by MLPA as test diagnosis for patients with congenital malformation and disability intellectual

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Alterations in copy number variation (CNV) represent about 3-8% of potential cause of delay development, dysmorphic featuresand congenital abnormalities in subtelomeric regions. In these regions there are genes flanked by short repetitive sequences of nucleotides that are highly unstable. The detection of abnormal copy numbers in subtelomeric regions should be done carefully because the subtelomeric copy number changes can also occur in unaffected individuals and the effect of a deletion or duplication will depend on the genes involved. The detection of abnormal copy numbers in subtelomeric regions should be done carefully because the subtelomeric copy number changes can also occur in unaffected individuals and the effect of a deletion or duplication will depend on the genes involved. Nowadays, there are many methods to study deletions and duplications in the whole genome as fluorescent in situ hybridization (FISH), Multiplex Ligation-dependent Probe Amplification (MLPA) and whole-genome array screening. The study of deletion and duplications in subtelomeric regions by MLPA technique is an alternative more accessible and suitable for quantification of CNV. The aim of this study was to validate two P036 and P070 MLPAkits to investigate CNV in subtelomeric regions of the 23 pairs of chromosomesin a clinical laboratory.

The samples of 31patients of both sexes, with age birth to adulthood were collected at Institute of Education and Research of Santa Casa de Belo Horizonte - IEPSCBH and tested with the kit P036 and P070 Human Telomere in parallel with Laboratory of Human Genetics - Institute Hermes Pardini. The MLPA reaction was carried out following manufacturer's instructions. GeneMarker® software was used to analyze the electropherograms. The Kappa statistic was used to compare the results.

Our results were concordant in 100% of analyzes.The kit P036 found three alterations, in the chromosomes 4p16.3, 8p23.3 and 22q 11.21 (9.67%) and the kit P070 found two alterations, in the chromosomes 4p16.3 and 8p23.3 (6.45%) in the samples of 31 patients.The difference in detection of chromossome 22 is because the region investigated by P070 is other (22q13.33), so the use of both kits concomitantly can improve detection rate of alterations.

We concluded that the kit MLPA P036 and P070 could be used in the laboratory's routine and we recommended the use of both concomitantly to improved detection rate of the alterations.

B-207

Association between polymorphisms of microRNA-binding sites in integrin genes and gastric cancer in Chinese Han population

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Background: Single nucleotide polymorphisms (SNPs) in the microRNA-binding sites within 3′ UTR region of target genes may cause the level change of target gene expression and then influence the susceptibility to diseases including cancer. Recently the expression of integrin has been reported highly elevated in a variety of malignant tumors. This study aimed to investigate the association between polymorphisms of microRNA-binding sites of integrin genes and gastric cancer (GC) in Chinese Han population.

Methods: Five SNPs of the microRNA-binding sites in the 3′ UTR region of integrin genes (rs1062484, rs17664, rs3809865, rs743554 and rs2675) were genotyped in 472 GC patients and 521 unrelated controls in Chinese Han population, using high resolution melting (HRM) method on LightCycler 480 machine.

Results: The genotype distributions of the five SNPs in GC patients and controls were all within Hardy-Weinberg equilibrium (HWE).The polymorphism of SNP rs2675 was associated with susceptibility of GC (allele: P=0.02, OR=0.75, 95%CI = 0.59-0.95; genotype: P=0.03). No significant differences of genotype distributions or allelic frequencies of the other four examined SNPs were found between patient and control groups.

Conclusion: The findings of this study suggest that the polymorphism of rs2675 within microRNA-binding sites in the 3′ UTR region of integrin is associated with GC susceptibility in Chinese Han population. More well-designed studies with larger sample size have to be conducted to confirm our conclusion.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Pediatric/Fetal Clinical Chemistry

B-208

Effect of Multiple Transfusions on Lipid Peroxidation in Preterm Infants

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Multiple blood transfusions are commonly used in the course of neonatal intensive care unit (NICU) stay in very low birth weight (VLBW) infants. The number of transfusions received has been associated with the development of prematurity complications (retinopathy, necrotizing enterocolitis, bronchopulmonary dysplasia, intraventricular hemorrhage, and periventricular leukomalacia). The severity of the illness can increase the number of transfusions required, resulting in iron overload which may increase the release of reactive oxygen species. The aim of this study was to determine the relationship between blood transfusions, ferritin levels and oxidative stress in preterm infants.

Preterm infants (n=23, gestational age 28.43±3.50 weeks; birth weight 1180±471g) admitted to the NICU were enrolled. Five of them (21.7%) were never transfused, while 10 cases (43.5%) were transfused less than 5 times, 2 cases (8.7%) 6-10 times, and 6 cases (26.1%) were transfused more than 10 times. Venous blood samples were taken when they were at least 20 days of age in a period free of infection according to clinical signs and laboratory test results. Serum malondialdehyde (MDA) levels were measured by HPLC (Ultimate 3000, Thermo Dionex, USA) with a flourescence detector. Within-run precision values were 1.8-5.5% and between-run precision values were 6.5-9% for 0.40-1.55 µmol/L MDA, according to manufacturer's claim. The lower detection limit was 0.02 µmol/L. Serum iron and iron binding capacity were measured colorimetrically (Cobas 8000 Modular Analytics, Roche Diagnostics, Germany). Ferritin levels were measured with an immunometric test with electrochemiluminescence detection (Modular Analytics E170, Roche Diagnostics, Germany).

There was a significant difference in serum ferritin levels between transfused (median: 457ng/mL, range:108-2717) and non-transfused (median: 203ng/mL, range:102-268) infants (P=0.017). There was a statistically significant correlation between serum ferritin and MDA levels (P<0.001; r=0.693). Also, the correlation between the number of transfusions and serum ferritin levels was statistically significant (P=0.016; r=0.558). Serum MDA levels were significantly higher in infants with serum ferritin levels >450 ng/mL (P<0.001). When the infants were grouped according to prematurity related complications; transfusion numbers, serum ferritin, and MDA levels of those with two or more complications were significantly higher when compared to cases without complications (P<0.001, P=0.001, and P=0.019, respectively).

In conclusion, iron status of VLBW infants has to be monitored to detect iron deficiency and also transfusion-related iron overload. Ferritin can be used to assess the iron status of preterms. Ferritin levels can also reflect lipid peroxidation as we have shown its correlation with MDA, the levels of which were higher in infants with two or more prematurity-related complications. It is important to use restrictive transfusion guidelines in order to protect preterms from iron overload and oxidative stress. Further research is necessary to determine a cut-off level for ferritin to decide when to start iron prophylaxis.

B-209

An Evaluation of the HemoCue Assay for the Rapid Assessment of Plasma Free Hemoglobin in Pediatric Patients Undergoing Extracorporeal Membrane Oxygenation (ECMO)

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Background: Extracorporeal membrane oxygenation (ECMO) therapy on newborns is associated with increased risk of hemolysis which can lead to hemoglobin associated nephropathy and, in some cases, acute renal failure. Accordingly, ECMO patient care guidelines stress the importance of early detection of ECMO associated hemolysis *via* measurement of the levels of plasma free hemoglobin.

Objective: To verify the accuracy and precision of the HemoCue spectrophotometric method for assessment of plasma free hemoglobin (PfHb) on ECMO patients with a focus on values in the lower range of detection.

Methods: HemoCue (HemoCue, Brea, CA) PfHb results were compared to those obtained on the well-established Abbott Architect (AA) c4000 analyzer (Abbott Laboratories, Chicago, IL). The HemoCue method 1) oxidizes hemoglobin to methemoglobin with nitrite, then 2) after conversion to azidemethemoglobin, absorbance is measured at 570 and 880 nm. Potential interferences were assessed by performing a spectrophotometric scan on our Cary 100 (Agilent, Santa Clara, CA) in order to determine the degree of spectral overlap with bilirubin and lipoprotein, the latter being partially corrected using the 880 nm absorbance reading. In addition, analysis for bilirubin and triglyceride was performed. Also, as an adjunct marker for hemolysis, lactate dehydrogenase was measured. Precision studies were based on replicate analysis on patient plasma samples. Assays were also performed on ultracentrifuged samples with results compared to those obtained on neat and filtered samples. Serial dilution recovery studies were performed on a lysed sample with a known/measured hemoglobin value (Unicel DxH800, Beckman Coulter, Brea, CA).

Results: Linear regression analysis showed a significant proportional error with results 29 percent lower on the Hemocue method (Hemocue=0.71*AA + 4.8; R^2 = 0.70, N = 22). Two samples with triglyceride > 400 mg/dL showed spuriously high Hemocue values. No statistical difference in mean values was observed between the two methods (t Stat = 0.165, P = 0.870). Serial dilution recovery on the Hemocue method was 106 percent (Hemocue (7.2 g/dL) versus DxH800 (6.8 g/dL)). The serial dilution curve plot showed an excellent fit with an expected log relationship (Hemocue = -111.5*ln(Dilution Factor) + 594.3). Precision studies showed CV% at seven levels ranging from 9.7- 4.2 percent. LDH showed positive correlation with PfHb values obtained on the AA method (AA = 5.9*LDH + 290; R^2=0.44). As expected, bilirubin levels showed a positive PfHb interference, the latter increasing slightly with the bilirubin level. Neat samples tested versus filtered samples showed no significant difference whereas ultracentrifuged samples consistently gave lower PfHb values.

Conclusions: The HemoCue method provides rapid and reasonably accurate measurements of plasma free hemoglobin. However, our study suggests that levels in the 10 to 30 mg/dL range should be interpreted with caution. Triglyceride and bilirubin both interfere in a proportional manner and must be considered when interpreting results on unfiltered samples. Adding LDH as a surrogate hemolysis marker appears to be of marginal value.

B-210

Transference of CALIPER Pediatric Reference Intervals to Beckman Coulter AU Clinical Chemistry Assays

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Objective: The CALIPER program has established a comprehensive database of pediatric reference intervals largely using the Abbott ARCHITECT biochemical assays. To expand clinical application of CALIPER reference standards, transference studies have been initiated to transfer data from Abbott assays to other common clinical chemistry platforms based on the CLSI guidelines. Here, we report a transference study aimed to transfer CALIPER reference intervals from the Abbott ARCHITECT to Beckman Coulter AU assays.

Design and Methods: Approximately 200 pooled patient serum specimens were measured on both the Abbott ARCHITECT c8000 and the Beckman Coulter AU systems. Beckman coulter offered more than one assay for the majority of tested analytes. Data analysis and transference were performed in accordance with the CLSI documents C28-A3 and EP9-A2. R^2 values were determined using linear or Deming regression, and quantile-quantile, standardized residual, and Bland Altman plots were used to assess the correlation of the data between the two systems. Analytes with an R^2 value <0.70 were deemed non-transferable. For stringent validation, 100 reference samples from the CALIPER cohort of healthy community children were assayed on the Beckman Coulter AU system. Transferred reference intervals were considered verified when ≥90% of CALIPER values fell within the 95% confidence intervals of the calculated intervals.

Results: Results from the vast majority of Beckman Coulter AU assays (82%; 51/62) strongly correlated (R^2≥0.70) with the corresponding Abbott ARCHITECT assays. Only bicarbonate and calcium results showed poor correlation between both systems. Abbott ARCHIITECHT reference intervals were transferrable to all 51 Beckman Coulter assays. Transferred reference intervals were, in general, similar to previously published CALIPER reference intervals. The vast majority of the transferred reference

intervals were sex-specific. Most [80% (40/51)] of the transferred reference intervals were verified using healthy children reference samples from the CALIPER cohort. This percentage increased to 94% (48/51) if we set the verification cutoff to 80% of CALIPER samples falling within the 95% confidence intervals of the calculated reference intervals. It is important to note that the comparisons performed between the Abbott ARCHITECT and Beckman Coulter systems make no assumption as to which system is more accurate.

Conclusion: The majority of CALIPER reference intervals were transferrable to Beckman Coulter AU assays allowing the establishment of a new database of pediatric reference intervals. This further expands the utility of the CALIPER database to clinical laboratories using the AU assays and should help improve test interpretation in the clinical setting. Laboratories using the assay-specific CALIPER reference intervals reported in the present study should perform further validation on their own testing platform using reference specimens from healthy children in the local population as recommended by CLSI.

B-211

Lecithin-sphingomyelin ratio and phosphatidylglycerol are not superior to lamellar body count when assessing risk for respiratory distress syndrome

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Background: In our practice, lamellar body count (LBC) is the initial laboratory testing for assessing the maturity of the fetal lungs. LBC <15,000 is indicative of an immature fetus, while LBC >39,000 is indicative of a mature fetus. LBC 15,000 – 39,000 is considered indeterminate. Lecithin-sphingomyelin ratio (L/S ratio) and Phosphatidylglycerol (PG) by thin layer chromatography (TLC) used to be considered confirmatory testing for assessing the risk of respiratory distress syndrome (RDS). LBC is easy to perform with a quick turn-around time, and is available 24 hours per day, while the L/S Ratio and PG takes approximately 6 hours and requires tedious sample preparation. The important L/S ratios as related to fetal lung maturity are divided into two categories: immature (L/S<2.0) and mature (L/S ≥2.0). A PG positive result is indicative of mature lung. Recent literature casts doubt on the values of L/S ratio test. We hypothesized that L/S ratio and PG are not better indicators than LBC for assessing the risk of RDS. **Design:** Amniotic fluid was collected via standard clinical practice. LBC was run immediately after sample collection and samples with LBC 15,000-39,000 were performed for L/S ratio and PG at the time of clinical care. Leftover samples with LBC >39,000 and <15,000 were kept at -70°C for later L/S ratio and PG testing. Collection of leftover patient samples and clinical data for this study was approved by the Institutional Review Board. **Results:** Of the 113 samples, 72 samples had LBC >39,000, while 5 had LBC <15,000, and 36 had LBC between 15,000 and 39,000. 29 samples with positive or negative LBC results were randomly selected and analyzed for L/S and PG. In total, there were 64 samples with complete data for LBC, L/S, and PG. Of the 64 patients, 7 babies were born with RDS. Their LBCs ranged from 1,000 – 38,000 (2 had LBC <15,000, the remaining 5 had LBC 15,000 - 39,000). The L/S ranged from 1.4 – 3.4, while 4 out of the 7 samples were negative for PG. 93% of LBC gave correct diagnosis (<15,000 with RDS and >39,000 without RDS), while 80% of L/S ratios gave correct diagnosis (<2.0 with RDS and ≥2.0 without RDS), and only 63% PG results gave correct diagnosis (negative with RDS and positive without RDS). For LBC in indeterminate range (15,000 to 39,000), 77% of L/S ratios gave correct diagnosis, and only 44% of PG results gave correct diagnosis. **Conclusion:** L/S ratio and PG are not superior to LBC for predicting RDS. However, L/S ratio may be used as a follow-up test for patients with indeterminate LBC results.

B-212

Validation of Minimum Volume Blood Gas Collections

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Background:

We evaluated minimum collection volume in the Smiths Medical Portex 1 mL Line Draw Arterial Blood Sample Syringe kit (Smiths Medical, Keene NH) that would produce reliable arterial blood gas (ABG) and electrolyte results.

Methods:

We collected 0.3 mL blood through an arterial catheter from adult inpatients in a 1 mL Smiths Medical blood gas syringe with a Smiths Medical Filter-Pro device to remove air bubbles; and compared ABG results to those obtained from a full 3 mL Smiths Medical Portex syringe (with Filter-Pro). Analytes measured included pO2, pCO2, pH, hemoglobin, ionized calcium, sodium, potassium and glucose on Radiometer ABL825 and Radiometer ABL90 (Radiometer, Bronshøj, Denmark) blood gas analyzers. We also compared ABG results between minimum volume samples hand-carried to the laboratory vs. those sent via pneumatic tube. Finally, we evaluated 0.3, 0.4, and 0.5 mL collection volumes with mixing of samples for 2 minutes (rather than 30 seconds) prior to analysis on the ABL90 analyzer.

Results:

Minimum volume (0.3 mL) samples analyzed on the ABL825 demonstrated a mean (SD) hemoglobin bias of -0.4 ± 0.3 g/dL, with 3/20 samples demonstrating hemoglobin results > 0.5 g/dL different from the matching 3 mL syringe value. In contrast, minimum volume samples (n=14) analyzed for hemoglobin on the ABL90 demonstrated a mean (SD) bias of -0.1 ± 0.2, with 13/14 within 0.5 g/dL of the matching full syringe value.

pO2 values (n=20) from the 0.3 mL collections demonstrated a mean (SD) bias of 31 ± 25 mm Hg compared to full 3 mL syringe values, with 17/20 failing to meet crosscheck criteria (within 10 mm Hg at pO2 < 100 mm Hg and within 10% at pO2 ≥ 100 mm Hg). Hand-carrying 0.3 mL samples did not significantly impact this bias (n=10), with a mean (SD) bias of 35 ± 38mm Hg and 7/10 failing crosscheck criteria.

Comparison of 0.3, 0.4, and 0.5 mL collection volumes in the 1 mL syringe demonstrated mean (SD) pO2 bias of 9 ± 14 mm Hg (0.3 mL), 12 ± 23 mm Hg (0.4 mL), and 3 ± 11 mL (0.5 mL) when samples were mixed for 2 minutes prior to analysis (rather than 30 seconds). 8/20 (0.3 mL), 7/19 (0.4 mL), and 4/19 (0.5 mL) samples failed crosscheck criteria for pO2 when samples were mixed 2 minutes prior to analysis on the ABL90. No other blood gas or electrolyte analytes demonstrated significant differences between sample volumes or analyzers.

Conclusion:

Oxygen tension and hemoglobin demonstrated sensitivity to sample volume. Use of the ABL90 (rather than ABL825) improved accuracy of hemoglobin measurement for reduced sample volumes. For pO2, significant bias and variability was seen when less than 0.5 mL was collected into a 1 mL syringe. Increasing mixing time to 2 minutes (from 30 seconds) mitigated this bias, though collection of volumes < 0.5 mL still resulted in ± 20 mm Hg variability in pO2 values. Neonatal practices using minimum volume collections should be aware of the potential for variability in pO2 values.

B-213

L-2-Hydroxyglutaric aciduria: a case report

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Introduction: L-2-Hydroxyglutaric aciduria (L-2-HGA) (OMIM #236792) is an autosomal recessive neurometabolic disease. Since its first description by Duran in 1980, only few cases have so far been reported. It occurs mostly in childhood and characterized by slowly progressive neurological dysfunction with cerebellar ataxia, pyramidal signs, intellectual decline, seizure, and extrapyramidal symptoms. Characteristic magnetic resonance imaging findings include signal intensity abnormalities of the subcortical cerebral white matter, putamen, and dentate nucleus. We report two siblings who were diagnosed to have L-2HGA.

Case report: An 11-year-old boy was referred for extrapyramidal movements and learning disabilities. He was born to 2nd degree consanguineous parents and had an uneventful perinatal period. He had normal development until the age of 5 years, when he presented with afebrile seizures and social withdrawal. This became progressively worsened. On examination, he had extrapyramidal movements consisting of ataxia, tremors and dyskinetic movements. He was able to speak short sentences with meaning. Cranial nerve examination was normal but had mild spasticity of all four limbs. He had normal occipito-frontal circumference. Blood counts, renal and liver function tests were normal. Cranial MRI showed generalized polymicrogyri and white matter changes involving the cerebrum and cerebellum with a subcortical distribution and changes of the basal ganglia. Electroencephalogram showed frequent beta activity diffusely but within normal limits. Urinary organic acids done by gas chromatography/mass spectrometry (GC-MS) showed elevated 2-OH glutaric acid levels with normal levels of glutaric acid, ethyl malonic acid and isovaleryl-glycine. His older sibling had similar neurological manifestations but with milder learning disabilities. Urinary organic acid profile of the older sibling also revealed elevated levels of 2 hydroxyglutaric acid. Acylcarnitine profile, plasma amino acids and chromosome study were normal. In view of the clinical picture and elevated levels of 2-hydroxy glutaric aciduria, enantiomeric analysis was done and that confirmed the diagnosis of L-2-HGA. Molecular analysis confirmed the homozygous mutation

c.844C>T of the L2HGDH gene. Our patients were started on Riboflavin and follow-up have shown improvement in the dystonia.

Conclusion: L-2-Hydroxyglutaric aciduria is a neurometabolic disorder which should be considered as a differential diagnosis in patients with neurodevelopmental regression, extrapyramidal signs and characteristic MRI findings.

B-214

Is the use of pancreatitis associated protein (PAP) as a second tier in newborn screening of Cystic Fibrosis justified?

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Background:

New born screening (NBS) for cystic fibrosis (CF) in Ireland was incorporated into the national newborn bloodspot screening programme from the 1st of July 2011. The programme adopted an IRT/DNA strategy.

Use of DNA technology is inevitably associated with the risk of incidental findings. In the context of NBS for CF this corresponds to a detection of carriers and variants of uncertain significance, the number being dependent on the initial IRT cut-off and the number of CFTR mutations screened.

In order to mitigate the potential for harm, some NBS programmes have incorporated a second tier PAP measurement on the bloodspot samples with raised IRT, instead of or in combination with DNA testing. This allows either no or less CF carriers to be detected, depending on the regulatory standards of the individual country.

Study Objective:

The objective of this study was to establish

1. Whether the use of PAP as a second tier screening test is justified to reduce the number of carriers detected or could the same improved performance be achieved by simply raising the bloodspot IRT cut-off concentration.

2. The optimal IRT/PAP/DNA protocol in the Irish setting.

Methods:

Screening protocols

The performance characteristics and the screening cost of three different screening protocols were compared with the existing strategy, one of which was IRT/DNA with a top 0.5 percentile IRT cut-off and two of which were IRT/PAP/DNA protocols, each with different IRT and PAP cut-offs. IRT/PAP/DNA strategy protocols considered were:

1. IRT/PAP-fail safe (FS) IRT/DNA strategy. Samples with an IRT above the top one percentile were analysed by PAP and those above a single PAP cut-off were referred for DNA analysis. The protocol included a fail-safe, in that all samples with an IRT above the top 0.1 percentile, irrespective of the PAP concentration, were referred directly for DNA analysis.

2. IRT/PAP/DNA strategy using two IRT and two PAP cut offs.

Study population

The 12 month prospective observation study was performed in the National Newborn Bloodspot Screening Laboratory, in Temple Street Children's University Hospital, Dublin.

Results:

During the one year study period, 72,177 babies were screened. The IRT/PAP/DNA strategy with two PAP cut offs, would have resulted in 298 samples being referred for mutation analysis and the parents of 47 babies referred for genetic counselling (as opposed to 796 and 80 respectively with our existing IRT/DNA strategy). The strategy would detect 25 infants with classic CF, no equivocal diagnosis and 22 carriers, reduced by almost 60% in comparison with the existing strategy. One CF case Phe508del + 2-2657+3insA would be missed. The significance of the latter mutation is however of uncertain significance.

Conclusion:

IRT/PAP/DNA protocol incorporating two IRT and two PAP cut-offs had the best performance characteristics, namely a much better PPV, compared to the other strategies. This strategy significantly reduced the number of carriers detected by more than 50%, from approximately 1.9% of carriers born per year to just 0.8% and did not detect any variants of uncertain phenotypic significance. It also resulted in significant cost savings of €147,000 per 100,000 babies screened ($165,000).

B-215

Utility of full gene analysis in the diagnosis of cystic fibrosis

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Background:

Over 1500 mutations in the CFTR gene have been identified. The current gold standard for diagnosing cystic fibrosis (CF) is the sweat test. Additionally, multiple genetic testing options are available including several mutation panels as well as a full gene analysis (CFTR sequencing). The advantage of full gene analysis over mutation analysis, especially in patients that have indeterminate sweat chloride results, has not been well documented. Additionally, the correlation between genotype and phenotype is extremely variable.

Methods:

This was a single-institution, retrospective clinical study. We identified all sweat chloride tests ordered at St. Louis Children's Hospital from July 1, 2012 through June 31, 2014. For each patient with a sweat chloride >30 mMol/L or higher, a chart review was conducted to obtain genetic testing results and final diagnosis.

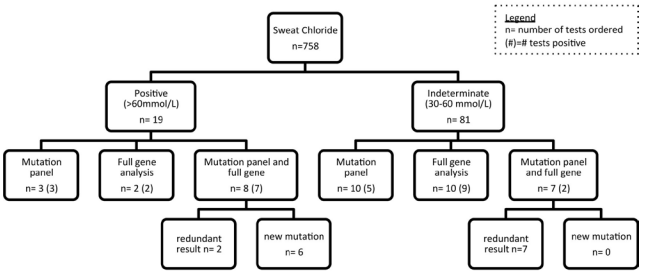
Results:

Of the 758 sweat tests conducted within the 2 year period, 19 (2.5%) resulted positive (>60mmol/L) and 81 (10%) indeterminate (30-60mmol/L). Of the 19 patients that had positive sweat chloride, 13 underwent genetic testing. 12 (92%) were positive for CFTR mutations. In this group, full gene analysis identified a new mutation that was classified as clinically significant in 3 of the 10 cases (S489X, E1371X, and I618T); however their identification was not diagnostically or therapeutically useful.

Of the 81 patients that had an indeterminate sweat chloride, 27 underwent genetic testing. 16 (59%) patients were positive for CFTR mutations. All 7 instances were both a full gene and mutation panel was requested resulted in redundant results. In this group, full gene analysis identified a new mutation in 2 out of 17 cases (I1139V and P750L); however this knowledge did not aid in diagnosis.

Conclusion:

At this point, full gene analysis does not seem to offer a diagnostic advantage in both sweat chloride positive and indeterminate patient populations.



B-216

A six-month survey of meconium and umbilical cord drug testing results between July and December of 2014

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Background: The concern of substance-exposed newborns and neonatal abstinence syndrome is rising. Meconium and umbilical cord are unique neonatal specimen types that provide longer detection window for drug testing than maternal/neonatal urine. Due to ethical considerations, data of drug deposition and concentration in neonatal matrices addressing *in utero* substance exposure is limited.

The objective was to survey the drug classes found in unmatched meconium and umbilical cord samples from at-risk newborns submitted to our laboratory for forensic purposes.

Methods: Meconium and umbilical cord specimens were aliquoted at 0.5g for drug screening by validated immunoassays. Presumptive positive samples were then aliquoted again for confirmation by validated chromatography-mass spectrometry-based methods to identify and quantify drugs and metabolites. Results were collected between July and December 2014 and summarized in the Table.

Results: During the 6-month period, 8,169 meconium samples and 16,985 umbilical cord samples were received. The two matrices showed similar positivity rates or

prevalence ranking for most drug classes. Cotinine, carboxy-THC, opiates (codeine, morphine, hydromorphone, and hydrocodone), and buprenorphine (BUP) are the four classes with highest positivity rate seen in both matrix types, followed by other pharmaceutics oxycodone/oxymorphone (OXY), methadone, and benzodiazepines (BZP). Amphetamines and cocaine are the next two prevalent illicit drug classes found in both matrix types. Some discrepant positivity rate of each drug class between the two matrices may be due to distinct sampling sizes, analytes of choice, and analytical sensitivity. For example, oxazepam was the only BZP analyte confirmed in meconium, whereas nordiazepam, diazepam, alprazolam, midazolam, temazepam were additionally confirmed in umbilical cord. BUP and OXY had doubled positivity rate in umbilical cord compared with meconium, possibly due to much lower limits of quantitation.

Conclusion: We showed that cotinine, THC, several opioids, and benzodiazepines are the most commonly seen drug types tested in both meconium and umbilical cord.

Positivity rate and minimum-maximum (median) concentrations of drugs in meconium and umbilical cord			
Meconium positivity rate	Min-Max (median) ng/g	Umbilical cord positivity rate	Min-Max (median) ng/g
Cotinine 20.9% (14/67)	11 - 133 (55)	Cotinine 49.9% (384/769)	2.2 - 505 (58)
THC 16.1% (1315/8169)	40 - 3,033 (156)	THC 15.2% (2581/16985)	0.04 - 75 (0.9)
Opiates 8.8% (719/8169)	41 - 16,806 (239)	Opiates 12.2% (2071/16985)	0.2 - 414 (2.6)
Buprenorphine 6.6% (134/2031)	8.6 - 5,250 (238)	Buprenorphine 11.4% (956/8405)	0.13 - 61 (2.6)
Methadone 4.7% (254/5428)	84 - 141,256 (6,027)	***Oxycodone 4.6% (554/11994)***	0.2 - 1,826 (2.8)
Amphetamines 4.1% (337/8169)	41 - 36,829 (979)	Methadone 3.7% (529/14236)	0.8 - 403 (57)
Cocaine 2.6% (215/8169)	40 - 6,226) 279	***Benzodiazepines 3.1% (414/13234)***	0.9 - 384 (4.6)
Oxycodone 1.9% (64/3437)	92 - 1,631 (385)	Amphetamines 2.9% (495/16985)	2.0 - 2,877 (42)
Tramadol 1.3% (44/3437)	65 - 25,136 (1,872)	Cocaine 1.9% (322/16985)	0.2 - 6,435 (15)
Meperidine 1.2% (41/3437)	47 - 1,531 (325)	Barbiturates 1.7% (247/14236)	1.9 - 7,989 (237)
Barbiturates 1.0% (53/5428)	263 - 28,889 (1,708)	Meperidine 1.3% (161/11994)	1.0 - 545 (33)
Benzodiazepine 0.1% (5/4333)	62 - 310 (203)	Tramadol 1.2% (148/11994)	1.4 - 2,384 (115)
PCP 0.09% (7/8169)	34 - 1,112 (258)	PCP 0.02% (3/16985)	7.4 - 34 (9.7)
Propoxyphene 0.02% (1/4333)	(1,405)	Propoxyphene 0.01% (1/13234)	(82)

B-217

Serum amyloid A (SAA) values in healthy newborns and infants

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Background: Serum amyloid A (SAA) is important diagnostic biomarker including peadiatric diseases. The aim of the study was to evaluate the reference intervals of SAA in healthy newborns and infants.

Methods: Serum levels of SAA were investigated in group of 50 healthy children aged from 1 day to 1year (30 boys and 20 girls), CRP (C reacting protein) less than 3 mg/l. We used commercially available immunonephelometric assay on an Beckmann Immage 800 analyzer. Children were divided into three groups according to the age (1st group - 10 newborns with the age 1 - 30 days; 2nd group - 30 children with the age 31 - 180 days; 3rd group - 10 children with the age of 181 days - 1 year). Simple nonparametric bootstrap procedure was used for the evaluation of the reference ranges (2.5th and 97.5th percentile) in selected groups.

Results: Serum amyloid A (SAA) reference ranges in groups of newborns and infants were as follows (expressed as 2.5th and 97th percentile): 1st group: 1.00 - 8.93 mg/l; 2nd group: 0.99 - 10.56 mg/l, 3rd group: 0.99 - 3.73 mg/l.

Conclusion: We evaluated SAA reference intervals in healthy newborns and infants.

B-218

Evaluation of Cotton Balls for Urine Collection for Measurement of Homovanillic Acid and Vanillylmandelic acid Acid

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Background: Homovanillic acid (HVA) and vanillylmandelic acid (VMA) are measured in the diagnosis and follow-up of neuroblastoma that are most common cancer type in infants and young children. Urine is a preferred sample for the measurement of HVA and VMA. Although 24 hour urine sample collection is probably the best, random urine collection with normalization of HVA and VMA results by creatinine concentration are acceptable for both diagnosis and follow-up of neuroblastoma. Urine collection in children could be challenging and it often needs use of bags for sample collection. This method is cumbersome and time consuming. Alternate ways of sample collection such as urine collection on filter paper have been used. We investigated the possibility of urine collection on cotton balls as they are easy to use and widely available. We evaluated 4 different types of cotton balls as an alternate way of urine collection for the measurement of HVA and VMA.

Methods: Four different cotton balls were evaluated: Walgreens Studio 35 Beauty, Wal-Mart White Cloud, Target Up &Up and Kendall Curity. A total of 22 patient urine samples, commercial controls purchased from Bio-Rad Diagnostics and spiked urines were used for this study. These samples were tested for creatinine, HVA and VMA concentrations prior to the addition of cotton balls. One cotton ball from each source was saturated with 2-5 mL of each patient, control and spiked urine and then processed at 6 hour and 18 hour intervals for creatinine, HVA and VMA analyses. Creatinine was measured using a Syva V-twin chemistry analyzer. HVA and VMA were extracted from urine using ethyl acetate. The extracts were derivatized, and HVA and VMA were measured by gas-chromatography mass spectrometry using deuterated internal standards. HVA and VMA concentrations were expressed as mg/g creatinine.

Results: No significant difference was noted either in creatinine or HVA and VMA concentrations in the samples incubated with cotton balls as compared to straight samples. Mean creatinine concentrations were 121, 123, 122, 124 and 122 mg/dL for direct sample, and samples incubated with cotton balls from Walmart, Walgreens, Target and Kendall respectively. Also, no significant difference was found in HVA and VMA concentrations among direct samples or samples incubated with cotton balls. Mean HVA concentrations (mg/g creatinine) were 15.2, 15.1, 15.0, 15.3, and 15.4 respectively. Mean VMA concentrations were 12.7, 12.7, 12.8, 12.9, 12.9 mg/g creatinine respectively.

Conclusion: The cotton balls tested demonstrated no adverse affect on HVA, VMA or creatinine concentrations, and, therefore, can be used for urine collection as necessary for the measurement of HVA and VMA.

B-219

CLSI-based Transference of CALIPER Pediatric Reference Intervals to the Roche Cobas 6000 and the Roche Modular System

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Background: Correct interpretation of laboratory tests requires

accurately established reference intervals (RIs). Many gaps in pediatric

reference intervals currently exist and CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) has begun to address these limitations by establishing pediatric age- and gender-specific RIs for over 80 biochemical markers on the Abbott ARCHITECT system. However, this database was only directly applicable for Abbott ARCHITECT assays. In 2013, CALIPER expanded the scope of this database by transferring RIs to biochemical assays from other major manufacturers, including the Roche Cobas 6000, as well as Beckman, Ortho, and Siemens systems. This current study further broadens the application of the CALIPER database by performing further transference and validation studies for additional analytes on the Roche Cobas 6000, and the Roche Modular System.

Methods: Approximately 200 serum samples from pediatric outpatients attending SickKids Hospital (Toronto, Canada) were analyzed on the Roche Cobas 6000, the Roche Modular System, and the Abbott ARCHITECT ci8200 systems. Statistical analysis was performed using Excel (Microsoft) and R software. According to

CLSI C28-A3 and EP9-A2 guidelines, CALIPER RIs established on the Abbott ARCHITECT were transferred to assays performed on the Roche Cobas 6000 and the Roche Modular System. Specifically, the correlation between the analyzers was assessed and the line of best fit was calculated by the least squares approach or Deming regression, depending on the r^2 value. The appropriateness of the linear model was assessed using Q-Q, standardized residual, and Bland-Altman plots. The equation of the line of best fit was then used to transfer the CALIPER RIs to the Roche systems. 95% confidence intervals were calculated using the root of the mean-squared error (RMSE), calculated as reference limit ± 1.96*RSME. Calculated RIs were validated on these systems using 100 reference specimens from the CALIPER biobank of healthy children. RIs were considered validated if >90% of the reference samples fell within the transferred RIs, inclusive of the 95% confidence intervals.

Results: Most assays were transferable from the Abbott ARCHITECT to the Roche Cobas 6000 (12 out of 16 analytes) and the Roche Modular System (31 out of 36 analytes). Carbon dioxide and magnesium were not transferable to either system due to poor correlation ($r^2 < 0.70$). The hsCRP assay was not transferable to the Roche Cobas 6000 due to failure to meet criteria of the normality plots. Eight of the 12 transferred reference intervals were verified following analysis of reference specimens from healthy children on the Roche Cobas 6000, and 19 of 31 transferred reference intervals were verified on the Roche Modular System.

Conclusion: This study extends the utility of the CALIPER pediatric reference interval database for laboratories using the Roche Cobas 6000 and the Roche Modular Systems, enabling further implementation of CALIPER reference intervals across Canada and worldwide. CALIPER RIs for different analytical platforms can later be collectively analyzed by future studies in an attempt to develop common RIs across all clinical chemistry instruments and standardize laboratory test interpretation in diagnosis and monitoring of pediatric disease.

B-220

Copeptin in pediatric patients

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Background: Copeptin is a carboxy-terminal peptide cleaved from pre-pro-vasopressin (AVP). It is a stable surrogate biomarker for AVP. It is produced in a 1:1 ratio with AVP, has no known physiological function, a longer plasma half-life, and is more stable in serum/plasma. In patients with heart failure (HF), elevations are associated with increased risk of death or need for cardiac transplantation independent of B-type natriuretic peptide (BNP) and cardiac troponin concentrations. Gender differences in copeptin values have been reported in healthy adults, newborns and patients with myocardial infarction. Gender differences have not been described previously in a large pediatric population.

Objective: We sought to determine reference intervals among pediatric patients.

Methods: Sera from 240 healthy children (40 each male and female in three age groups: 2-6 years, 7-12 years and 13-17 years) were identified from an institutional pediatric biobank and obtained in compliance with the Institutional Review Board. Any patient with a diagnosis of anemia, autoimmune disease, hematologic disease/bleeding, circulatory/heart failure, kidney or liver disease, malignancy, malnutrition, diabetes, or pregnancy were excluded. Copeptin was measured using the B.R.A.H.M.S Kryptor Compact Plus (Kryptor / Thermo Fisher, Waltham, MA) with Copeptin Ultra-Sensitive (US) Immunoassay kit (802R.050) Non-parametric analysis was used to establish the 95th percentile reference interval between genders.

Results: The overall mean serum copeptin (±SD) was 14.6±43 pmol/L. Concentrations were not significantly associated with age. The large variation in normal values prompted a more detailed investigation into medication histories. Active fentanyl prescriptions were identified in 48 (20%) subjects. Serum copeptin was significantly elevated among these patients (39.9±91 pmol/L vs. 8.3±5.4 pmol/L; p=0.0102). Patients prescribed fentanyl had diagnoses of digestive disorders (n=15), infectious respiratory disorders (n=13), skin concerns (n=4), urinary problems (n=4) and musculoskeletal complaints (n=15) but there was no association between fentanyl and specific comorbidities. Copeptin concentrations also were not associated with any specific comorbidity. After excluding patients prescribed fentanyl, the mean serum copeptin was significantly higher in boys (9.3±5.9 pmol/L) compared to girls (7.3±4.8 pmol/L; p =0.0116). The 95th percentile cutoff for normal was 18.4 pmol/L (95CI 16.2 - 38.2) for boys and 20.0 pmol/L (95CI 15.1 - 21.3) for girls. In our adult validation, copeptin was similarly significantly higher among men (7.18±5.53 pmol/L) than women (4.46±2.43 pmol/L, p=0.003) in a healthy cohort aged 23 - 80 (n=230). The 95th percentile cutoff for normal among adults was higher at 13.0 pmol/L (95CI 2.9 - 23.2) for men and 8.3 pmol/L (95CI 6.5 - 10.0) for women.

Conclusions: Serum copeptin is significantly but modestly higher among boys compared to girls. The considerable overlap of confidence intervals at the 95th percentile may limit the diagnostic importance of this gender difference. Serum copeptin is elevated in patients receiving fentanyl. Additional studies are required to determine if fentanyl is the reasons for this effect but its use must be taken into account when calculating normal values.

B-221

Drug Excretion into Breast Milk: are all drugs contraindicated for breastfeeding?

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Background:

Epidemiological research provides strong evidence for health benefits associated with breastfeeding, including reduction in infant mortality, infection and development of chronic diseases, as well as positive impacts on cognitive development. Studies have shown that 66-80% of women are on medication during the postpartum period. Although not all drugs may be considered contraindicated while breastfeeding, there remains little data on this topic. Methotrexate(MTX) is the first line of treatment for rheumatoid arthritis(RA), which has a high incidence in women of childbearing age. We developed a sensitive and specific LC-MS/MS method to quantitate MTX and its metabolite in human milk and applied it to patient samples. We also calculated the relative infant dose of MTX to determine the risk to the infant.

Methods:

A simplified drug extraction method using hexane, methanol and acetonitrile facilitated efficient drug extraction from breast milk. Methotrexate was measured using an IONICS 3Q 210 mass spectrometer. Detection was performed by multiple reaction monitoring mode using electrospray ionization in positive ion mode. Settings: ESI Voltage 5000; Nebulizer Gas, 400; Drying Gas, 120; Heating Gas, 350; Source Temp(°C), 250; MTX MRM 455.1/308.0 and 455.1/134.0. Liquid chromatrographic separation was performed on a Shimadzu Prominence UFLC. A 5 µL sample was injected into an Imtakt C8 column (2.0x75 mm, 3 µm) at room temperature. The method was fully validated in terms of selectivity, linearity, accuracy, precision, stability and recovery according to standard clinical laboratory protocols. Comparison using patient samples was also performed. Patients receiving MTX therapy for RA were recruited through the SickKids Motherisk Program for the DLAC Project or through the Rheumatology Clinic at Southlake Regional Health Centre in Newmarket, Canada. Whole breast milk samples were aliquotted and stored at -20°C until sample preparation, extraction and analysis.

Results:

Results from the method validation will be presented. Pharmacokinetic profiling of methotrexate and its metabolite in breast milk were determined following a subcutaneous dose of 25 mg/mL of methotrexate, once weekly. Breast milk samples were obtained at the following 7 timepoints: pre-dose (time zero), 1 hr, 12 hrs, 24 hrs, 48 hrs, 72 hrs and 96 hrs post-dose. Both foremilk and hindmilk were measured. We found that MTX is excreted into breast milk, but with no notable differences in drug concentrations between foremilk and hindmilk. The highest drug concentrations occurred between 1-12 hours post-dose; the concentration steadily decreased between 12 - 48 hours, with small but detectable levels from 48 - 96 hrs. Methotrexate is excreted into breast milk at significant concentration within the first 24 hrs post-dose. However, no notable differences in drug concentrations between foremilk and hindmilk were observed

Conclusion: Due to the difficulty in obtaining foremilk and hindmilk, this is the first study to measure and compare drug levels in this sample type. This data provides the foundation to establish a TDM system for measuring drug concentrations in breast milk, with the aim to carry out population-based pharmacokinetic analysis to determine safety guidelines on drug excretion into breast milk as well as breast feeding guidelines.

B-222

Free light chains in the response assessment of celiac disease patients under gluten free diet

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Background:

Celiac disease (CD) is a chronic immune-mediated small intestinal enteropathy, triggered by exposure to dietary gluten in genetically predisposed individuals and frequently diagnosed during childhood. Confirmatory duodenal biopsy can be avoided if suggestive clinical symptoms are accompanied by positive tests for CD-specific antibodies. New CD-biomarkers would increase confidence on the diagnosis and the number of patients exempting biopsy. Increased serum free light chain levels (sFLC) have been observed in patients with auto-immune diseases making it a potential new test for CD diagnosis and response assessment after initiation of the gluten-free diet (GFD). We seek to assess the utility of sFLC levels as markers of intestinal mucosa alterations in CD patients.

Methods:

165 CD patients with serum samples at diagnosis, of which 21 had follow-up samples at 6 months post-GFD initiation. As control group, 52 patients with initial suspicion of CD that was later ruled out were included. Serum biomarkers: antibodies IgA anti-transglutaminase (TG2) and anti-endomysial (Menarini diagnostics), and FLC (Freelite®)

Results:

CD patients showed median levels of κ+λ sFLC significantly higher than the control group (30.2mg/L vs 18.0mg/L, p<0.0001, Fig.1). Additionally, samples obtained 6 months post-GFD show a significant decrease of summated sFLC levels compared to those at diagnosis (33.6mg/L vs 19.3mg/L, p=0.0016): median decrease of 1.5 fold (0.9-3.6). In fact, after GFD initiation, there is no longer a statistical difference between this group and the non-CD control group (18mg/L vs 19.3mg/L, p=0.28). Finally, 19 of the 20 follow-up samples with available TG2 data show a reduction of its values at 6 months of GFD.

Conclusion:

The statistical difference between the studied groups shows that summated serum FLC levels are good indicators of disease response, possibly reflecting normalization of the intestinal mucosa. The decrease of the TG2 values upon GFD initiation supports this hypothesis but validation from patients with available biopsy is necessary.

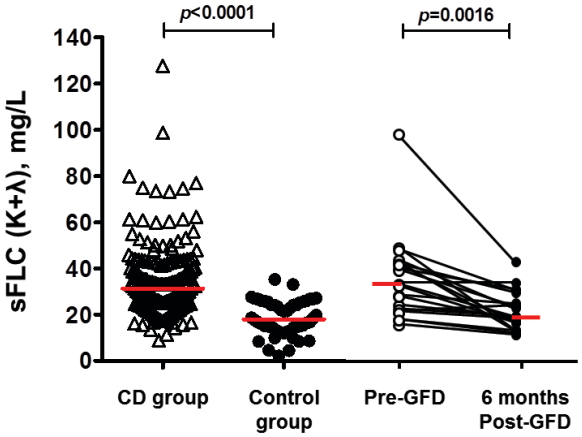

Figure 1. Summated serum free light chain values (sFLC) of 165 CD patients and 52 controls. CD: Celiac disease; GFD: Gluten free diet. The horizontal red lines represent median values. Mann-Whitney statistical test.

B-223

An improved meconium extraction that significantly reduces turn-around-time and enhances laboratory efficiency

S. W. Cotten. *Ohio State University, Columbus, OH*

Background:Meconium is currently the specimen of choice for identification of neonate drug exposure during pregnancy. Discharge from the hospital can occur within 24-48 hours for vaginal delivery, however meconium collection to result times for the laboratory often exceed 24 hours. This extended analysis time coupled with inadequate screening often resulted in missed collections and delayed discharge or social services intervention at our institution. Mapping the entire testing process from identification of at-risk mothers to result acknowledgment revealed two pre-analytical steps needing improvement in addition to the extended analysis time.

Methods:The projected evaluated a faster extraction to reduce the meconium toxicology screening time. A total of 38 meconium specimens that were positive or negative for THC, Methadone, Benzodiazepines, Opiates, or Cocaine, were measured using the laboratories current methanol extraction and using a supported liquid extraction (SLE). For the SLE procedure, 0.5 g of meconium was homogenized for 5 minutes followed by centrifugation at 13,000 rpm for 5 minutes. Clarified extract was applied to a 1.5 mL SLE column (Biotage) and target compounds eluted with 5 mLs of ethylene acetate:isopropanol (90:10). Extracts were dried under nitrogen and resuspended in 1.0 mL of water followed by immunoassay analysis on a Beckman Coulter AU680 chemistry analyzer

Results:Agreement between the methods was calculated for each drug class: THC (87%), Methadone (100%), Benzodiazepines (97%), Opiates (97%), Benzoylecgonine (100%)

Conclusion:The new SLE extraction detected 2 new THC positive and 1 new opiate positive meconium specimens. The laboratory's average receipt to result turn-around-time for meconium testing was reduced from 22 hours to 9 hours. Additional efficiency was also gained by elimination of approximately 1 hour of phone time per week for toxicology staff fielding calls related to delayed meconium results

B-224

CAPILLARY BLOOD SAMPLING KIT FOR HBA1C VERSUS VENOUS PUNCTURE ON CAPILLARYS 2 FLEX PERCING

C. Bendavid[1], L. Peltier[2], C. Letellier[3]. *[1]INSERM U991, Rennes1 University, Rennes University Hospital, rennes, France, [2]INSERM U991, Rennes1 University, Rennes University Hospital, Rennes, France, [3]Rennes University Hospital, Rennes, France*

Background:

Capillary blood sample collected from finger prick presents many advantages over venous puncture: low volume, less invasive for the patient, better patient's compliance with monitoring recommendations. The present study was designed to compare the measurement of capillary blood hemoglobin A1c levels with venous blood hemoglobin A1c levels using the Capillarys 2 Flex Piercing system (C2FP) (Sebia, France) on a large range of HbA1c values and with different storage conditions.

Methods:

Data was collected from samples of 60 volunteer patients and covering a wide range of HbA1c values (4.7% - 14% NGSP). Both venous and capillary blood samples obtained simultaneously from each subject were tested using the C2FP system. After an initial assessment of venous HbA1c at J0, capillary and venous samples were stored at room temperature (Room T°) and 4°C respectively, away from light, and re-analyzed together at J5 on the same C2FP system in duplicates. To test stability, 4 different samples were simultaneously taken from venous puncture and finger prick, and stored at different T° (-20°C, 8 days; 2-8°C, 8 days; Room T°, 8 days; 30°C, 3 days). Respective duplicates values were compared to capillary and venous (reference) result at J0.

Results:

The trendline of J5 values using mmol/mol IFFC units (slope: y=0.9904x + 0.1387; R2=0.997) or %NGSP units (slope: y=0.9896x + 0.046; R2=0.997) showed a good correlation. Bland Altman plots showed a 0.4mmol/mol IFCC and 0% NGSP mean differences. All values were included in the recommended +/-6% bias on the bias plot.

Room T° storage during 5 days resulted in a small additional peak of degradation but HbA1c value was still accurate. Reproducibility was assessed using the mean biases between the NGSP duplicates and showed the same 0% for venous and capillary results. Stability study on low, medium and high HbA1c levels showed that ideal conservation was 4°C. Room T° and -20°C give rise to degradation without alteration

of HbA1c result. After 3 days at 30°C, only one sample result was slightly out of uncertainty of measurement.

Conclusion:

The Sebia capillary sampling kit offers full automation and full positive ID. We have demonstrated a good correlation with venous sample results. Storage study showed a sufficient robustness for usual sample delivery to central laboratory.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Point-of-Care Testing

B-225

Evaluation of GEM4000® whole blood bilirubin as a screening tool for neonatal hyperbilirubinemia

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Background: Whole blood bilirubin is proposed as an alternative method for neonatal hyperbilirubinemia screening. It provides fast results with small sample volume and can measure co-oximetry and other tests.

Objectives: The objectives of the study were to compare whole blood bilirubin results on the GEM4000® (Instrumentation Laboratory) against plasma bilirubin results on the Vitros 5600® (Ortho Diagnostics), and to examine whether measurement is impacted by various pre-analytical, analytical or clinical factors.

Methods: 440 consecutive samples obtained from newborn babies in both outpatient clinics and postpartum wards, who had bilirubin testing as per usual care, were included. The inclusion criteria were babies who were less than 14 days postnatal age and their samples had sufficient volume for both the whole blood and plasma bilirubin analyses. Bilirubin was measured on specimens in the central laboratory, first on the whole blood using the GEM4000®, and then on the Vitros 5600® using plasma obtained from the remaining blood. Statistics were performed with SPSS, R, and Stata software.

Results: 378 samples (87.5%) with both whole blood bilirubin and co-oximetry results were included in the analysis. 62 (12.5%) were excluded because of an incalculable instrument error for either bilirubin or co-oximetry on the GEM4000®. The demographic data of the babies were: Mean gestational age, 38.8 weeks; Mean birth weight, 3274.2 grams; Postnatal age, 48 hours; Male:female ratio, 52:48; Delivery, 60% vaginal, 40% C-section. Passing-Bablok regression of GEM4000® versus Vitros 5600® results revealed a negative bias at low levels of bilirubin and a positive bias at higher levels (y=1.438x - 63.14). The Bland-Altman plots found an overall negative bias with a mean difference of -2.23 mmol/L (95% CI: -86.99 to 82.52 mmol/L). Stata-multivariate regression revealed that the degree of hemolysis (H index) and the hemoglobin level accounted for approximately 86% of the observed variation between the results on the different methods. Applying the Bhutani nomogram 40th percentile (95th percentile) to categorize risks, the results by the GEM4000®, in comparison to the Vitros 5600®, exhibited a false positive rate of 20% (63%) and false negative rate of 23% (37%).

Conclusions: An imperfect correlation was observed between whole blood bilirubin measured on the GEM4000® and plasma bilirubin on the Vitros 5600®. The major contributors to the differences were specimen hemolysis and the accuracy of total hemoglobin by GEM4000®, the latter of which affects the calculation of plasma-equivalent bilirubin. Additionally, the lack of standardization of total bilirubin calibration between the two instruments, particularly in newborn specimens, may account for some of the disagreement in results. The present work does not support the application of whole blood bilirubin measured on the GEM4000® for neonatal hyperbilirubinemia screening.

B-226

Using Point-of-Care Glucose Meters in the Critically Ill: Assessing Meter Performance in the Clinical Context

D. B. Schmolze, G. L. Horowitz, N. V. Tolan. *Beth Israel Deaconess Medical Center, Boston, MA*

Background: Point of care (POC) glucose meters are widely used in hospitals to aid in monitoring blood glucose levels. Recent publication of FDA draft guidelines, setting stringent accuracy requirements for manufacturers of POC glucose meters to be used in hospitals, has again raised concerns of off-label use, particularly in "critically ill" patients.

Methods: In order to evaluate the accuracy of results obtained from meters in our institution by our end-users, we matched six months of POC glucose results using the Precision *Xceeed* Pro Blood Glucose POC system (Abbott Diabetes Care Ltd., Alameda, CA) with central laboratory glucose data that was obtained by either the Roche Cobas Modular P Gluco-quant Glucose/Hexokinase (Roche Diagnostics, Indianapolis, IN) or the RAPIDLab 1265 Blood Gas (Siemens AG, Munich, Germany). In an effort to reduce the likelihood of changes in blood glucose concentrations due to clinical interventions occurring between POC and lab glucose samples, we minimized the difference between the collection times and restricted our analysis to samples collected no more than 10 minutes apart. We evaluated the correlation between the POC and lab glucose pairs using Thiel-Sen linear regression analysis and interpreted the differences in a clinical context using the Clarke Error Grid (CEG). We also evaluated the performance in the critically ill, based on location (non-ICU vs. ICU) and other relevant laboratory results (sodium, bicarbonate, lactate, hematocrit, pO_2) collected within 24 hours of the POC/lab glucose pair. Finally, as a quality assessment of each individual glucose meter in use at our institution, we also prepared CEGs by serial number for those meters with at least ten POC/lab glucose pairs.

Results: Our final dataset comprised 860 records, obtained from 41 unique hospital locations, 97 unique glucose meters, and 452 unique patients. From an analytical perspective, the agreement within the POC/lab glucose pairs was far from ideal, where the correlation of the POC glucose results to the laboratory concentrations was described by the equation: y=0.93x+15.27, and r^2=0.65. However, when analyzed with the CEG, the overwhelming majority (802/860, 93.3%) of these discrepancies were found to be clinically insignificant. No relationship was found between severity of illness and degree of discrepancy, by non-ICU or ICU location ($p>0.5$) nor abnormalities in the additional laboratory results obtained ($p>0.1$). Finally, no significant biases were observed for any particular meter and all displayed a predominance of POC/lab glucose pairs that fell within Clarke zones A and B.

Conclusions: Evaluating POC glucose meter performance in a clinical context, rather than in a strictly analytical manner, offers a more robust determination of the accuracy necessary to effectively manage hospitalized patients, especially in the absence of "tight glycemic control" protocols. Our data suggests that the meters used in our institution are performing well, allowing for the advantages of real-time blood glucose monitoring and outweighing the limited instances of potential clinical errors. Another interesting aspect of this performance evaluation is that it provides a mechanism for ongoing quality assurance and would identify specific meters that may not be performing optimally.

B-227

Glucose Connectivity Meter Evaluation in Intensive Care Unit

A. Ivanov. *Tartu University Hospitals, Tartu, Estonia*

Background: The CLSI POCT12-A3 guideline states that a) 95% of meter measurement results should be within 0,67 mmol/L for glucose <5,55 mmol/L and within 12,5% for glucose ≥5,55 mmol/L and b) 99% of values should fall within 0,86 mmol/L for glucose <4,2 mmol/L and within 20% for glucose ≥4,2 mmol/L comparing with laboratory.

We assessed the performance of a point-of-care (POC) glucose meter by using the spreadsheet program is designed for estimating the bias between two methods using patient samples.

Methods: The study was performed over a three week period using samples obtained from the intensive care unit of Tartu University Hospital. Method correlation was performed by analyzing 120 whole blood specimens on the Stat Strip glucose connectivity meter (Nova Biomedical) compared to ABL blood gas analyzer (Radiometer). Sample collection was performed by arterial Disposable Pressure Transducer Kits and Safeset Closed Blood Sampling/Conservation System (Philips). Mean glucose concentration was 7,31 mmol/L (range 3,8-26 mmol/L).

Results: The imprecision for glucose meter of the 3 levels was 6,1/3,4/5,4 %(mean values: 3,3/6,4/16,1 mmol/L) and for ABL was 3,7/1,1/1,1% (mean values: 1,6/5,6/14,1 mmol/L).

The linear regression analysis demonstrated a slope 0,99, intercept -0,38 and R^2 -0,988. The glucose meter had the lowest mean biases (-0,151 mmol/L) compared with laboratory method (ABL) ($p<0,001$). Mean relative difference was 5,93 %.

114 (95%) of glucose meter results was within 12,5% and 11 of them (glucose value <5,55 mmol/L) was within ±0,67 mmol/L. Furthermore, 120 (100%) of glucose meter results was within 20%.

Conclusion: Stat Strip glucose connectivity meter met POCT 12-A3 performance criteria and demonstrated a close correlation to the laboratory method.

B-228

Development of a Novel, Lab-on-Cartridge based Point-of-Care Device for the Measurement of Clinical Diagnostics Tests

V. MATHUR, V. Pandey, R. Mamdapurkar. *DIASYS DIAGNOSTICS INDIA PVT. LTD, MUMBAI, India*

Background:

Point-of-care testing for common clinical diagnostics tests (immunoassays, clinical biochemistry, electrolytes/blood gases & coagulation) requires multiple dedicated instruments that are often unreliable, expensive and need frequent maintenance. It is not possible for small labs & clinics in developing countries to afford the instrument infrastructure needed to provide healthcare diagnostics. In order to provide healthcare diagnostics to ALL at affordable costs, there is a need for a device that can do all common diagnostic tests reliably and is low in cost (device and consumable cost).

We have developed the world's first portable lab-on-cartridge based point-of-care device (**QDx InstaLab**) for quantitative measurement of all common diagnostics tests typically used in lab. **QDx InstaLab** incorporates an innovative, high performance, inexpensive microfluidic cartridge for rapid quantitative measurement of diagnostic tests in whole blood /plasma/serum samples. Our proposed methodology utilizes a novel, patented nanomaterial based plastic electrochemical biosensor /immunosensor that uses chronoamperometry / differential pulse voltammetry technique to provide a sensitive and accurate result in ~3 min for clinical biochemistry tests and in ~10 min for immunoassays. The device is simple, easy-to-use and reliable as the measurements are carried out at a constant temperature of 37°C. The **QDx InstaLab** is capable of performing a single test or multiplexed tests from a fingerpick with 10 microliters of whole blood sample per parameter.

Methods:

We evaluated the **QDx InstaLab** using patient samples for linearity, precision, interference and cartridge stability for all the clinical biochemistry profiles such as metabolite profile (glucose, lactate), kidney profile (urea, creatinine), diabetic profile (HbA1c, Hb), lipid profile (total cholesterol, triglycerides), electrolyte profile (Na/K/Cl), liver profile (ALT, AST, bilirubin) and coagulation profile (PT-INR). Interference study was done against hematocrit variation of 30% to 60% and with ascorbic acid at 3 mg/dL at two different analyte concentrations with samples run in triplicate. Accelerated stability testing was done at 2-8°C and at 45°C for 2 weeks for the assays during which linearity samples were run on 0, 4, 7, 14 days respectively.

Results:

Data analysis indicates that the assays have a CV < 3%, with $R^2 > 0.95$, interference bias of < 10% and the cartridges are stable up to 9 months at 2-25°C storage temperature based on preliminary extrapolated data.

Conclusion:

The developed technology platform for **QDx InstaLab** is reliable and meets all the performance specifications of a lab. Hence, it can be easily adapted for low cost, sensitive and rapid measurement of common diagnostics tests in low resource settings such as in urban, semi-urban and rural areas in the developing countries.

B-229

A comparison of values on point-of-care instruments in patients with EBOV

E. L. Ryan[1], N. A. Brammer[2], R. Powers[2], K. Jenkins[2], C. Evans[2], J. C. Ritchie[1]. *[1]Emory University School of Medicine, Atlanta, GA, [2]Emory University Hospital, Atlanta, GA*

Background: The ongoing epidemic of Ebola Zaire (EBOV) in West Africa has mandated a handful of patients with the disease being treated in western medical facilities. Four patients with confirmed EBOV were admitted to the specialized isolation unit at Emory University between August and October 2014. All laboratory values were generated within the dedicated isolation laboratory. Anecdotal differences between instrumentation were noted.

Methods: Sodium, potassium, chloride, glucose, hemoglobin, and hematocrit were measured on more than one instruments in our laboratory. The electrolytes (Na, K, Cl) and glucose were measured via Chemistry analyzer (Abaxis Piccolo Xpress [ABAXIS, Inc, Union City, CA]) and Blood-gas analyzer (GEM Premier 4000 [Werfen, Barcelona, Spain], while hemoglobin and hematocrit on Blood-gas analyzer (GEM Premier 4000 [Werfen, Barcelona, Spain] and Hematology analyzer (pocH 100i [Sysmex Corporation, Kobe, Japan]). All instruments went through verification studies prior to patient testing and met expectations. Samples for the blood gas analyzer and chemistry analyzers were lithium heparin while those for the hematology analyzer potassium EDTA.

Results: Results from paired samples (drawn at the same time) across all 4 patients were looked at along with the reported indices for each sample, where appropriate. There were 44 paired results for the chemistry values (Na, K, Cl, and glucose) and 38 for hemoglobin and hematocrit. Overall there was no significant bias between instruments for any of the analytes. However, when looking at hemolysis there were differences in bias for potassium and glucose. The bias among hemolyzed samples (H≥1+) was 0.37±0.07 while it was 0.16±0.05 among non-hemolyzed samples(p=0.0155). The trend was reversed for glucose with hemolyzed samples having a bias of 4.7 ±1.1 and 8.7 ±0.8 with non hemolyzed values. Both of these trends continued to be significant when accounting for those samples that were arterial rather than venous. Chloride showed no bias and Sodium showed a bias among lipemic samples although it was no longer significant when accounting for arterial sampling. Hemoglobin and Hematocrit showed no bias between instruments.

Conclusion: When using values from point-of-care instruments to inform clinical decision making precision and accuracy of the methods used should be taken into consideration. The usual performance characteristics of test results may be different. The laboratory professional must be aware of these issues and communicate with the clinical team to ensure interpretation of test results is appropriate.

B-230

Assessment of Interference from Hemolysis, Lipemia and Icterus on the Abaxis Piccolo Liver Panel Plus Reagent Disc

C. M. Kazmierczak, D. E. Kazmierczak, S. C. Kazmierczak. *Oregon Health & Science University, Portland, OR*

Background: Interference due to hemolysis, lipemia or icterus in samples tested at the point-of-care can be problematic, especially in critically ill patients who tend to produce samples containing these endogenous interferents. Although manufacturers provide data on interference effects, these claims are often too vague to be of use. Other aspects of interference effects often not considered include the number and permutations of different analyte and interferent concentrations that are investigated when performing interference studies. In an attempt to more accurately assess the effect of endogenous interferences on the Piccolo Xpress analyzer (Abaxis, Inc., Union City, CA), we conducted a comprehensive study on interference effects from hemolysis, lipemia and icterus.

Methods: We evaluated interference on analytes included in the liver panel plus reagent disc: total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), alkaline phosphatase (ALP), amylase (AMY), and total bilirubin. We prepared two separate plasma pools obtained from remainder patient samples. One pool contained increased activity (3-5 times the upper limit of normal) of enzyme activities while the other pool contained normal enzyme activities. We purchased an interference test kit containing plasma hemoglobin, bilirubin and triglycerides of human origin (Sun Diagnostics, LLC., New Gloucester, ME). We divided each plasma pool containing either normal or abnormal enzyme activities into two equal aliquots. To one aliquot we added hemoglobin, triglyceride or bilirubin interferent solution to produce interferent concentrations of approximately 600 mg/dL, 1700 mg/dL and 15 mg/dL, respectively. We added an equal volume of saline to the other aliquot of plasma to produce a pool with negligible interferent. Next, we created five intermediate pools by making admixtures of the low and high interferent pools. Thus, for each interferent, we created seven aliquots containing interferent across a broad range of concentrations, and testing both normal and increased enzyme activities. Each of the aliquots was measured in quadruplicate. Interference affects were considered to be clinically significant on the basis of expected within-lot precision based on quality control data. If the measured analyte value differed by more than +/- 2.8 standard deviations of the expected quality control imprecision, the interference effect was considered to be significant.

Results: Icterus had no effect on any of the analytes evaluated. Hemolysis demonstrated interference with measurement of total bilirubin, with a decrease of approximately 0.5 mg/dL in measured concentrations at plasma hemoglobin concentrations of 600 mg/dL. AST, when present at normal activity, was affected when plasma hemoglobin concentrations exceeded 600 mg/dL. Lipemia affected ALT, although the effect was less pronounced when ALT was present at high activities. Total protein showed a decrease of up to 50% when triglycerides were greater than 1500 mg/dL and total bilirubin showed significantly decreased measured concentrations when triglycerides were greater than 1000 mg/dL.

Conclusions: The Piccolo Xpress has very good specificity when endogenous interferents are present, even at very high concentrations of interferent. Use of the Piccolo in critically ill patients who tend to produce samples with a higher likelihood of endogenous interferents is warranted.

B-231

Clinical evaluation of mobiLab, a smartphone-enabled microfluidic NAAT platform for Chlamydia trachomatis screening

D. Shin, P. Athamanolap, L. Chen, J. Hardick, C. A. Gaydos, T. Wang. *Johns Hopkins University, Baltimore, MD*

Chlamydia trachomatis is the most common notifiable disease in the United States with over 2.8 million diagnosed cases and direct medical cost estimated at $516.7 million in 2008 [1]. Affordable and highly sensitive point-of-care diagnostics has the potential to reduce the social and economic costs associated with Chlamydia infections. We report the development of a low-cost mobile nucleic acid analysis platform (mobiLab) utilizing a smartphone-enabled microfluidic device for streamlined analysis of biological samples. By using magnetic particles as a mobile solid phase for DNA capture and transport, fluidic processing is simplified to particle translocation on a robust and scalable cartridge. Process integration facilitated by Bluetooth-enabled microcontrollers enables full control of the instrument by the user with a smartphone application.

The mobiLab platform consists of three discrete units: a droplet microfluidic cartridge, a battery-powered instrument for droplet manipulation and amplification, and a smartphone for user interface, data acquisition and processing. The microfluidic cartridge design utilizes open-surface magnetofluidic manipulation [2] which enables bioassays requiring multiple buffer exchanges to be performed without complex instruments. Each cartridge costs less than $2 using off-the-shelf components at retail price, which is an order of magnitude cheaper than $9.98/test for a subsidized Cepheid GeneXpert cartridge [3]. The instrument utilizes a microcontroller which controls the rotary bead manipulator, thermal incubation and Bluetooth-based communication with the smartphone application. Each assay consumes approximately 10% of the battery capacity, allowing up to 10 assays to be performed consecutively without access to a power outlet.

We designed a single-stream loop-mediated isothermal amplification (LAMP) assay to operate in tandem with the mobiLab platform. In this assay, polyhistidine-coated magnetic particles capture DNA targets from sample lysate via electrostatic interaction. The affinity between particles and nucleic acids is maintained at acidic pH, which is reversed when particles enter LAMP buffer. The basic pH of LAMP mixture is compatible with the elution conditions for nucleic acids, enabling seamless integration between DNA extraction and amplification. We tested the single-stream assay using plasmid targets and were able to capture and amplify 10^3 copies of gene targets. Specificity of the assay for Chlamydia trachomatis was tested, and the absence of cross-reactivity with human or other bacterial genomic DNA was verified.

The mobiLab platform was validated by testing Chlamydia trachomatis infection from patient-collected vaginal swab samples. Volunteers enrolled in an internet-based Chlamydia screening program, where two sets of swabs were self-collected and mailed back to our lab [4]. One set of swabs was analyzed using the gold standard Gen-Probe AC2 CT assay, while the second set was tested using the mobiLab platform. The two results were in agreement for 20 out of 20 samples after 30-minute incubation, demonstrating that the droplet assay performance is comparable to the gold standard for the samples tested. To our knowledge, this abstract presents the first smartphone-based NAAT platform that integrates sample preparation, amplification and data processing.

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B-232

Viscoelastic Coagulation Monitoring: Current use of TEG, ROTEM, and Sonoclot

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Objective: Viscoelastic hemostasis assays (VHAs) including thromboelastogram (TEG), rotational elastometry (ROTEM) or sonoclot are FDA cleared tests that are used to assist in the administration of blood and transfusion products. These assays have the potential of altering the practice of transfusion medicine and management of blood utilization in critical and surgical care settings by improving long-term patient outcomes in time-sensitive fields such as trauma. The goal of this study was to survey members of the National Academy of Clinical Biochemistry (NACB) to estimate: How many hospitals in the United States are using the three VHA assays; what hospital areas are using information from these tests; the type of personnel performing VHAs and how the information is delivered to caregivers..

Design and Methods: An on-line survey was conducted among all members of the AACC/NACB. Data were reported as descriptive statistics

Results: Respondents from 51 institutions, having bed size from 50 to 1500 participated in the survey. 67% of these hospitals used either TEG or ROTEM. Use of Sonoclot was not reported by any of the responding institutions. 65% of those using VHAs were able to view results in real time, while the testing was being conducted. 65% of TEG/ROTEM testing was found to be performed exclusively by laboratory technologists. Additionally only 39% of TEG users and 13% of ROTEM survey respondents used the device in a trauma center clinical environment.

Conclusion: This survey of AACC/NACB members showed that VHAs are currently used in a variety of hospitals. VHA testing at most centers was available for decision making in real time. Use of VHAs for patient management is now part of transfusion guideline algorithms, and it is likely that use of TEG/ROTEM in time sensitive areas such as trauma will increase.

Viscoelastic coagulation assays in hospital areas	
Hospital Area	% Responding institutions (n=31)
Cardiac Surgery	65% (20/31)
Emergency Room	23% (7/31)
Hepatic Surgery	48% (15/31)
Intensive Care Unit	35% (11/31)
Liver Transplantation	Liver Transplantation61% (19/31)
Obstetrics	19% (6/31)
General Surgery	29% (9/31)
Trauma	42% (13/31)

B-233

Differences between concurrent central laboratory and point-of-care glucose measurements: a comparison between distributions from critical care units and non-critical care units using the Roche Accu-chek Inform II analyzer

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Background: The Centers for Medicare and Medicaid Services (CMS) recently issued a notice to state survey directors to identify use of waived blood glucose monitoring systems (BGMS) in critically ill patients as non-compliant unless the system has been specifically cleared for such use. Consequently, many hospitals are investigating how best to define critically ill patients, and documenting analytical performance characteristics of BGMS within this population. One option is to define critically ill patients by location within critical care units. Our objective was to evaluate differences between point-of-care glucose meter measurements (POCT) and concurrent central laboratory glucose measurements (LAB), specifically to compare distributions of such differences between patients in critical care (CC) vs. those in non-critical care (N-CC) units.

Methods: Retrospective POCT data (capillary, Roche Accu-chek Inform II) and LAB data (serum, Roche Cobas) were obtained for a one-month interval (November 2014). LAB and POCT measurements were defined as concurrent if recorded times-of-collection were within 15 min of each other. Among 38,489 POCT measurements and 23,549 LAB measurements, 1221 measurement pairs were concurrent. These data were divided according to location from among 23 CC locations (500 pairs, 41% of total) and 58 N-CC locations (721 pairs, 59% of total) for comparison.

Results: Correlations of results between POCT and LAB were very similar for CC and N-CC. Results within ±30 mg/dL showed overall 1:1 numerical correspondence: for C (88.4% of results), POCT(CC) = 0.991 LAB (r2 = 0.962); for N-CC (88.6% of results), POCT(N-CC) = 1.00 LAB (r2 = 0.976). Differences (D) between POCT and LAB within ±30 mg/dL were normally distributed: for CC, D = -0.53 ±11.5 mg/dL; for N-CC, D = 1.84 ±12.2 mg/dL. Whereas means of these distributions were numerically distinct ($p<0.01$), they were indistinct from a clinical perspective. For D >30 mg/dL, LAB>POCT (A) outnumbered LAB<POCT (B) for both CC and N-CC: for CC, A/B = 2.05 (A+B = 11.6% of total results); for N-CC, A/B = 1.16 (A+B = 11.4% of total results). Such outliers are known historically to include circumstances of interventions between reportedly concurrent POCT and LAB draws, but such circumstances cannot be discerned from retrospective data. Review of ICD-9 diagnoses associated with each CC and N-CC patient showed that CC and N-CC were distinct populations with

respect to critical illness. Within CC, 28% of diagnoses were unique to CC (i.e., not represented within N-CC); within N-CC, 54% of diagnoses were unique to N-CC (i.e., not represented within CC). Unique diagnoses among CC were, expectedly, of far greater acuity/morbidity than those among N-CC.

Conclusions: Distributions of POCT vs. LAB glucose measurements for CC and N-CC units were essentially equivalent. To the extent that critical illness can adversely affect POCT, it is unlikely, based on review of diagnoses, that such circumstances should occur with equal prevalence in both CC and N-CC populations. Our interpretation of comparison data is that use of the Roche Accu-chek Inform II glucose meter for capillary POCT in CC (non-waived) is analytically equivalent to its predicate use for capillary POCT in N-CC (waived).

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Performance Characterization of a Liver Function Panel on the Abaxis Piccolo Xpress

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Objectives: Laboratories that choose a point of care approach for liver function testing in patients undergoing evaluation for Ebola virus disease (EVD) have few options to choose from. The primary objective of this study was to conduct a performance characterization of a CLIA-waived liver function panel on the Abaxis Piccolo® Xpress chemistry analyzer. Secondary objectives were to evaluate multiple specimen types, characterize whole blood specimen stability, and validate disposable exact transfer pipettes. Our final objective was to assess instrument airflow from a biosafety perspective.

Methods: An instrument validation, including imprecision, linearity, comparison to Roche Cobas c502 and c702 methods, reference interval verification, and specimen type evaluation was conducted using the Liver Panel Plus reagent discs, which included albumin (ALB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), amylase (AMY), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total bilirubin (TBIL), and total protein (TP) assays.

Results: The Piccolo Xpress demonstrated excellent imprecision with total CV's less than 6% over ten days of testing. All assays were linear over suitable analytic ranges. Method comparison studies showed good correlation for all assays though notable biases were seen for ALP (average bias, -18.6%), AMY (average bias, -29.1%), and TBIL (constant bias, approx. +0.3 mg/dL). Pre-programmed reference intervals were verified except for the ALP (male and female) and ALT (female) assays, which had greater than ten percent of results below the programmed ranges. Results for sample types (PST whole blood- uncentrifuged, PST plasma-

centrifuged and SST serum) when compared to lithium heparin whole blood were overall quite consistent. It was noted, however, that AST trended lower in PST whole blood (-11.6 ± 14.8%; p=0.062), PST plasma (-14.8 ± 17.7%; p=0.048), and serum (-15.9 ± 17.4%; p<0.025). GGT results in serum were significantly higher (33.0 ± 37.2%; p <0.001) and TP results were significantly lower (-4.9 ± 1.7%; p <0.001) in serum. Whole blood stability results showed no clinically or statistically significant differences over five hours of ambient storage. Results using disposable exact transfer pipettes were comparable to results using a standard fixed volume pipette. Airflow studies suggested that, in the context of EVD protocols, instrument placement in a biosafety level (BSL) 2 cabinet or greater is justified.

Conclusion: Given its analytical performance and ease of operation, the Piccolo Xpress was transferred to a BSL 2 cabinet in our BSL 3 suite for use in our hospital's diagnostic protocol for providing liver function testing in patients undergoing evaluation for EVD.

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Diagnostic Utility of Leucocytes Esterase and Nitrites in Detecting Urinary Tract Infection in Tertiary Care Hospital

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Background: Urinary tract infections are very common illness in outpatient setting. Urinalysis and microscopy is one of the most requested test in those type of patient's population. The diagnostic accuracy of in dipstick's leucocytes esterase and nitrite in this population has been evaluated in comparison to urine culture. Our objective is to reduce unnecessary urine culture.

Methods: A total of hundred consecutive urine samples from outpatients were evaluated. Each sample underwent testing using urinalysis and urine culture. Each matched pair of urinalysis and urine culture was considered as the unit of measurement. We evaluated the diagnostic performance and calculated the sensitivity, specificity, negative predictive value, positive predictive value and overall accuracy.

Results: Of 100 patients, 73 (73%) were female. The presence of bacterial growth was confirmed in 83% of the samples. There were fifteen different types of bacteria were identified. *Escherichia coli* (35%) were the major bacteria detected followed by *Klebsiella pneumonia* (21%) and *Streptococcus agalactiae* (8.4%). Using Leucocytes Esterase as marker had sensitivity and specificity of 55.4% and 64.7% respectively with overall accuracy of 57%. The presence of nitrites on dipstick had high specificity (100%) but very poor sensitivity (8.4%) with overall accuracy of 24%. The positive and negative predictive values were 88.5% and 22.9% respectively for Leucocyte Esterase and 100% and18.3% for nitrites respectively.

Conclusion: The study concludes that combined Leucocyte esterase-nitrite dipstick test is not sufficiently sensitive and specific to be used for routine screening of bacteriuria in replace of laboratory culture, though it has an excellent indicators for infection and may be more cost effective in low resource settings.

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Hemoglobin A1c Screening using ADx100 Dried Blood Spot Collection Cards on the Trinity Primus Affinity Ultra2 Analyzer

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Introduction: The use of the Dried Blood Spot (DBS) card as a screening method for various endogenous compounds is not a new concept in laboratory medicine; it has been used for a number of years in various capacities for numerous tests. Blood spotted onto DBS cards has been found to be a suitable collection medium for direct to consumer testing for a number of assays. Compared to traditional venipuncture, the DBS sample can be collected by non-phlebotomists in non-clinical settings; the cards are relatively inexpensive and can be stored and transported more conveniently. The goal of this study was to create a simple extraction and testing method for Glycated Hemoglobin testing (HbA_{1c}) that could be easily integrated with the current DBS test offerings employed in our laboratory, optimized on existing instrument platforms and provide a quality screening result comparable to the whole blood plasma methods.

Methods: In our study, 148 previously tested patient samples spanning the clinically diagnostic range were analyzed over 19 separate runs. No distinction was made as to the clinical diagnosis, gender, race or the age of the patient. The DBS samples were tested against fresh, previously run EDTA whole blood samples and the data analyzed using Microsoft Excel and Data Innovations EP Evaluator. Advance Dx100 Technology (ADx) cards were selected as a result of their ability to separate the cellular material from the serum component of whole blood in a cellulose matrix; this property creates a region of concentrated cellular material well suited for testing the A1c component. The cards were inoculated with 4 to 5 drops of peripheral blood (approximately 200 µL) collected from a finger stick and a ¼ inch (6.4 mm) punch is taken from the inoculation area of the card. The punched spot is added to 1.5 ml of a hemolysing reagent, vortexed and incubated at room temperature for 30 - 60 minutes. Following the incubation the residual paper punch is removed from the lysate reagent and the samples are loaded onto the Trinity Primus Affinity Ultra2 Analyzer and analyzed using boronate affinity chromatography.

Results: In order to determine the Glycohemoglobin A1c results from the ADx100 DBS card a correlation calculation had to be extrapolated as no true 1:1 relationship exists to correlate whole blood A1c results from recovered dried blood spot cards. Our final calculation provided us with a >90% correlation across the analytical range (3.2% - 28.7%). Linearity studies performed from pooled patient materials provided us with a slope of 0.991 and an observed error of 5.7%. Inter- and intra-assay precision data showed that we could reproduce results: CV% = 2.0% - 5.7%. In our final correlation studies, whole blood EDTA samples were compared to the corresponding DBS A1c samples (n=108) and demonstrated a correlation coefficient of 0.9182 with a slope of 0.9490 and a negative bias of -0.20.

Conclusion: Preliminary validation studies have demonstrated that it is possible to quantify Glycohemoglobin A1c from DBS specimens for the purposes of screening patients.

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Patient identification errors in 20 point-of-care blood gas analyzers

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Background: At La Paz University Hospital, there is a Point-of-Care Testing (POCT) project including 20 blood gas analyzers. Various components comprise the functional organization of the connectivity system: Analyzers, a data manager system (DMS), and laboratory information system (LIS) / hospital information system (HIS). Briefly, after a sample measurement, a trained operator identifies the patient with the medical record number (MRN) using barcodes. Then, patient demographics are transferred by DMS/LIS/HIS and an accession number (AN) is created by the LIS. The AN is added to the patient results and the record is sent to the LIS. The identification (ID) procedures must be correctly implemented and followed because errors put patient safety at risk.

The aim of this study was to evaluate the type and frequency of patient identification errors in the point-of-care network.

Material and Methods: The measurements performed on all POCT analyzers (ABL90/ABL80; Radiometer Aps) over 4 months were evaluated with DMS Radiance (Radiometer Aps) and LIS LabTrak (Intersystems). We assessed the type and rate of incidents related to patient ID errors.

Results:

Setting (Number of analyzers)	Samples (n)	No patient ID (%)	Patient ID with operator number (%)	Patient ID with patient name (%)	Patient ID with unknown number (%)	Total errors (%)
Delivery room (3)	3.724	11.7	2.0	0.5	2.0	16.1
Pediatric intensive care unit (1)	3.090	2.4	0.2	0.6	1.3	4.5
Pediatric reanimation unit (1)	2.252	2.6	0.2	0.1	0.7	3.6
Pediatric hemodynamics unit (1)	212	5.7	0.5	0.0	0.0	6.1
Neonatal intensive care unit (3)	5.955	6.4	0.6	7.1	3.1	17.3
Coronary intensive care unit (1)	913	9.7	0.2	0.3	0.4	10.7
Reanimation unit (3)	7.492	4.2	0.2	0.1	1.3	5.8
Intensive care unit (1)	3.850	3.6	0.8	2.1	0.2	6.6
Burn unit (1)	1.793	3.5	0.4	0.2	1.1	5.1
Surgical suites (3)	1.199	3.6	2.0	0.0	0.7	6.3
Pulmonology offices (2)	649	0.6	0.0	0.0	3.9	4.5
Total (20)	31.129	5.2	0.7	1.8	1.5	9.2

Conclusions: The samples with "no patient ID" (5.2%) could not be measured due to clots, air bubbles or insufficient sample volume. The other 3 types of errors (4%) included the use of a patient ID different from the correct MRN. These results were not transferred to the patient electronic medical record, with a possible impact on patient care. Moreover, if a patient ID with an erroneous MRN is used, the blood gas results could be assigned to another patient medical record. It is important to evaluate and monitor the type and frequency of these errors in each setting. Due to the high incidence observed and despite having an adequate identification procedure, specific training for POCT operators is critical to ensuring correct patient identification.

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Evaluation of a POC Glucose Meter for High Complexity Testing in the ICU and NICU for Critically Ill Patients at Centura Health Network of Hospitals

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Background: Several Point-Of-Care (POC) glucose meters state "not for use with critically ill" in the package insert. Using these meters to monitor the effectiveness of diabetes control in critically ill patients is considered off-label high complexity testing according to Centers for Medicaid and Medicare Services (CMS). The American Diabetes Association (ADA) recommends monitoring of blood glucose levels in these patients in order to avoid hypo and hyperglycemic events which has been shown to reduce mortality and improves outcomes. Measurement with a POC device allows for rapid assessment with results available at the bedside in 20 seconds, leading to faster intervention than with main lab methods. Centura Health has defined critically ill as all patients in the Intensive Care Units (ICU) and Neonatal Intensive Care Units (NICU). In order to determine if this meter can be used as a high complexity test system for critically ill patients in the ICU and NICU we followed CLSI guidelines for evaluation of a high complexity test, and additionally wanted to evaluate the diagnostic sensitivity and specificity of the meter for this patient population.

Objective: The objective of this study was to calculate the diagnostic sensitivity and specificity of the POC glucose meter for critically ill patients in the ICU and NICU.

Methods: A total of 80 NICU and 379 ICU patients were included in the study. Glucose was measured on both the POC glucose meter and the chemistry lab analyzer within 5 minutes. Each data pair was then tagged as True Positive (TP), True Negative (TN), False Positive (FP) and False Negative (FN) for hypo and hyperglycemia. Hypoglycemia was defined as a lab value of <40mg/dL and <70mg/dL for NICU and ICU respectively. Normoglycemia was defined as any lab value between 71-180 mg/dL, and hyperglycemia was defined as any lab glucose >180 mg/dL for ICU patients (no instances of hyperglycemia from the NICU were observed). From this, diagnostic sensitivity, specificity, PPV and NPV were calculated using standard formulas. Data pairs were then plotted using EP Evaluator® for Two Instrument Method Comparison to determine the percentage of results that met CLSI guidelines for values <75mg/dL and >75mg/dL.

Results: Diagnostic sensitivity and specificity of POC glucose measurements for NICU patients were 97% and 89% respectively for hypoglycemic events within 24 hours of birth (PPV=87%), (NPV=98%). Sensitivity and specificity of POC glucose measurements for ICU patients were 93% and 97% respectively for hypoglycemic events (PPV=97%),(NPV=95%), and 97% and 96% respectively for hyperglycemic events (PPV=95%),(NPV=98%). Two method comparison studies showed that 98% of values across the AMR were within a TEa of 15mg/dL for values <75mg/dL and 20% for values >75mg/dL.

Conclusions: Blood glucose measured by the POC glucose meter has an acceptable diagnostic sensitivity and specificity for critically ill patients at Centura Health facilities. This information in addition to separate studies for accuracy, precision, reportable range, reference range, analytical sensitivity and analytical specificity show that it can continue to be used in ICU and NICU for monitoring the effectiveness of diabetes control programs at Centura Health facilities.

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Accellix Automated Flow Cytometry

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Background:

Medical flow cytometry (FC) provides diagnostic answers by detecting the presence and concentration of cell populations, and/or by measuring concentrations of cell surface markers expressed on cells. Currently, FC is limited to high complexity labs by time consuming pre-analytical steps, requiring highly trained technologists. Inter-instrument and inter-operator variability limit broad acceptance of IVD FC. Finally, interpretation of FC results requires highly trained professionals typically available only during business hours. The Accellix compact table top multicolor flow cytometer automates the 3 step process required for population identification and/or cell surface marker measurement. Sample preparation and reading are performed in a dedicated disposable cartridge. Analytical data processing utilizing proprietary algorithms provides answers directly to the user.

Methods:

Accellix Cartridge - This disposable cartridge-based platform provides 24/7 availability in a moderate complexity lab - ultimately CLIA waved setting - by implementing sample preparation using three reagent blisters. With different reagents in the blisters the same cartridge structure can be used for multiple applications. The 3 Accellix CD64 cartridge blisters contain staining cocktail of conjugated monoclonal antibodies, lysis buffer, and reference beads respectively. Once sample processing is complete, the sample flows through a dedicated reading channel where data is acquired.

Applications implemented on Accellix:

•Sepsis diagnosis and monitoring based on upregulated CD64 expression on neutrophils.

•HIV monitoring based on determining T cell subsets: proportion of T helper cells (CD4) to cytotoxic T cells (CD8) compared with total T cells (CD3).

•Population analysis of cells: differentiating T cells, B cells, NK cells and monocytes based on cell surface marker expression.

•Measuring sepsis induced immunosuppression via HLA-DR expression on circulating monocytes.

Results:

In a demonstration of cell surface marker quantitation a comparison study of 53 blood samples showed a correlation coefficient of 0.91 for Accellix determined neutrophil CD64 compared to those determined using a FACS. In a study to identify lymphocyte subsets a comparison study of 5 samples (run in triplicates) showed a correlation coefficient of 0.99 for Accellix determined T cell differentiation based on CD4/CD8 ratio, and correlation coefficients of 0.94 and 0.99 for lymphocyte population analysis to determine B cells (CD19/CD45) and NK cells (CD56/CD3-/CD45) compared to FACS.

Conclusions:

These initial studies show that the cartridge-based Accellix system can determine the presence and concentration of cell populations as well as determine the concentration of cell surface markers. Thus, implementation of a wide range of fully automatic IVD assays with results in 30 minutes or less is possible using Accellix.

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Analytical Validation of Point-of-Care Emergency Tests on the PATHFAST System in Comparison with Automated Laboratory Analyzers

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Background:

The PATHFAST system consists of an automated analyzer that uses single cartridges containing reagents for quantitative measurement in whole blood (WB), serum and heparinized, citrated or EDTA plasma. The turn-around-time (TAT) lies within 16 min. We evaluated the determination of the 6 emergency parameters cardiac troponin I (cTnI), high sensitivity C-reactive protein (hsCRP), myoglobin (Myo), CK-MB, NT-proBNP, and D-Dimer in comparison with Roche E 170 and cobas Integra 800.

Methods:

Intra- and inter-assay imprecision were evaluated using BioRadLiquicheK Cardiac Markers Control, patient plasma and WB samples. Linearity, analytical and functional sensitivity, limit of blank (LoB) were determined by using predefined samples and zero calibrators. The method comparison with Roche E 170 and cobas Integra 800 was performed using patient samples with marker concentrations comprising the whole measurement range.

Results:

Coefficients of variation (CVs) of intra- and inter-assay imprecision ranged between 3.3% and 8.0%. All assays showed recovery between 90% and 110% and complete linearity across the total range. The LoB was determined by measurement of 10 replicates of the zero calibrator and of the lowest non-zero calibrator in parallel. Sample matrix evaluation was performed using WB and plasma samples. All assays showed high comparability between WB, serum, heparinized, citrated plasma or EDTA plasma. The results method comparison with Roche E 170 and cobas Integra 800 are displayed in the table.

Conclusion:

Method comparison revealed high concordance of the PATFAST system with the Roche E 170 and cobas Intregra 800 analyzer. POC testing on the PATHFAST analyzer allows measurement of whole blood samples within 16 min after blood drawing in the point-of-care setting providing comparable results with the central laboratory.

PATHFAST imprecision data and results of method comparison

	WB samples Mean (n=20)	Intra-assay CV (%)	Inter-assay CV (%)	Comparison	Intercept	Slope	r
CKMB (µg/L)	2.51	6.2	8.2	E-170	-1.278	0.9221	0.988
cTnI (µg/L)	0.19	6.1	5.6	E-170*)			
D-Dimer(µg/L)	0.69	4.8	5.5	Integra	0.3133	1.005	0.977
hsCRP(mg/L)	0.63	6.4	6.6	Integra	0.0334	0.883	0.998
NTproBNP(ng/L)	411	4.0	6.9	E-170	196.3	1.265	0.967
Myoglobin(µg/L)	37.7	5.6	6.1	Integra	-1.037	1.050	0.991

*) Overall agreement at 99th% cut-off 77% and at 0.264 µg/L 97%

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Analytical Performance of the Abaxis Piccolo Xpress Point of Care Analyzer in Whole Blood, Serum, and Plasma

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Background: The Abaxis Piccolo Xpress is a portable bench top analyzer that performs multiple simultaneous assays with a single-use reagent disc. The analyzer is designed to accept whole blood, serum, or heparinized plasma, which is directly added to the plastic reagent disc. The analyzer is able to report a total of 31 separate analytes, including lipids, electrolytes, liver function tests, renal function tests, and general chemistry assays. There has been much interest in these devices due to a recent Ebola outbreak, with many hospitals establishing policies restricting clinical laboratory testing on confirmed or suspected Ebola patients to point-of-care testing. Despite much interest in this device, there have been limited comprehensive studies published in the literature that have critically examined the analytical performance of this device in all three matrices. The primary objective of this study was to examine the analytical performance of 14 comprehensive metabolic panel (CMP) analytes on the Abaxis Piccolo Xpress point-of-care analyzer in serum, plasma, and whole blood. Methods: Precision was evaluated by running two levels of control material. Linearity was evaluated using material provided by the manufacturer and the College of American Pathologists (CAP) linearity surveys. Accuracy was evaluated by comparing the results from 60 patient specimens on the Piccolo with the Ortho Vitros 5600 analyzer. The method comparison was performed on all three specimen types intended for use on the Piccolo; serum, heparinized plasma, and whole blood. Results: High precision was noted for all the analytes with the exception of TCO2, which had a CV of almost 16%. Linearity was found to span the clinically useful range for all analytes. The method comparison demonstrated significant proportional bias (slope <0.85 or >1.15), poor correlation(R2 < 0.85), or both, for sodium, ALT, albumin, total protein, and total CO2 in all matrices. Furthermore, significant proportional biases and or poor correlations were noted for calcium, total bilirubin, and urea nitrogen in whole blood and plasma. Conclusion: The Piccolo Xpress allows for the delivery of CMP results in a footprint small enough to be stored in a biological safety cabinet, while providing satisfactory performance for the majority of analytes.

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Combination of the Mortality in Emergency Department Sepsis (MEDS) Score with PATHFAST Presepsin Improves Outcome Prediction of Sepsis

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Background: Assessment of disease severity at presentation could be helpful in the management of patient with sepsis. The POC test PATHFAST Presepsin has been shown to provide powerful prognostication. We thought to evaluate PATHFAST Presepsin (PSEP) for outcome prediction in combination with the MEDS score.

Methods: 121 septic patients were included. MEDS score, PSEP and procalcitonin (PCT) were determined at admission to the ED. Primary endpoint was death. The combined endpoint (MAE) consisted of the primary or at least one of the secondary endpoints ICU, mechanical ventilation or dialysis. **Results:** 21 patients died and 34 patients exhibited MAEs during 30 day follow up. The number of decedents and patients with MAEs were 2 (3.2%) / 5 (8.1%), 8 (21.6%) / 15 (40.5%) and 11 (50,0%) / 14 (63.6%) in patients with sepsis (n=62), severe sepsis (n=37) and septic shock (=22), respectively. Median values of MEDS score and PSEP in sepsis (n=62) were 8 and 738 ng/L compared to 11 and 1407 ng/L (p<0.0001) in severe sepsis or septic shock (n=59). 30-day mortality was 17.4 %, ranging from 0 % in the 1st to 43.3 % in the 4th quartile of PSEP concentration. ROC analysis revealed AUC values for MEDS score and PSEP of 0.851 and 0.810, respectively, compared to 0.549 of PCT. The combination MEDS+PSEP revealed an AUC value of 0.909.

Conclusion: MEDS score and PSEP demonstrated strong relationship with disease severity and outcome in patient with sepsis in the ED. The combination of MEDS score and PSEP provided a higher predictive value than both markers alone. The PATHFAST system allows early determination of PSEP from whole blood in the ED in addition to MEDS score and may improve the management of sepsis.

Results of ROC analysis					
		AUC	SENS (%)	SPEC (%)	Cutoff
MEDS	Death	0.851	81.0	85.0	>12
	MAE	0.806	82.4	65.1	>9
PSEP	Death	0.810	81.0	70.0	>1179 ng/L
	MAE	0.736	97.1	39.5	>608 ng/L
MEDS+PSEP	Death	0.909	95.2	83.8	>0.122
	MAE	0.860	97.1	60.5	>0.041

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Development of a new rapid assay for quantitative measurement of H-FABP in whole blood and plasma.

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Introduction: H-FABP (Heart-type Fatty Acid Binding Protein), a member of the FABP group, is present in the cytoplasm of cardiac muscle cells and leaks rapidly into blood circulation after suffering a myocardial injury. Therefore, H-FABP is used for diagnosis of acute myocardial infarction. We have developed a new rapid and quantitative assay kit for H-FABP that works on the principle of lateral flow immunochromatography. The test requires only 10 minutes and 120µL of whole blood or plasma. After application of the sample onto the ready-to-use cartridge-type reagent, immunoreactions and test report will automatically be performed by our easy-to-use immuochromato-reader "Rapidpia™ (SEKISUI MEDICAL Co., LTD.)".

Principle: The test strip housed in the cartridge contains two monoclonal antibodies which react with H-FABP. One of the antibodies is labeled with colloidal gold and the other coats the detection zone membrane. While flowing on the test strip, the labeled antibody forms a complex with the H-FABP. Following the separation of the red blood cells, the labeled H-FABP in the plasma react with the antibody coated membrane and form a reddish line while passing through the detection zone. The intensity of the line increases depend on the concentration of the H-FABP. Rapidpia™ measures, and converts the signal intensity to a quantitative report.

Performance: The lower detection limit for H-FABP was 2.0 ng/mL, and upper quantitation limit was 160 ng/mL. No prozone effect was observed in H-FABP samples of concentrations from 160 through 2,300 ng/mL. The within-run C.V. (n=5) at 7.2 ng/mL, 37.8 ng/mL, and 108 ng/mL was 5.7%, 3.8%, and 6.7%, respectively. The between-run C.V. (n=5) at 7.7 ng/mL, 38.8 ng/mL and 119 ng/mL was 2.5%, 1.3% and 2.3%, respectively. Comparison of our assay kit with an approved IVD reagent, the principle of which is latex-enhanced immunoturbidimetry, yielded a correlation coefficient of 0.993 and an equation of Y (present method) = 0.99X + 0.00 (n = 88 heparinized plasma samples). Furthermore, a high correlation was also observed in the comparison of heparinized plasma and whole blood (R : 0.996 ; Y = 1.01x - 0.60).

Conclusion: This newly developed assay kit is accurate, precise and easy to use for the measurement of H-FABP in whole blood and plasma. We believe that this assay kit will be a useful tool in emergencies.

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Point of Care Testing Expansion in Public Health; One County's Experience

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Background:

The Marion Co. Public Health Department Laboratory increased the quality and quantity of point of care (POCT) in health department clinics in 5 years. This mirrors a national trend to decentralize clinical testing, which has the potential to improve healthcare delivery, and public health in particular1. Decentralized testing along with improved connectivity of information and more robust testing devices also supports a greater responsiveness to emergency response essential for public health. 2

Methods:

We made the improvements through continuous quality improvement projects, Lean management practices and without increased staffing. Over 18-24 months, and through the Clinical Manager and Quality Manager staff turnover we implemented CLSI standards and put in to place CMS suggestions3 to assure the accuracy and reliability of POCT (waived) testing through implementation of structured staff training and competency assessment. Successes with this approach and expansion of the WIC and Refugee/Foreign Born (RFB) programs resulted in increased need for the expanded lab support of POCT.

Results:

The number of WIC sites grew from 12 to 13, and the RFB programs began to offer POCT, including HIV screening, urinalysis and urine pregnancy tests. Substance Use Outreach Services department also began rapid HIV screening. Only the School-Based Clinic sites contracted from 4 to 1 sites in the same time. Overall the number of non-lab personnel using POCT rose in the past 3 years from 77 to 122, and the test count increased from 1 to 4, increasing the number and complexity of training and competency assessment materials needed for that training. By 2014 a large shift had occurred, nearly a quarter (23%) of the results from waived tests were produced outside the lab by staff who were trained and had competency assessed by the lab QA program. Improvement continues. In 2014 we created the Laboratory Support Division, managed by the QA Manager to better serve not only POCT, but also in-lab testing QA.

Conclusion:

The Lab working together with clinical departments has been able to make great strides in improving the amount and quality of clinical testing that occurs near the public health client. This allows rapid clinical decision-making in a single patient visit, minimizing the impact of losing patients to follow-up while waiting for test results to be returned; a particular problem in economic challenge areas. This POCT-QI program has empowered more health department employees to gain competency in POCT. This in turn results in improving Analytical/Assessment Skills and Public Health Sciences Skills across the health department.

References:

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B-245

Performance of Estapor® Microspheres and Hi-Flow™ Plus Membranes in a Lateral Flow Assay for Human Chorionic Gonadotropin (hCG)

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Background:

Available in a range of sizes, latex microspheres are versatile detector particles which can, depending on their composition, be detected by colorimetry, fluorometry, or paramagnetism, and with functional chemistries be covalently attached to antibodies and other molecules.

In lateral flow assays particles must move through a porous membrane. Since particle size and membrane flow rate will impact particle mobility, assay manufacturers must optimise these parameters to achieve their desired assay sensitivity.

In this study, we examine the relationship between microsphere size, membrane flow rate, and assay sensitivity, using Estapor® carboxyl-modified dyed microspheres and Hi-Flow™ Plus membranes.

Methods:

Lateral flow test strips for the detection of hCG were assembled using blue Estapor carboxyl-modified microspheres with diameters ranging from 0.185 to 0.478µm, and Hi-Flow Plus lateral flow membranes with flow rates ranging from 75s/4cm to 180s/4cm. Microspheres were conjugated to anti-hCG antibodies using a two-step EDC/Sulfo-NHS covalent coupling procedure and resuspended to a concentration of 1% (w/v) in 50mM Tris (pH. 8.0), 0.5% casein. For conjugate pad application, conjugates were diluted to 0.065% (w/v) in 50mM Tris (pH 8.0) containing 0.5% casein, 2.5% trehalose, 10% sucrose, and 0.5% polyvinylpyrrolidone. A 1µL microsphere suspension was applied to each pad (0.5 x 30 cm), yielding 10.8µg of microspheres per test. Hi-Flow Plus membranes were striped with ß-hCG antibody at a concentration of 1mg/mL in 50mM MES (pH 6.0) and goat anti-mouse IgG, as a control antibody, at 1mg/mL in Milli-Q® water.

Results:

After assembly, test strips were run with hCG samples of known concentration and test line signal intensities evaluated colorimetrically. Microspheres of 0.185µm produced weakest signals regardless of the membrane's flow rate. Microspheres of intermediate diameters produced comparable signals. While microspheres of 0.478µm diameter produced the most intense signals, they also exhibited nonspecific binding as evidenced by measureable test line signals with hCG-negative controls.

To eliminate nonspecific binding, modifications to the conjugation procedure were investigated. Reducing the amount of ethanolamine by 50% and changing the blocking agent from 0.5% (w/v) casein to 1% (w/v) fish skin gelatin were most effective.

Optimized 0.478-µm microspheres were re-evaluated on Hi-Flow Plus 75 (HF075), Hi-Flow Plus 135 (HF135), and Hi-Flow Plus 180 (HF180) membranes. The signal intensity on HF075 was reduced at all concentrations. On HF135 and HF180, reduced signal intensities were observed at concentrations below 1600mIU hCG/mL. Above that, the optimized and standard microspheres produced similar signal intensities. No signals were detected with hCG-negative controls.

Conclusion:

By pairing microspheres of varying diameters with membranes of varying flow rates, we show quantitatively that microspheres of 0.478µm diameter produced the highest signals. Microspheres with diameters of 0.228, 0.413, and 0.422µm produced intermediate signal intensities that were similar across membranes. Microspheres with a diameter of 0.185 µm produced the lowest signals. False positives with negative controls observed on test strips run with 0.478µm microspheres were eliminated through conjugation optimization. Thus, using hCG detection as a model system, we have demonstrated the efficacy of Estapor microspheres in lateral flow test strips manufactured on Hi-Flow Plus membranes.

B-246

Comparison of Nova StatStrip Xpress and Roche Advantage glucose meters for use during hyperbaric oxygen treatment

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Background:

Blood glucose testing is commonly performed in a hyperbaric chamber (HBC) for patients undergoing hyperbaric oxygen treatment (HBOT). The Roche Advantage glucose meter, a device evaluated for use during HBOT, is no longer being manufactured. We compared Roche Advantage (Roche Diagnostics, Indianapolis IN) and Nova StatStrip Xpress (Nova Biomedical, Waltham MA) for use during HBOT.

Methods:

Accuracy evaluation was performed outside HBC using residual lithium heparin whole blood samples (n=47) on the Advantage, Xpress, and Radiometer ABL825 (Radiometer Medical ApS, Bronshøj, Denmark). Immediately following analysis, samples were centrifuged and plasma glucose measured using a Roche cobas c501. Advantage and Xpress glucose results were compared to the average of ABL825 and c501 values (reference).

Accuracy under 2 atmospheres (ATA) of pressure in HBC was assessed by dosing both meters with specimens (n=49) that had glucose measured on Radiometer ABL90 blood gas analyzer (reference method outside of HBC). Within 15 minutes of analysis on ABL90, syringes were transported to HBC where samples, test strips, and meters equilibrated at 2 ATA for up to 15 minutes prior to testing. Additionally, 25 samples were tested in duplicate on Xpress under 3 ATA and compared to ABL90 performed outside HBC. Glucose meter results were compared to reference glucose by calculation of median (interquartile range, IQR) bias and comparison of number of outlier results obtained in HBC.

Results:

Outside HBC, median (IQR) bias between Xpress and reference glucose among the 47 samples (range 12-525 mg/dL) was -1- (-6 to 3) mg/dL; compared to median (IQR) bias of 6 (1 to 16) mg/dL for Advantage ($p < 0.0001$). 44 of 47 Xpress results and 37 of 47 Advantage results fell within current CLSI POCT12-A3 accuracy guidelines.

Among the 49 samples (range 25-456 mg/dL) tested under 2 ATA in HBC, median (IQR) bias on Xpress was -12 (-23 to -6) mg/dL, compared to median (IQR) bias on Advantage of -25 (-42 to -9) mg/dL ($p=0.0232$). Median bias on both devices was impacted by several outliers. 8 of 49 Xpress and 23 of 49 Advantage results differed from reference result by more than 20 mg/dL (for Radiometer glucose < 100 mg/dL) or 20% (for Radiometer glucose ≥ 100 mg/dL).

When 25 lithium heparin whole blood samples were analyzed in duplicate on Xpress under 3 ATA in HBC, median (IQR) bias was -3 (-10 to 1) mg/dL, with only 2 of 50 measurements resulting in an outlier.

Conclusion:

Nova StatStrip Xpress is a suitable replacement for Roche Advantage for measuring whole blood glucose during HBOT. Xpress had better accuracy outside HBC and produced fewer outliers under 2 ATA inside HBC. It is unclear whether glucose meter outliers observed under 2 or 3 ATA were due to pre-analytical effects on the lithium heparin whole blood samples or whether outliers may occur when capillary whole blood is analyzed during HBOT.

B-247

Comparison of Blood Collection Via Fingerstick and Venipuncture Methods for Near Patient/Point of Care Testing: An Evaluation of Sample Quality and Subject Pain Perception

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Background. Near patient/point of care (NP/POC) testing can provide faster turnaround time, which may increase clinical efficacy and contribute to improved outcomes. However, the results provided must be accurate. NP/POC testing currently uses a variety of sample types, such as venous, arterial and capillary, which can be tested as either whole blood or plasma. In all cases, sample quality is important and issues, such as hemolysis, can affect test results. Little data are available regarding the sample quality of fingerstick blood collections.

Objective. In this study, visual hemolysis in samples collected by fingerstick under controlled conditions (trained phlebotomists, healthy adult subjects, best practices) was compared to that in samples collected by antecubital venipuncture. The study also assessed pain perception during collection from participating subjects.

Methods. Samples were obtained from 30 adult subjects via fingerstick using a BD Vacutainer® Contact Activated Lancet into Fisher Brand Micro-Hematocrit Capillary Blood Collection Tubes with Heparin and via venipuncture using a BD Vacutainer Push Button Blood Collection Set into 2 mL and 4 mL lithium heparin tubes. Collections were divided equally among three phlebotomists. Blood from each venipuncture was transferred to a plain, non-anticoagulated Micro-Hematocrit Capillary Blood Collection Tube. Venous and fingerstick samples in the Micro-Hematocrit capillary tubes were centrifuged in a Clay Adams AutoCrit Ultra 3 at 13,700g and then observed for visual hemolysis. Percent and frequency of occurrences of visual hemolysis (none, trace, moderate, gross) were tabulated. Each subject also evaluated their perception of pain after each procedure using a 15-cm Absolute Visual Analog Scale. Pain perception ratings (from no pain [0 - low end of scale] to excruciating pain [15 - high end of scale]) were compared between methods to determine differences in performance.

Results. There were no significant differences in visual hemolysis observed between the collection methods or the draw volumes (venipuncture only). 100% ratings of "none" were observed in the samples collected via fingerstick. For the samples collected via venipuncture, one 4 mL lithium heparin tube had a rating of "moderate". For pain perception, collection by fingerstick had a lower average pain rating than venipuncture. The average method comparison difference in maximum recorded pain for fingerstick versus venipuncture was -0.73 with 95% confidence interval (-1.25, -0.20).

Conclusion. In this study, samples collected by fingerstick capillary blood collection demonstrated no significant difference in quality (i.e., hemolysis) when compared to samples collected by venipuncture. The fingerstick capillary collection method also elicited less pain than the venipuncture method. For low volume requirements, which are common for many NP/POC tests, fingerstick capillary collection, when performed according to best practices, is a valid alternative to venipuncture and may offer some advantages, such as patient comfort and lower volume sampling.

B-248

Using urine dipstick as a presumptive result for urinary tract infection.

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Background:

Urine dipstick (UD) point of care (POCT) is a quick and inexpensive alternative to urine screening that reduces the time of patients in emergency department (ED). The aim of this study is to evaluate the sensitivity and specificity of two specific parameters in UD compared to the urine sediment and urine culture.

Methods:

We performed retrospectively from January to March 2014, 2261 patients who performed concomitantly the three tests: Urine dipstick in ED was performed in Uryxxon 300 (Macherey Nagel®), the sediment in iQ Sprint (IRIS®) and urine culture performed in chromogenic CPS culture media (Biomerieux®). For positive culture when applicable, identification and sensitivity were obtained through Vitek 2 (Biomerieux®).

Results:

When was compared UD (nitrite and esterase) with urine culture it was observed a sensitivity of 45% and a specificity of 99%. When another parameter was included such a presence of proteins the sensitivity increased to 73%. On the other hand when compared the quantification of leukocytes in the sediment and urine culture we observed a sensitivity of 93% and a specificity of 75%.

Conclusion:

We conclude that the UD nitrite and esterase has high negative predictive value for urinary tract infection when compared to urine culture. Thus, when these parameters are negatives in UD it almost excludes the possibility of urinary tract infection according urine culture. However there is a lack of sensitivity that could possibly be improved using an additional parameter, as positive protein. The sensitivity of positive protein, nitrite and esterase increase 28% the sensitivity of the test compared with positives nitrite and esterase only. As an alternative for resources management the use of UD to exclude urinary tract infection could be useful in emergency department once 68,9% (1552/2261) of the patients will have nitrite and esterase negative of the total.

B-249

How Much Training is Required on the epoc® Blood Analysis System to Produce Clinically Accurate & Reproducible Results in a Point-of-Care Setting?

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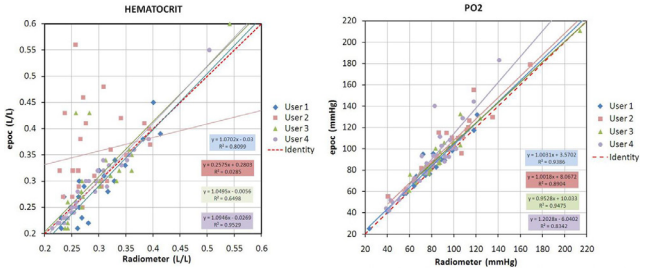
Introduction: To reduce Emergency Department visits Alberta Health Services is introducing a new Emergency Medical Services (EMS) program which brings diverse medical services, including point-of-care testing, to continuing care facilities. The suitability of the Alere epoc® blood analysis system was studied to determine whether non-laboratory staff with minimal training could produce clinically accurate & precise results.

Methods: The evaluation was performed last year at University of Alberta Hospital. Two EMS workers and two nurse practitioners performed all testing after having received 2.5 hours of epoc® training. Precision was estimated by analyzing either hematology or chemistry quality control material in duplicate each day at two (hematocrit) or three levels (pH, pCO_2, pO_2, sodium, potassium, ionized calcium, glucose, lactate). Each staff member ran 25 patient samples on the epoc® using a Radiometer ABL825 as the comparative device. Each patient sample was first run on the Radiometer; leftover sample was then analyzed on the epoc®. Acceptability criteria for bias and imprecision were based on CLIA '88, biological variation, or other resources.

Results: Initial analysis of the complete data set revealed acceptable performance for pH, pCO_2, potassium, glucose, and lactate. The performance of pO_2, sodium, ionized calcium, and hematocrit was more variable. The figure shows a user-dependent variation in accuracy for hematocrit and pO_2; similar user-dependent variation was identified for sodium and ionized calcium. Exclusion of just one user's results yielded acceptable overall performance for all analytes.

Conclusion: Our results suggest that the 2.5 hour epoc® training program is sufficient for many non-laboratory users to generate clinically accurate & reproducible test results. However, our results also indicate that select users require additional training. Point-of-care programs should design training sessions carefully in order to identify such individuals and to provide them with additional support as necessary.



B-250

Development of Multiplex Rapid POC Test for Current, Persistent, and Recurring Syphilis Infections Based on the Simultaneous Detection of Treponemal and non-Treponemal Antibodies in Human Blood Specimens

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Background: Syphilis, caused by Treponema pallidum, is an STD with serious consequences if left untreated. Despite availability of diagnostic tests and effective treatment, it remains a global health problem. Correct diagnosis requires clinical evidence combined with results of serological tests for antibodies against: nTP (cardiolipin) and TP antigens. These two serological tests are complementary, and widely used for screening and confirmation of syphilis. There is an unmet need for a solution that combines the two in a POC test that can be performed while a patient waits. MedMira's Multiplo Rapid TP/nTP Antibody Test (Multiplo) is a manually performed, visually interpreted assay that can be completed in three minutes and provides screening and confirmatory results based on the presence of nTP and TP antibodies.

Methods: Preclinical studies were undertaken by testing commercially obtained specimens with Multiplo (per manufacturer's instructions). Multiplo contains two test zones; a TP zone and an nTP capture antigens zone, and a control zone. The TP test zone contains optimized TP recombinant antigen. The nTP test zone contains a proprietary cardiolipin-based antigen. Antibodies from patient specimens, if present, are captured through their respective immobilized antigens, and are subsequently visualized in the distinct test zones through binding to protein A gold conjugate. Two syphilis mixed titer panels (n=23) and a dilution panel were tested to assess sensitivity. Specificity was assessed using 22 plasma and 42 whole blood specimens. Interference/cross-reactivity was assessed using a set of 95 specimens collected from individuals with unrelated conditions. Results in each test zone was recorded and compared to available reference results.

Results: Within performance panels, there was 100% concordance between Multiplo and reference assays for both TP and nTP antibodies. Serial dilutions of three highly positive (both TP and nTP antibodies) specimens into negative specimens illustrated reactive results for TP and nTP in all dilutions tested (1/2 to 1/8) confirming the high sensitivity of this assay. Specificity was evaluated for the TP portion by testing a set of 22 specimens certified as non-reactive for HIV, HBV, HCV, and TP antibodies by FDA approved assays and an additional 42 whole blood samples. 100% specificity of Multiplo TP was observed. Interference/cross reactivity was evaluated using a 95 member panel. nTP results were positive in 34 of the 95 specimens, reporting a variety of disease states. At the time, reference nTP results were not available for the 95 specimens. Observed Multiplo nTP results are likely biologically false positives. More significantly, there was no interference observed in the TP test zone of Multiplo in this panel.

Conclusion: Multiplo, built on MedMira's Rapid Vertical Flow (RVF) technology, has been designed to meet the ASSURED criteria established by the WHO. Sensitivity and specificity of the TP portion Multiplo TP/nTP were both calculated to be 100%. Normal state specimens showed approximately 5% nTP biological false positives compared to 36% for the diseased state specimens. Additional studies will be conducted to further assess performance in a larger scale trial.

B-251

Diagnosis of Clostridium difficile diaherra using the Xpert C. difficile PCR assay for detection in fecal specimens as a point of care test in a tertiary care hospital in São Paulo, Brazil.

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Background:

Toxin-producing *Clostridium difficile* is the most common cause of health-care-associated diarrhea. *C. difficile* infection (CDI) diagnosis is defined by positive laboratory test detecting toxigenic *C. difficile* in the stool (toxins A and B). In the last years, the epidemic strain of *C. difficile* producing a third toxin called the binary toxin, referred to either NAP1, BI, or 027, has been associated with several outbreaks. Although culture followed by toxin detection remains the gold standard for diagnosis of CDI, alternative tests has been used in clinical practice, including detection of *C. difficile* toxins in stool samples by enzyme immunoassays (EIAs), and real-time PCR assays, with variations in sensitivities and specificity.

Methods:

From July 2014 to January 2015, a total of 63 stool samples from 63 patients attending a private hospital in São Paulo, Brazil, with CID suspected were tested by Clostridium toxin by Xpert *C. difficile* assay, and 34 out of 63 samples were also tested by EIA for detection of the GDH antigen. Xpert *C. difficile* PCR assay detects the toxin B gene (*tcd*B), the binary toxin gene (*cdt*), and the *tcd*C gene deletion at nt 117.

Results:

A total of 63 stool samples from 63 patients were studied; 13 samples were Xpert *C. difficille* assay positive and 1 sample was also positive for toxigenic *C. difficile* 027-NAP1-BI. A total of 34 fecal specimens were tested by both methods PCR and Immunoassay; only 2 out of 13 positive samples by Xpert C. difficile were positive by immunoassay. The turnaround time for Gen Xpert C. difficile assay was less than two hours.

Conclusion:

Xpert *C. difficile* assay had a higher sensitivity for screening toxigenic *C. difficile* in fecal specimens compared to EIA. The rapid diagnosis based on Xpert permits a faster result since the clinical suspicion of CDI and laboratory investigation, which is important to appropriate management of the patients infection control practices. In addition, the detection of NAP1-B1 is important to epidemiological surveillance and adequate healthcare policies.

B-252

Evaluation of Bias of Glucose Measurement Among Multiple Analytical Systems in Various Specimen Types

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Background:

Glucose measurement is one of the most frequently ordered tests in clinical settings. Multiple methods are available for point-of-care (POC) and central laboratory glucose testing using plasma, serum, or whole blood (WB) specimens. However, bias may exist among various systems when various specimen types are used. The objective of this study is to determine the bias in glucose measurements among POCT devices, critical care analyzers, and central clinical chemistry laboratory analyzers when different specimens are used.

Methods:

Abbott FreeStyle glucose meter is based on the glucose dehydrogenase-nicotinamide adenine dinucleotide method (GDH-NAD reaction), while Abbott i-STAT, Radiometer ABL800 FLEX analyzer, and Beckman Coulter UniCel®DxC 800 analyzers measure glucose amperometrically by oxidation of glucose using glucose oxidase. Venous whole blood (VWB) samples (n=26) collected in lithium heparin (LH) tubes were analyzed for glucose by Radiometer, FreeStyle and i-STAT in duplicates. Then, these WB samples were spun down and the plasma was measured for glucose by Beckman Coulter UniCel®DxC800 in duplicates immediately. Arterial whole blood (AWB) samples (n=25) in LH syringes were used to analyze glucose by Radiometer, FreeStyle and i-STAT. The overall average percent bias was calculated between i-STAT-DxC, FreeStyle-DxC and Radiometer-DxC for VWB vs plasma comparison employing DxC as a reference. For VWB vs VWB and for AWB vs AWB, average percent bias was calculated between i-STAT and Radiometer, FreeStyle and Radiometer using Radiometer as a reference, also FreeStyle and i-STAT was compared using i-STAT as a reference.

Results:

For the VWB vs plasma comparison, -12% and -4% biases were observed between FreeStyle and DxC and between i-STAT and DxC, respectively, whereas no significant bias (-0.1%) was observed between Radiometer and DxC. For VWB vs VWB in LH tubes, -4% and -12% biases were observed between i-STAT and Radiometer and between FreeStyle and Radiometer, respectively, and a -8% bias was observed between FreeStyle and iSTAT. For AWB vs AWB from LH syringes -3% and -3% biases were observed between i-STAT and Radiometer and between FreeStyle and Radiometer, respectively, and no significant bias (-0.2%) was observed between FreeStyle and iSTAT.

Conclusion:

Overall for VWB vs plasma comparison, FreeStyle shows the most significant negative bias (-12%). FreeStyle also has significant negative bias in VWB when compared to Radiometer (-12%) and i-STAT (-8%). However, in AWB, the negative bias of FreeStyle decreases to an insignificant level when compared to radiometer (-3%) and i-STAT (-0.2%). It is unclear why FreeStyle demonstrates different bias in venous and arterial blood samples. However, since the oxygen content in venous blood is much lower than that in arterial blood, the difference in the oxygen content between arterial and venous blood might play a role in the better correlation in arterial samples. Further studies are to be conducted to determine the underlying factors contributing to this difference in bias of glucose measurement by FreeStyle meters between arterial and venous blood samples.

B-253

Glucometer performance in critically ill patients: a correlation of the Roche Accu-chek Inform II vs the Roche Accu-chek Inform I, Abbott i-STAT and Beckman Coulter AU 680

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Background: Historically, the Food and Drug Administration(FDA) evaluated glucometers used in hospitals and at home by patients by the same methods and standards. Since over-the-counter "home use" glucometers are classified as waived testing, hospitals have used these meters for convenient, rapid bedside glucose testing. However, it has become increasing clear that these two settings are different, with critically ill hospitalized patients having physiological derangements that could potentially affect glucometer performance. The FDA's draft guidelines call into question the waived status of glucometers currently in use at many hospitals and deems hospital blood glucose testing as "off label" use, which would nullify the glucometer's waived status, categorize it as "high complexity" testing, and subject glucometers to much more stringent testing personnel, competency, and validation requirements. Also recommended is that manufacturers label their glucometers with a statement regarding use in critically ill patients, which would make it very difficult to perform rapid bedside glucose monitoring in hospital intensive care units, operating rooms, emergency rooms, and ambulances.

Methods: We defined "critically ill" as the patient population in intensive care or critical care units. Samples were collected over a 3½ month period at four hospitals. Glucose measurements were performed on the Roche Accu-chek Inform I, Roche Accu-chek Inform II, Abbott i-STAT, and Beckman Coulter AU680.

Results: Samples from 34 patients, of which 21 were female and 13 were male, ranging in age from 36 hours to 89 years, were obtained. Due to available blood volume limitations on newborn and pediatric patients, results on all four instruments were available for 23 patients, three instruments for 8 patients, and two instruments for 2 patients. Mean blood glucose concentration ranged from 61mg/dL to 307mg/dL. When data was available for all four instruments, the linear regression line was (Inform II) = (0.8677)*(i-STAT) + (-0.0996)*(Beckman AU) + (0.2165)*(Inform I) +(-1.6299), with an R^2 of 0.9900 and a standard error of 6.009. Looking at the Inform I, i-STAT, and AU680 vs the Inform II individually, the slopes were 0.9634, 1.0329, 1.0143, the intercepts were -0.8815, -3.4617, -1.5020, and the standard errors were 8.48, 7.26, 6.46, respectively. The R^2 values were all greater than 0.9. The paired t-test (two-tailed) were less than t critical for the i-STAT and AU680, with p-values of 0.64 and 0.80, respectively, while that of the Inform I was larger than t critical, with a p-value of 0.001.

Conclusion: The t-test difference between the Inform I and Inform II was somewhat surprising; however, the Inform II was developed because of issues with interferences and to improve accuracy of test results. Limitations of this study include the small sample size and issues with not having enough specimen for testing to be performed on all four instruments. For both pediatric and adult, a larger sampling of different kinds of critically ill patients would have been preferable, as would additional hypo- and hyperglycemic specimens. However, the Accu-chek

Inform II, despite having the limitation regarding use in critically ill patients, performed relatively similarly to the Inform I, i-STAT, and AU680.

B-254

Comparison of the Abaxis Piccolo Xpress to core-laboratory automated chemistry analyzers

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Background:

The Abaxis piccolo Xpress is a small disc-based centrifugal analyzer designed to separate plasma from whole blood, and test plasma for acute-care clinical chemistry panels. Its compact size, minimal volume requirements (0.1 mL) and high test yield

make it appealing for small laboratories, point of care applications, and use inside biological safety cabinets. As little published data exists on its performance, we sought to compare it to two popular core-laboratory random access chemistry analyzers.

Methods:

A minimum of forty anticoagulated whole blood specimens were sampled each from core laboratories at the University of Alberta Hospital (Edmonton, Alberta, Canada) and Alberta Children's Hospital (Calgary, Alberta, Canada). Specimens were split and immediately tested on the Piccolo Xpress , the Beckman UniCel 800 (Edmonton) and the Roche Cobas 6000 (Calgary) for the comprehensive metabolic panel (Na, K, Cl, Ca2+, CO2, glucose, creatinine, urea, total protein, albumin, ALT, AST, ALP, total bilirubin). Proportional bias (slope), constant bias (intercept), coefficient of determination (R2) and Syx as a proportion of the mean were calculated for each comparison using linear regression. Total error of each Piccolo assay was determined based on stated precision claims and bias versus main analyzers, and contrasted to allowable error set by the College of American Pathologists.

Results:

The Piccolo Xpress proportionally underestimated results in 80% of tests versus main analyzers (up to 37% difference), but 85% had constant overestimations that tended to resolve many of these differences. Most R2 values were above 0.80, suggesting that each analyzer ranked specimen results similarly for most tests. Total error remained below allowable error for most tests. However in comparisons with the Roche Cobas and the Beckman Unicel, the R2 for Sodium was 0.57 (slope = 0.84, intercept = 22) and 0.63 (slope = 0.67, intercept = 50) respectively. Allowable error was exceeded for both comparisons, and was exceeded for creatinine in comparison to the Beckman UniCel, and Syx as a % of the mean was 19% in comparison to the Roche Cobas.

Conclusions:

The Piccolo Xpress does not yield identical results as core-laboratory chemistry analyzers for several important clinical chemistry tests. While correction factors may be applied to harmonize results with core-lab analyzer tests, this should only be done if correlations between tests are high. Further, it may not be necessary to drop individual tests that exceed allowable error thresholds. Poorly performing tests may instead be re-purposed for semi-quantitative or qualitative testing in different clinical scenarios. For highly correlated Piccolo tests which differ from core laboratory results by clinically important proportional or constant differences, we encourage Abaxis to allow the user to set correction factors within the Piccolo software interface.

B-255

Clinical evaluation of point-of-care assay for urinary NGAL & TIMP-2 as early biomarker of acute kidney injury in ICU patients

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Introduction: Acute kidney injury (AKI) is a major public health problem affecting millions worldwide that deserves more awareness [1]. AKI is associated to poor outcomes, including decreased survival, increased progression to chronic kidney disease (CKD), as well as higher susceptibility to other complication such as bacterial infection [2]. Using the RIFLE for AKI definition, a study [3] examined 325,395 ICU patients and determined 22% developed AKI with a mortality rate of 10%. Recent publications [4] have shown that biomarker neutrophil gelatinase-associated lipocalin (NGAL) is an early predictor of AKI in emergency department and ICU patients. In contrast, tissue inhibitor of metalloproteinases-2 (TIMP-2) [5,6] in urine has been tested in subsets of ICU patients, such as those who underwent cardiac surgery, or with complication in respiratory or cardiovascular system. In this study, we investigated a general ICU population by evaluating the performance of urinary NGAL, TIMP-2 individually, and in combination of the two biomarkers.

Methods: In 43 ICU patients at a AAA hospital in China, we measured the concentration of both biomarkers using immunoassay point-of-care testing at the following time points after ICU admission: 0, 2, 4, 8, 12, 24 hour. Patient serum creatinine level was monitored daily after ICU admission. AKI was defined by applying the Kidney Disease: Improving Global Outcomes (KDIGO) classification.

Results: Out of the 42 patients, 28 individuals experienced AKI based on the KDIGO definition. NGAL, TIMP-2, and combined markers demonstrated modest AUC of 0.64, 0.68 and 0.66, respectively. When we examined those who enrolled into ICU within 24 hours from emergency department (n=31), the AUC of NGAL, TIMP-2, and NGAL x TIMP-2 were improved to 0.83, 0.77, and 0.86, respectively. Analysis of other parameters, including sensitivity, specificity, positive and negative predictive values from both biomarkers were equivalent to published data on NGAL and TIMP-2 [4,5].

Conclusion: In addition to identifying AKI in subgroup of ICU patients, this study illustrated that TIMP-2, similar to NGAL, also have strong predictive value of AKI in the general ICU patients who enrolled into ICU within 24 hour from emergency department. The data also supports future exploration of the clinical utility of NGAL and TIMP-2 in patients at risk for AKI in the emergency department using point-of-care testing.

B-256

Validation of a hand-held point of care device for lactate in adult and pediatric patients: analytical and clinical considerations for use in the pre-clinical setting

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Background: Sepsis and trauma are leading causes of mortality in adults and children in the US and worldwide. Delayed diagnosis and treatment are associated with significant mortality and there is a need for prognostic biomarkers in early disease. Advanced stages of these conditions are characterized by poor organ perfusion and elevated lactate. The pre-hospital use of lactate by emergency medical systems (EMS) could enhance the process of triaging patients in need of specialized care and decrease morbidity. The availability of handheld devices for lactate measurement makes such studies feasible but analytical validation is needed in the context of the intended use. Objective: We aimed to evaluate the analytical performance of the StatStrip Lactate Xpress Hospital Meter (Nova Biomedical, NJ) in samples from critically ill adults and children. Methods: This study used residual heparinized arterial and venous whole blood samples from critically ill adults (n=50) and children (n=50) to compare the test method to the Radiometer ABL 800 (Radiometer, Denmark), which was used as the reference method in two independent hospitals. Statistical analyses consisted of linear regression and difference plots. The bias observed was evaluated in the context of risk stratification and impact in serial measurements. Results: The ranges of concentrations in the ABL800 were 1.0-17.5 and 0.6-18.5 mmol/L in the adult and pediatric populations, respectively. Linear regression analysis between the StatStrip and the ABL800 resulted in slopes of 0.806 in adult and 0.718 in pediatric samples. The mean bias ± SD (% bias) between methods was −0.6 ± 0.7 (18.1%) in adult and -0.6 ± 1.1 (21.1%) in pediatric samples. For both populations, the StatStrip Lactate Xpress method demonstrated a negative bias, proportional with increasing lactate concentrations. Lactate underestimation in the StatStrip Lactate Xpress method was most dramatic >5 mmol/L. There were some discrepancies between methods when samples were classified into 3 risk categories defined using evidence-based cut-offs for sepsis and/or trauma as follows: low (≤2.0 mmol/L), intermediate (2.1 - 3.9 mmol/L) and high (≥4 mmol/L). One pediatric and three adult samples classified as intermediate risk by Radiometer were classified as low risk by the Nova meter. Three adult samples classified as high risk by Radiometer were classified as intermediate risk by the Nova meter. At lactate concentrations ≥4 mmol/L, results between the methods were 10% to 55% different. Conclusions: For concentrations ≤5 mmol/L, which comprise the cut-offs for medical decisions in sepsis and trauma, the StatStrip Lactate Xpress Hospital meter showed a slight low bias but overall acceptable comparability with the ABL800. The negative bias above >5mmol/L was >20% in the test method. The discrepant risk classification observed in our study suggests that pre-hospital stratification models must be derived from data generated using the field method. Since the two methods studied demonstrated intrinsic bias, measurements of patient samples across these methods to determine lactate clearance will be misleading. The meter evaluated here is not currently approved by the US Food and Drug Administration, but its compact size and ease of use warrants evaluation for lactate measurement by EMS in the pre-hospital setting.

B-257

Demonstration of a Urine Dipstick Control in a Revolutionary Single-Use Pouch with Extended Room Temperature Stability Ideal for Point-Of-Care Testing (POCT).

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Background: Since refrigeration is not always available near the site of patient care, many point-of-care test (POCT) devices are designed to be stored and operated at room temperature (RT). Quality control materials that are used to verify the performance of the POCT devices would ideally also have extended RT stability. Urinalysis using multi-analyte dipsticks is one of the most commonly performed POCT. These dipsticks contain reagent pads to assay analytes such as bilirubin, blood,

creatinine, glucose, hCG, ketones, leukocytes, microalbumin, nitrite, pH, protein, specific gravity, and urobilinogen in a urine sample. They are typically stored at RT and require testing with control materials to verify performance. An ideal quality control material would therefore have extended RT stability, allow for full dipstick immersion, and be packaged in a single-use design that minimizes contamination risk.

Objective: To formulate a two level urine dipstick control in a single-use thermoplastic pouch with RT stability of at least 90 days.

Methods: Two levels of a simulated urine control were formulated using proprietary stabilizers and excipients. Level 1 was formulated to test as negative/normal while Level 2 formulated to test as abnormal/elevated for bilirubin, blood, creatinine, glucose, hCG, ketones, leukocytes, microalbumin, nitrite, pH, protein, specific gravity, and urobilinogen. Using a custom form-fill-seal machine, 1.5 mL was dispensed into single-use thermoplastic polymer pouches. RT stability was evaluated by maintaining pouches at 25ºC for up to 60, 90, and 120 days while real-time stability will be followed for up to 3 years at 2-8ºC. SG Roche Chemstrip® 10MD, Siemens Multistix® 10SG, McKesson 10SG, and Henry Schein Urispec® 11-way dipsticks were used to evaluate bilirubin, blood, glucose, ketones, leukocytes, nitrite, pH, protein, specific gravity and urobilinogen. Siemens Clinitek® Microalbumin 2 dipsticks were used to evaluate creatinine and microalbumin. Quidel QuickVue® cartridges were used to evaluate hCG. All tests were performed in triplicate.

Results: All tests performed on Level 1 maintained the appropriate negative/normal level for every analyte and every test method for up to 120 days at 25ºC and up to 600 days at 2-8ºC. All tests performed on Level 2 maintained the appropriate abnormal/elevated level for every analyte and every test method for up to 120 days at 25ºC and up to 600 days at 2-8ºC, with only one exception of the Urispec dipsticks failing to detect ketones only after 120 days at 25ºC.

Conclusion: The RT stability of at least 90 days for all analytes across several urine dipsticks exceeds the RT stability of all other urine controls formulated with native ketones on the market. The pouch design allows the user to visually verify that the dipstick is fully immersed into the control solution. The single-use feature minimizes the risk of contamination while the slim pouch design minimizes the volume required for testing. Lastly, the elongated rigid pouch design allows for a full dipstick immersion which directly simulates the dipping method utilized on patient samples. All of these features make the new Quantimetrix urinalysis dipstick control in the revolutionary single-use pouch the ideal companion for urinalysis POCT.

B-258

Lactate, procalcitonin, white blood cell, neutrophil and immature granulocyte count as biomarkers of sepsis, and severe sepsis or septic shock

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Background: We compared lactate, procalcitonin, white blood cell (WBC), neutrophil, and immature granulocyte (IG) count for the prediction of sepsis, and severe sepsis or septic shock in patients presenting to the Emergency Department (ED) with suspicion of sepsis.

Methods: We prospectively enrolled 501 patients presenting to the ED at St Marys Hospital (Rochester, MN) with suspected sepsis who had a sepsis panel (includes lactate, WBC and neutrophil count) ordered. WBC, neutrophil, and IG were measured on a Sysmex XT-2000i analyzer (Sysmex America) and lactate was measured by i-STAT (Abbott Diagnostics). Procalcitonin was later measured (Brahms Kryptor; Thermo Scientific) using frozen (-70C) EDTA plasma from the initial sepsis panel sample. Patients were classified as having sepsis if within 24 hours (of sepsis panel) they had 2 or more of the following: WBC>12 or <4, respiratory rate >20 (two consecutive measurements) or pCO2<32 mmHg, temperature>38C or <36C, or heart rate>90 (two consecutive measurements); ***and*** either a positive sterile site culture or suspected site of infection noted in ICU transfer or discharge records. Severe sepsis was defined as sepsis ***and*** development of blood lactate >4mmol/L or increased creatinine >0.5mg/dL within 5 days of sepsis panel. Septic shock was defined as severe sepsis ***and*** systolic blood pressure <90mmHg despite 30mL/kg fluid resuscitation. Univariate ROC sensitivity analysis was performed to determine the odds ratio (OR) and AUC for prediction of sepsis (no sepsis vs. any sepsis), and prediction of severe sepsis or septic shock (no sepsis or sepsis vs. severe sepsis or septic shock). Recursive partitioning and multivariable ROC analyses were used to determine whether a multi-marker strategy provided benefit over any single biomarker.

Results: There were 267 patients without sepsis, 199 patients with sepsis, and 35 patients with severe sepsis or septic shock. Lactate had the highest OR (1.44, 95th% CI 1.20-1.73) for sepsis prediction; while WBC, neutrophil number and percent (neutrophil/WBC) had OR >1.00 ($p<0.05$) by univariate ROC analysis. IG number and percent (IG/WBC) and procalcitonin had OR that did not differ from 1.00 ($p>0.26$). Lactate, WBC, and neutrophil number/percent did not differ significantly in AUC (0.59-0.69), or optimal cut-off sensitivity (55-63%) or specificity (57-63%) for prediction of sepsis. Multi-marker models could improve either sensitivity or specificity of sepsis prediction (but not both) compared to any single biomarker.

Initial lactate was the best biomarker for predicting severe sepsis or septic shock, with an odds ratio (95th% CI) of 3.07(2.29-4.11) and AUC 0.88(0.81-0.96). At the optimal cut-off (1.9 mmol/L), lactate had a sensitivity 82.9(67.3-91.9)% and specificity 81.4(77.6-84.7)% for severe sepsis or septic shock. Multi-marker models did not improve the AUC, sensitivity, or specificity for prediction of severe sepsis or septic shock over lactate alone. In a subset of patients (n=182) who had positive sterile site cultures, lactate remained the best predictor of severe sepsis or septic shock.

Conclusion: Lactate was the best biomarker for prediction of severe sepsis or septic shock in patients presenting to the ED. Lactate, WBC, and neutrophil (count and percent) all had some value in predicting sepsis.

B-259

Comparison of Cardiac Troponin Specificity between the Stratus CS Acute Care Diagnostic System and the VITROS 5600 Integrated System

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Background: This study compared the clinical specificity for troponin I of the Stratus® CS Acute Care™ Diagnostic System from Siemens Healthcare Diagnostics and the Ortho Clinical Diagnostics VITROS® 5600 Integrated System used in emergency departments. Given troponin's high myocardial specificity and sensitivity, it is recognized as the preferred biochemical marker for myocardial damage. The results of cardiac troponin testing serve as guidance for intervention; cardiac troponin l measurements can be used as an aid in diagnosis of acute myocardial infarction (AMI) and in risk stratification of patients with acute coronary syndrome (ACS).

Method: A comparison study was conducted at the Community Hospital of the Monterey Peninsula comparing the VITROS 5600 system to the Stratus CS system. Random specimens with results on the VITROS 5600 system that fell specifically within the clinical decision window for observation or further work-up (between 0 and 0.10 ng/mL) were immediately run in parallel on the Stratus CS system and the values compared. A "positive" result is defined as one above the manufacturer's 99th percentile upper reference limit (URL) (0.034 ng/mL for the VITROS 5600 system and 0.07 ng/mL for the Stratus CS system), whereas "negative" is below the URL. A random chart review was performed on a subset of discrepant results (over 50%, selected randomly) by a pathologist to assess for further work-up, prolongation of hospital stay, and the presence or absence of acute myocardial infarction.

Results: 12 specimens were excluded from comparison because they were high and obviously positive for acute MI. 34 of 47 (72%) and 5 of 47 (11%) of the remaining specimens were positive on the VITROS 5600 and Stratus CS instruments, respectively. All specimens positive on the Stratus CS system were also positive on the VITROS 5600 system. Thus, 29 of 34 results (85%) were discrepant (all positive on the VITROS 5600 system and negative on the Stratus CS system). Review of the charts on 16 of the 29 discrepant results showed further work-up and prolonged hospital stays but no evidence of acute myocardial infarction in any of the cases (false-positive results).

Conclusions: The findings suggest a significantly higher rate of false-positive results with the VITROS 5600 system and support the argument that the Stratus CS instrument has a higher positive predictive value and specificity than the VITROS 5600 system, which may eliminate additional costly follow-up studies and time in the hospital. The higher specificity does not appear to come at the cost of lower sensitivity.

Tracker artifact #A91DX-POC-150325-GC1-4A00

B-260

Icon Norma Hematology analyzer compared with Sysmex XE-2100; a preliminary evaluation

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Background. Complete Blood Count (CBC) in POCT in the past was less success compared to chemistry or gas analysis, since the marketed analysers were time

consumers showed less effective performance and yielded results less comparable to those provided by the analyzers used in main laboratory. Now a new generation hematology analyzer (based on volumetric impedance and microfluidics) is available: the aim of the study was to compare the analytical performance of the new hematology analyzer Icon to the XE-2100 Sysmex analyzers used in our laboratories.

Methods. We measured Red Blood Cells (RBC), Hemoglobin (Hb), Hematocrit (Ht), White Blood Cells (WBC), Platelets (PLT), Mean Cell Hemoglobin (MCH) Mean Cell Hemoglobin Concentration (MCHC) and Mean Cell Volume (MCV) in 105 consecutive samples received by Ravenna Laboratory. The analyses were carried out within one hour using Icon analyzer (Norma, Untertullnerbach, Austria) and XE-2100 (Sysmex, Kobe, Japan). Results were evaluated using Medcalc software (Ostende, Belgium).

Results. Comparison data yielded the following results: 1) RBC: Bland-Altman plot: Mean Difference %: XE-2100-ICON= 0.0 (+1.96=6.6;-6.6); Passing-Bablok regression analysis: ICON= 0.186+0.950 XE-2100; t-test: 0.936 (p=0.351); Wilcoxon test: p = 0.920; correlation coefficient r: 0.9915 (95% confidence interval = 0.9875-0.9942); 2) Hb: Bland-Altman plot: Mean Difference %: XE-2100-ICON= -0.7 (+1.96=6.2;-7.6); Passing-Bablok regression analysis: ICON= -0.368+1.040 XE-2100; t-test: -2.253 (p=0.0264); Wilcoxon test: p = 0.064; correlation coefficient r: 0.9821 (95% confidence interval = 0.9737-0.9878); 3) Ht: Bland-Altman plot: Mean Difference %: XE-2100-ICON = 0.6 (+1.96=8.1;-6.9); Passing-Bablok regression analysis: ICON=1.64+0.945 XE-2100; t-test: 1.995 (p=0.0487); Wilcoxon test: p = 0.068; correlation coefficient r: 0.9789 (95% confidence interval = 0.9691-0.9856); 4) MCH: Bland-Altman plot: Mean Difference %: XE-2100-ICON= 0.7 (+1.96 = 9.1;-7.8); Passing-Bablok regression analysis: ICON= 0.057+0.833 XE-2100; t-test: 2.163 (p=0.033); Wilcoxon test: p = 0.253; correlation coefficient r: 0.9717 (95% confidence interval = 0.9585-0.9807); 5) PLT: Bland-Altman plot: Mean Difference %: XE-2100-ICON=4.0 (+1.96 = 75.5;-83.4); Passing-Bablok regression analysis: ICON= 7,514+0.943 XE2100; t-test: 0.309 (p= 0.757); Wilcoxon test: p = 0.703; correlation coefficient r: 0.9919 (95% confidence interval 0.9881-0.9945); 6) WBC: for values >600 Bland-Altman plot: Mean Difference % XE-2100-ICON= 6.1 (+1.96 = 20.3;-32.6); Passing-Bablok regression analysis: ICON= 0.1614+0.9853 XE-2100; t-test: -2.456 (p=0.0163); Wilcoxon test: p = 0.0371; correlation coefficient r: 0.9899 (95% confidence interval 0.9841-0.9935).

Conclusions. The Icon analyzer not only yields results consistent with those obtained with XE-2100 particularly in RBC, Hb, Ht, PLT (for values > 20,000) counting, but also presents several features very important in a POCT environment such as extremely compact dimension (263 X 206 X 313 mm); limited weight (9.7 kg), high throughput (60 tests/hour), a two mode (open and close vial) functionality requiring respectively 9.8 and 15 uL sample volume, a totally touch screen interface with the operator production of histograms for WBC, RBC and PLT, all the reagents (diluent, lysant, etc) pack a single kit. The Icon is simple and practical to operate, very user friendly for nurses and Medical Doctors and demonstrated particularly suitable for POCT.

B-261

Evaluation of Whole Blood Basic Metabolic Panel Assay with Clinical Samples

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BACKGROUND: A Basic Metabolic Panel (BMP) is one of the most commonly ordered blood tests and it provides physicians with a quick assessment of patient electrolyte and fluid balance, blood sugar level and kidney function. A BMP cartridge based on electrochemical enzymatic creatinine and urea sensors for the GEM Premier analyzer (Instrumentation Laboratory) is currently in development. This is an addition to the electrolytes and metabolites currently offered on the GEM Premier analyzers. The goal of this clinical evaluation is to compare the whole blood (WB) analytical performance of GEM Premier BMP cartridge to the established reference methods.

METHODS: A total of 327 random samples were obtained from various sources (Emergency Department, Radiology, Critical Care Unit, Intensive Care Unit, Cardiothoracic and Outpatient). Both the heparinized WB and the plasma samples were analyzed on the GEM Premier analyzer (IL) with three BMP cartridges over the course of eight weeks. As reference methods, the WB samples were then assayed on a standard GEM Premier 4000 analyzer (IL) for Na+, K+, Ca++, Cl-, glucose, lactate, and hematocrit, the plasma was assayed on the ARCHITECT c8000 analyzer (Abbott Laboratory) for creatinine, BUN, and tCO2. A large portion of the patient samples were tested against the ARCHETECT as the focus was to evaluate the new assays under development.

RESULTS: The WB creatinine, BUN and tCO2 results from GEM Premier BMP cartridge compared well with those obtained from plasma on the reference analyzer across the ranges of the tested samples. Noticeable intercepts of Na+ and Cl- were due to the narrow ranges of samples tested. The correlation of the GEM Premier BMP cartridge vs. references is summarized in Table 1.

CONCLUSIONS: Good correlations were observed between the GEM Premier BMP cartridge and the reference methods. It can provide reliable WB BMP information with quick turnaround time in Point of Care environments.

Table 1. Correlation of the GEM BMP vs. References (a) ARCHITECT (N=665), (b) GEM 4000 (N=121)

Analyte	Slope	Intercept	R^2	Sample Range
tCO2 (a)	0.8622	3.1333	0.920	8-36 mmol/L
Crea (a)	0.9349	0.0806	0.937	0.28-10.32 mg/dL
BUN (a)	0.9024	1.4482	0.989	4-125 mg/dL
Na+ (b)	1.1145	-15.961	0.935	128-150 mmol/L
K+ (b)	0.9499	0.1763	0.984	2.7-5.9 mmol/L
Cl- (b)	0.8347	17.752	0.941	94-119 mmol/L
Ca++ (b)	0.894	0.0497	0.963	0.85-1.31 mmol/L
Hct (b)	1.0431	-0.1532	0.985	15-56 %
Glu (b)	0.9673	1.3615	0.996	9-219 mg/dL
Lac (b)	1.0312	-0.1512	0.995	0.6-14.4 mmol/L

B-262

Performance Evaluation Of Roche Cobas b 221 Point Of Care Blood Gas Analyzers Used In Intensive Care Units

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Background: Blood gas analyzers are important in assessing and monitoring critically ill patients. It is vital for the purpose of patient care to assure that all analyzers installed in the same unit give comparable results. Recently, 6 point of care Roche cobas b 221 blood gas analyzers were installed in neonatal , pediatric and adult intensive care units(ICU).The objective of this study was to evaluate the performance of these analyzers in measuring blood gases and whole blood electrolytes in the ICU setup.

Methods:A total of 60 blood gas samples were evaluated in this study for method comparison for analyzers that were installed in the same units,using heparinized arterial and capillary blood that were collected from adult, pediatric and neonatal ICU patients .Twenty samples were analyzed at each site immediately upon collection. Correlation for blood gases [pH, partial pressure of carbon dioxide(pC02), partial pressure of oxygen (pO2) and whole blood electrolytes [sodium(Na+), potassium(K+), chloride (Cl-),and ionized calcium(Ca2+)] were calculated for each machine. Regression analysis was used for the validation and the allowable systematic error.Precision study was done on quality control solutions at three different level concentrations for each test and coefficients of variation(CVs) were calculated. Linearity was done using 5 different concentrations spanning the analytical range for each test.

Results:All% CVs were consistent with those claimed by the manufacturer for all tests at all three concentration levels.% CVs of level 1 for pH, pC02, p02,Na+; K+,Cl-, and ionized Ca2 were less than or equal to 0.08, 2.72, 8.20,0.93,0.95,1.96,0.98 ,level 2 were less than or equal to 0.06,1.74, 5.38, 0.55,0.53,0.97,1.20,and level 3 were less than or equal to 0.07,2.74,4.51, 0.53,0.74,0.87,1.43 respectively .pH,pC02, pO2,Na+; K+, Cl- and ionized Ca2 were linear over a measured range of 6.87-7.70,12-126 mmHg,24-457 mmHg,90 -173 mmol/L ,2.0-8.8mmol/L,68-131 mmol/L,and 0.42-2.59 mmol/L, respectively. All analyzers located in the same unit showed satisfactory correlation between the results for all tests, the correlation coefficients were ≥0.975 for all tests.

Conclusion: Overall performance of cobas b 221 system was acceptable,it provided reliable results for all tests in all ICUs.

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Harmonization Verification of three Platforms for Assaying Patient Blood Glucose. A Practical Example Using Polynomial Regression Analysis.

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Background: In our hospital patient blod glucose is assayed with three different platforms, namely cobas c-501®, the laboratory method (LM), i-STAT® cartridges and ACCU-CHEK® *Inform*II, the point of care (POCT) methods. Evaluation of the performance of the two POCT methods versus that of the LM gives important information to the physicians regarding the harmonization and interchangeability of the results for patient care. We employed polynomial regression analysis to evaluate bias, linearity, and equality of the regression lines. M***ethods:*** Patient specimens were collected by venipuncture in green top test tubes (Becton-Dickinson) and analyzed with the three methods in parallel and within 15 minutes. The observations were transferred to Minitab® (version 16, Minitab Inc.) statistical software.Since previous experience had shown increase in variance for increasing values of blood glucose as determined with the three platforms, the values obtained with the POCT methods (dependent variables) were regressed against those obtained with the LM (independent variables) using a weighted polynomial regression model with coded variables (1 for ACCU-CHEK® *Inform* II, 2 for i-STAT®). ***Results*:** The weighted polynomial regression equation was: POCT= 1.7 + 0.98 LM + 0.87 POCT coded. The t-test for the beta of POCT methods showed that it was not statistically significantly different from zero (P=0.42). This indicated that there were no statistically significant differences between the regression lines of the two POCT methods. The analysis of the standardized deleted residuals (SDR) showed that they were quasi-normally distributed, that there were six potential outliers (SDR >|3|) and no statistically significant autocorreletion. The leverage and the Cook's distance did not show influential observations. For glucose blood values exceeding 450 mg/dL there was a curvilinear relationship.This was confirmed by the pure error test with data subsetting (P<0.001) and visually supported by the locally weighted scatterplot smoother(lowess) model. The plot of the differences between the POCT methods and the laboratory method showed that for values greater than 60 mg/dL the relative differences were within ±20% for ACCU-CHEK® *Inform*II and within ±10% for i-STAT; for values less than 60 mg/dL 85% (23/27) of the absolute differences were within ± 6 mg/dL. ***Conclusions*:** These results indicated that , for monitoring patient blood glucose,values obtained with the POCT methods within the interval 60-450 mg/dL may be used interchangeably with those obtained with the laboratory method and that values exceeding this interval should be confirmed with the laboratory method. This study also clearly showed that the availability of appropriate statistical software, such as Minitab®, using a multivariable weighted regression model supported by numerical and graphic diagnostic techniques, allowed the direct comparison of two POCT methods with a reference laboratory method.

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Evaluation of Liver Function Tests on Piccolo Xpress Chemistry Analyzer

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Background: Point of care testing (POCT) provides fast turnaround time of test results and easy use of assays for diagnosis and monitoring. We evaluated the analytical performance of the Abaxis Piccolo Xpress point of care chemistry analyzer using the Liver Panel Plus discs (provided by Abaxis) for our pediatric patient population.

Methods: Evaluations were performed on alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), amylase, aspartate aminotransferase (AST), Gamma glutamyltransferase (GGT), total bilirubin (TBIL) and total protein (TP) assays. Intra- and inter-assay precision studies were conducted using two levels of quality control materials as ten replicates. Linearity studies were performed using commercially available verification samples at three concentration levels as five replicates. Comparison studies were carried out on twenty samples using Piccolo Xpress chemistry analyzer and Vitros 5600 analyzer. Interference from hemolysis, lipemia and icterus were performed using nine different patient pool sera. Severe lipemic sample pools were treated with LipoClear to remove lipemia interference.

Results: The percent coefficient of variations (%CV) were less than 5% for intra- and inter-assay precision on all measured assays except TBIL which had %CV of 7.8% and 5.3% at low QC level for intra- and inter-assay precision, respectively. Results of linearity studies showed that all results were linear and accurate within the allowable total error. Results of comparison studies showed that ALP and TBIL did not have significant bias. Among others ALB, ALT, AST and TP had small but significant bias while AMY and GGT had significant bias (>10%) which could be due to differences in methodologies. None of the analytes were affected by hemolysis (p>0.05) up to interferent concentrations of 300 mg/dL. All eight analytes were not affected by icteric interference at concentrations of 7.5 mg/dL and 15 mg/dL. Five analytes (ALB, AMY, GGT, TBIL and TP) were not affected from mild, moderate and severe lipemia. AST and ALT was not affected by mild lipemia while both analytes were affected by moderate lipemia. ALT was the only analyte which was affected from mild lipemia. Treatment with LipoClear abrogated lipemic interference for all analytes except TP.

Conclusions: The analytical performance of the Abaxis Piccolo Xpress chemistry analyzer was acceptable for precision, linearity and interference from common substances. The good performance and capability of analysis for eight analytes using only 100 µL whole blood sample make the analyzer a good alternative for bedside analysis in infectious patients in the pediatric setting, as well as in remote areas.

B-265

Telehealth and Glucose Monitoring in Nursing Homes and Patient's Residence

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Fifteen years ago we proposed point-of-care (POCT) glucose monitoring away from the hospital environment, employing the PC and Internet for data transmission (Clin Chem

2000; 46(6): Suppl, p47, Abstract 22). Our efforts were a failure due to the lack of technical support at both ends, especially since physician offices were not wired at that time for an electronic health record (EHR). In the area of medical technology, the recent buzz words are Telehealth and Cloud (T&C). In simple terms, T & C is a replacement of expensive and bulky computer hardware with software applications that run on less expensive special computers and also meet cloud computing requirements. Realizing the need for increased self-management of diabetes, we have written a software app for this purpose using generic hardware (smart phones, androids, tablets, glucometer, etc.) and supplies (e.g., test strips). We have reduced patient identification errors by restricting the use of a glucometer to one person. If more than one person is using the same glucometer (e.g. nursing homes) the name and date of birth of the patient should appear on the screen for proper identification. For each user, under his or her name and date of birth, the latest prescription medications for diabetic control are also listed.

We have also introduced the concept of an upper limit of glucose (>160 mg/dL) and lower limit of glucose (<60 mg/dL) to modulate the dosage. Another feature of quality control is the repeat assay in case of glucose values > 160 mg/dL or < 60 mg/dL. The repeat assay must provide glucose results within ± 5 mg/dL. The glucose results are transmitted by a disconnected mechanism (e.g. WiFi) to the user's cell phone. The patient's glucose data is stored in a "Private" cloud using a de-identified mechanism to protect patient's identity. The patient's results are transmitted in real time basis into the EHR at the physician office. All abnormal results, e.g. > 160 mg/dL and < 60 mg/dL are flagged for the review of the physician. A weekly graph of the glucose values for the past 30 days is also provided to the physician for his or her review. Finally we envisage, as for any other medical device and procedure, a proper training of the user.

B-266

Accuracy assessment of three different cartridge lots of the LABGEO PT Hemoglobin A1c Test using reference materials

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Background: Since the point-of-care (POC) hemoglobin A1c (HbA1c) testing plays an important role for monitoring glycemic control and determining treatment strategies in patient with diabetes mellitus, both accurate measurement of HbA1c and minimal reagent lot-to-lot variability are essential for POC HbA1c assay. In this study, the accuracy of three different cartridge lots of the Samsung LABGEO PT HbA1c test (Samsung Electronics, South Korea) was investigated to determine whether the result of the LABGEO PT HbA1c test can be used for follow-up and screening of diabetes patients.

Methods: The Samsung LABGEO PT10 device and three different lots of LABGEO PT HbA1c test cartridge were used. A total of 7 levels of reference materials, 3

levels of certified reference materials and 4 levels of in-house reference materials, were measured using each cartridges in duplicate manner for three days. The bias (measured value minus reference value), within-laboratory precision, and total error were calculated, and medical-decision point analysis was performed.

Results: The mean bias was 0.23 for lot number 1, 0.11 for lot number 2 and0.06 for lot number 3. The mean percent bias was 3.3% (range, 0.7% to 5.0%) for lot number 1, 1.5% The correlation coefficient of lot number 1, 2 and 3 was 0.989, 0.987 and 0.977, respectively. The mean absolute bias (%), coefficient of variation (%) and total error (%) of each cartridge were 3.3%, 2.5% and 8.1%, respectively, for lot number 1; 1.9%, 2.6% and 7.1%, respectively, for lot number 2; and 2.7%, 2.8% and 8.1%, respectively, for lot number 3. The predicted value (95% confidence interval) of each cartridge at 6.5% of HbA1c was 6.74 (6.66-6.83) for lot number 1, 6.60 (6.51-6.70) for lot number 2, and 6.51 (6.39 -6.63) for lot number 3.

Conclusions: The performance of the LABGEO PT HbA1c Test showed within ±6.0% of bias, with less than 3.0% of imprecision and 9.0% of total error. In addition, the lot-to-lot variability of the LABGEO PT HbA1c Test was negligible. Therefore we thought that the LABGEO PT HbA1c Test could be used for monitoring of diabetic patients, and for diabetes screening with a false positive result in the doctor's office.

B-267

Development and Validation of a Paper-based analytical disc assay for rapid and multiplex blood typing

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Background:

Rapid blood typing plays a critical role in routine and emergency healthcare, but no point-of-care (POC) strategies are commercially available despite various blood typing tests have been put forward over 100 years.

Methods:

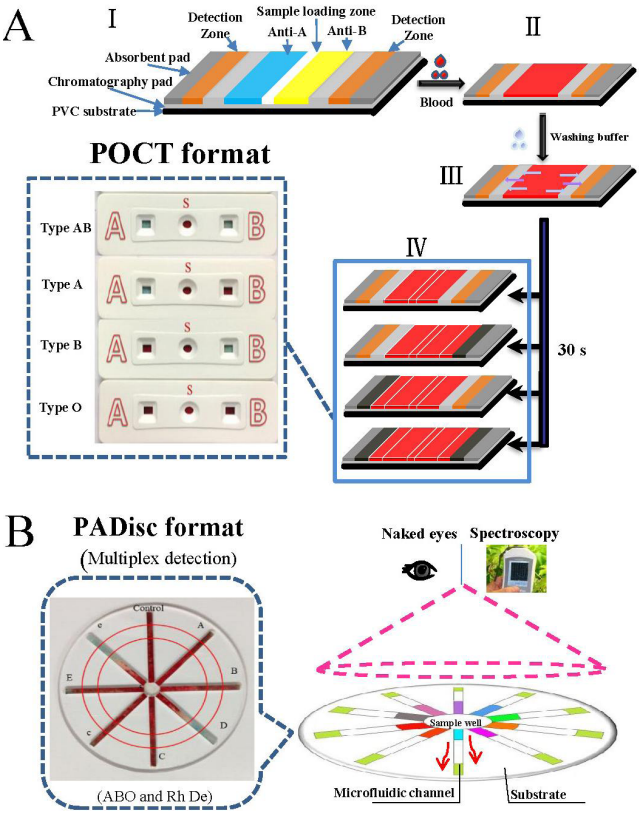
We proposed a simple, rapid, and reliable paper-based analytical disc (PADisc) assay for high-throughput determination of multiple antigens in ABO and Rhesus (Rh) blood systems. The PADisc embedded a series of disposable lateral flow strips on which both bromocresol green (BCG) and individual IgM antibody were immobilized. Only 100 μL fresh or anti-coagulated whole blood was needed for simultaneous typing of two antigens (A/B) in the ABO system and five antigens (D/E/C/c/e) in the Rh system. After rinsing using 50 μL washing buffer, any uncongulated erythrocytes and plasma would migrate to the test pad and interact with coated BCG to induce a color change (blue for plasma only and taupe for the whole blood), by which the distinct blood types could be visualized using naked eyes or spectroscopy.

Results:

No significant variations on the analytical results have been observed from moderate hemolytic samples in a wide haematocrit range (20.8%-76.7%), revealing its adaptability in various population. More importantly, the analytical specificity was guaranteed by taking BCG as inner quality control because any protein-free solution could be effectively discriminated. Additionally, automation is enabled by developing a portable three-wavelength spectrometer (560, 600, and 630nm) to discriminate albumin from whole blood according to their distinctive absorption peaks. Compared to gel column assay, the proposed PADisc assay demonstrated 100% accuracy in detecting 600 clinical blood samples (480 ABO and 120 Rh D/C/c/E/e) including 70 weak agglutination samples, verifying its analytical accuracy and applicability.

Conclusion:

The proposed PADisc assay permits simultaneous grouping several blood types within 1 min, brings approaches for improved emergency medicine and military medicine.



B-268

Roche Accu-Check® Inform II Blood Glucose Meter Reduces Maltose Interference and Risk to Patient Safety

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Background: Roche Accu-Check® Inform II blood glucose test strips were developed to replace Comfort Curve strips previously reported to give falsely elevated glucose levels in patients treated with maltose-containing or maltose-producing therapies e.g. some immunoglobulin preparations or peritoneal dialysis solution containing icodextrin. Strip chemistries susceptible to maltose interference can be misinterpreted and risk inappropriate insulin dosing and subsequent consequences of hypoglycemia. Inform II uses a variant of quinoprotein glucose dehydrogenase (Mut. Q-GDH enzyme) to eliminate maltose interference.

Objective: Verify that Inform II does not suffer interference from maltose up to 360 mg/mL and confirm assay performance compared to UniCel DxC800 (Beckman Coulter) analyzer used in our laboratory.

Methods: Clinical studies showed that plasma maltose concentration of up to 600 mg/dL could be expected in some individuals. We used plasma (lithium heparin) at low (46 mg/mL), medium (145 mg/mL), and high (243 and 364 mg/mL) glucose concentrations. To 1 mL of each sample, maltose powder was added to obtain the final concentrations of 280, 630, 990 mg/dL of maltose. Glucose concentrations were measured with Inform I & Inform II meters and Beckman DxC800, which is not subject to maltose interference.

Results: The difference in glucose concentrations between maltose-containing and maltose-free samples in patient specimens measured by the Beckman DxC800 glucose oxidase, Inform I, and Inform II methods are summarized below:

Initial Glucose concentrations with Inform I, Inform II, and DxC800, mg/dL	Maltose added, mg/dL	Glucose with DxC800, mg/dL	Glucose with Inform I, mg/dL	Glucose with Inform II, mg/dL	Δ Glucose with DxC800, mg/dL	Δ Glucose with Inform I, mg/dL	Δ Glucose with Inform II, mg/dL
Patient A (44, 51, 46)	A + 280	44	143	56	2 (5%)	99 (69%)	5 (9%)
	A + 630	43	268	71	3	224	20
	A + 990	44	431	80	2	387	29
Patient B (147, 159, 145)	B + 280	149	263	163	4 (3%)	116 (44%)	4 (2%)
	B + 630	149	388	174	4	241	15
	B + 990	150	529	182	5	382	23
Patient C (219, 227, 243)	C + 280	250	349	218	7 (3%)	130 (37%)	9 (4%)
	C + 630	255	516	233	12	297	6
	C + 990	252	qns	253	9	qns	26
Patient D (359, 417, 364)	D + 280	367	469	423	3 (1%)	110 (23%)	6 (1%)
	D + 630	371	qns	432	7	qns	15
	D + 990	370	qns	441	6	qns	24

Conclusions: No clinically significant interference by maltose up to 360 mg/mL was found with Inform II strips, confirming the manufacturers' claim. Maltose > 360 mg/mL increased glucose readings on Inform II, but the increase likely will not change the clinical decision to control blood glucose levels in these patients.

Recovery of glucose after spiking was consistent with CLSI EP7-A2 (< +/-10 mg/mL at glucose <100 mg/mL and < +/-10 % at glucose >100 mg/mL).

B-269

A point-of-care experience with GeneXpert MTB/RIF assay performance in detecting Mycobacterium tuberculosis and rifampicin resistance.

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Background:

Tuberculosis (TB) remains a major threat to public health, exacerbated by the emergence of multiple drug-resistant (MDR), defined as resistance to the first line anti-TB drugs such as isoniazid and rifampicin (RIF). About 9.4 million new tuberculosis cases and 2 million TB-related deaths occur every year. Rapid TB diagnosis and drug-resistance detection is important to reduce TB-related morbidity and mortality, especially in regions where TB is endemic.

Objective:

To evaluate the GeneXpert MTB/ RIF assay as a rapid point-of-care diagnosis of TB in a private hospital in São Paulo, Brazil, from July 2014 to January 2015.

Methods:

Clinical specimens from patients suspected to have TB were analyzed by GeneXpert assay at the site hospital laboratory. The test was used in hospitalized patients as the initial test for TB diagnosis with a turnaround of two hours. The GeneXpert MTB/ RIF assay (Cepheid, Sunnyvale, CA, USA) uses real-time PCR for simultaneous detection of *Mycobacterium tuberculosis* (MTB) *rpoB* gene and mutations associated with rifampicin resistance.

Results:

A total of 55 samples were tested, including 41 respiratory (sputum and bronchoalveolar lavage) and 14 other clinical specimens (biopsies, urine, synovial liíquido, pleural fluid, breast secretion and other). A total of 8 samples were positive - 5 respiratory, 2 bone biopsies and 1 synovial fluid specimen. Two specimens (one bronco alveolar fluid and one sputum) detected by GeneXpert were also positive by both smear microscopy and mycobacterial culture. Mutations associated with rifampicin resistance was not detect in any of the studied samples.

Conclusion:

GeneXpert MTB/RIF assay is more sensitive than smear microscopy and culture for detection of TB in respiratory specimens. Considering little hand-on time, easy to perform, easy to implement, point-of-care capability, the GeneXpert MTB/RIF assay is an excellent option for TB diagnosis. Rapid point-of-care diagnosis is needed to provide a better management of hospitalized TB patients and infection control procedures at the hospital setting. Investment in the TB diagnostics pipeline should remain a major priority for health care funders.

B-270

Comparison of Two Methods for Monitoring Compliance and Thoroughness of Glucose Meter Disinfection Practices

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Background:

Improper use of blood glucose test systems in healthcare environments has been linked with hepatitis B virus infection outbreaks in the United States. In 2010, the Centers for Disease Control and Prevention (CDC) released recommendations for cleaning and disinfecting glucose meters after every use if a glucose meter cannot be dedicated to a single patient; moreover, recommends periodic assessment of user compliance with disinfection practices. Previous studies have highlighted advantages and limitations in methods for monitoring disinfection practices in environmental health services; however, limited studies have compared methods for monitoring disinfections practices in point-of-care (POC) testing services. Fluorescent gel markers are used routinely to covertly monitor environmental cleaning in healthcare. With technological advances, select glucose meters can electronically capture user comments upon test result verification. The aim of this study was to compare fluorescent gel marker versus user entry of a "disinfection" comment for monitoring compliance and thoroughness of glucose meter disinfection practices within 1 institution.

Methods:

Study inclusion criteria included trained users of StatStrip glucose meter (Nova Biomedical, Waltham, MA) who performed routine patient testing within 7 wards at the University of North Carolina Hospital between October-November, 2014. All users (n>400) were trained to enter a "disinfection" comment within the glucose meter upon patient result verification, with immediate device disinfection using a Super Sani-Cloth (Professional Disposables International, Inc, Orangeburg, NY). Comments entered in glucose meters were automatically transmitted to Telcor data management system (TELCOR, Inc., Lincoln, NE) upon device docking. On 12 random days within the study period, 12 glucose meters located in the 7 wards were each covertly marked with Dazo Fluorescent Marking gel (Ecolab, St. Paul, MN) in 2 places; near the test-strip port and on the "OK" button on the device surface. Following a single patient test, the glucose meters were covertly examined for presence/absence of fluorescent marker using a black-light and (non)entry of the disinfection comment using Telcor software. Glucose meters not used for patient testing or performed > one patient test during the observation were excluded; 50 observations from 36 users performing routine testing were included in analyses. The % disinfection compliance rate using fluorescent marker=(# devices with ≥1 marker removed/50)*100. Disinfection thoroughness (%) =(# devices with both fluorescent markers removed/# devices with ≥ 1 marker removed)*100. The % disinfection compliance rate using "disinfection" comment =((#"disinfection" comment entries/50)*100).

Results:

98% compliance with glucose meter disinfection was captured using the "disinfection" comment method. Using the fluorescent marker approach identified 64% disinfection compliance, of which, 59% demonstrated thoroughness in glucose meter disinfection practices.

Conclusion:

Monitoring glucose meter disinfection using user entry of a "disinfection" comment versus covert fluorescent marker observation demonstrated poor concordance in compliance rate. Reasons for the discordance remain unclear. A limitation of the "disinfection" comment method is inability to assess the thoroughness of glucose meter disinfection, whereas the fluorescent marker method demonstrated poor thoroughness in disinfection practices within our study cohort. Altogether, this study highlights the need for active monitoring of glucose meter disinfection practices to proactively minimize risk of infection transmission.

B-271

Determination of Analytical Performance Characteristics of the RAMP® D-dimer Test

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Background: The RAMP® D-dimer test is a quantitative immunochromatographic test for the determination of the fibrinogen degradation product (FDP) D-dimer in human EDTA anti-coagulated whole blood. D-dimer is considered to be a marker of coagulation activation and is present in the circulation as part of the normal wound healing process. It is also valuable as a diagnostic marker for Disseminated Intravascular

Coagulation (DIC) and as an aid to the rule-out of Venous Thromboembolism (VTE), a spectrum of diseases that include Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE). The objective of these studies was to determine the analytical performance characteristics of the RAMP D-dimer test.

Methods: Detection limits, linearity, hook effect, repeatability, total precision, interference and cross-reactivity were determined for the RAMP D-dimer test according to methods outlined in CLSI guidelines, where applicable, using either plasma-based controls or EDTA whole blood samples.

Results: The limit of blank (LoB) and limit of detection (LoD) were determined to be 51 ng/mL FEU and 89 ng/mL FEU, respectively, using methods described in EP17-A. The 20% and 10% limits of quantitation (LoQ) were determined to be 419 ng/mL FEU and 839 ng/mL FEU, respectively, using total error estimates as described in EP17-A. Linearity was determined as per EP6-A on three lots of RAMP D-dimer tests, meeting a goal for total error of ≤ 20% from 100 to 5000 ng/mL FEU. No high dose hook effect was observed up to 250,000 ng/mL FEU.

Repeatability and total precision were determined as per EP5-A2 by testing three levels of frozen plasma control materials in duplicate, twice per day for 12 days on three lots of RAMP D-dimer tests. Repeatability and total precision CVs were 6.6 and 8.5% at 363 ng/mL FEU, 5.4 and 6.3% at 656 ng/mL FEU and 6.5 and 6.9% at 4044 ng/mL FEU, respectively. Repeatability and total precision were also determined for whole blood samples by testing three levels in triplicate, in five runs over three days on three lots of RAMP® D-dimer tests. Repeatability and total precision CVs were 19.7 and 22.6% at 174 ng/mL FEU, 10.3 and 12.3% at 465 ng/mL FEU and 7.8 and 10.0% at 2753 ng/mL FEU respectively.

As per EP7-A2, no interference was observed in the RAMP D-dimer test as the result of hemoglobin (200 mg/dL), bilirubin (conjugated 5 mg/dL, unconjugated 15 mg/dL), cholesterol and triglycerides (500 mg/dL each), gamma-globulins (60 mg/dL) or 45 common pharmaceutical compounds (3 x MRTD). No statistically significant cross-reactivity was observed with fibrinogen (1 mg/mL) or fragment E (20ug/mL); a bias of 0.009 ng/mL per unit was observed for fragment D.

Conclusion: The RAMP D-dimer test demonstrated acceptable analytical performance for the quantification of D-dimer, based on methods outlined in applicable CLSI guidelines.

B-272

Analytical validation of MRSA detection test through GeneXpert®.

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Background:

Healthcare-associated-infections affect hundreds of millions of patients worldwide every year and represent an important cause of morbidity and mortality. Methicillin-resistant *Staphylococcus aureus* (MRSA), now endemic in many healthcare facilities, is a leading cause of healthcare associated infections; patients in intensive care and surgical units are at increased risk for MRSA infections due to factors such as invasive procedures, antibiotic exposure and prolonged healthcare contact. Controlling MRSA is a primary focus of most hospital infection control programs. Currently, the standard surveillance method for detecting MRSA is culture, which is very laborious and time intensive. A rapid and more sensitive method for active surveillance of MRSA will represent a definite advantage for infection control programs.

Objective:

Validate the Xpert® MRSA assay (Cepheid) to identify MRSA using two comparative conventional tests: PCR and phenotypic identification by Vitek®2 (bioMérieux).

Methods:

Validation experiments were conducted with ten available laboratorial strains of MRSA previously detected by two conventional validated tests: phenotypic identification by Vitek®2 and an in-house real-time reaction. The GeneXpert Dx System is a polymerase chain reaction-based method for identifying *S. aureus* and methicillin resistance using an automated system. The system requires the use of single use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. The GeneXpert MRSA assays were performed according to the manufacturer's package insert, and results were interpreted directly from the report generated by the GeneXpert instrument. In-house PCR was based on the identification of mecA gene.

Results:

Among 10 available laboratorial strains, the Xpert® MRSA test showed agreement of 90% (within in-house PCR and Vitek®2 results). All MRSA strains identified by Vitek®2 (5/10) were suggestive of *mec*A, in accordance with in-house PCR results. One strain fell on the validation procedure, while the laboratory partner and Vitek®2 system had a negative result to MRSA and GeneXpert had a positive result. The turnaround time of GeneXpert was faster compared with the other tests, taking 69 minutes from beginning to result.

Conclusion:

We found that GeneXpert Dx System had a good performance to identify MRSA compared to in-house PCR and Vitek and it was a rapid and accurate tool with a good turnaround time. The good sensibility and faster speed in delivering the final results has a potential benefit in clinical practice, permitting to reduce length of stay, unnecessary hospital admissions and antimicrobial therapy. Also, GeneXpert can optimize effectiveness of infection control programs.

B-273

Linearity Standards for Automated Urinalysis Systems

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Background: Currently urinalysis systems are not required to undergo calibration verification, reportable range, and new instrument performance validation using linearity standards. Linearity standards have long been used to perform calibration verification, reportable range, and new instrument performance validation of quantitative methods in the clinical laboratory as required by CLIA 88 regulations. Traditionally, linearity standards were not required for urinalysis because it was performed visually by operators using urine test strips. Increasingly, urinalysis is performed by automated and semi-automated instruments, but the use of linearity standards for these devices has not yet been incorporated into standard practice. As the clinical laboratory environment becomes more regulated, requirements for linearity standards testing may be applied to urinalysis systems in the future. This study is designed to show that linearity standards can be formulated for urinalysis systems and that they can be used for calibration verification, reportable range, and new instrument performance validation of these systems. **Method:** Liquid ready to use linearity control solutions were prepared at Quantimetrix Corporation from human urine with added reagents to simulate positive human samples for each of the analytes on the Multistix® 10 SG strips; leucocytes, nitrite, urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin, and glucose. Analyte concentrations were targeted to the mid-point of each test pad on a Siemens Multistix10 SG reagent strip. Each linearity standard was tested by performing ten measurements on four different Siemens Clinitek Status® or Status Plus readers, for a total of 40 measurements per test point. Testing was performed as per the Siemens Multistix 10 SG and Siemens Status or Status Plus operating instructions. **Results:** All standard solutions met the requirements of the study 1) ≥70% of the results were on the targeted pad 2) no test results greater than 1 pad away from the targeted pad. **Conclusions:** The solutions prepared in this study produced results on the Multistix 10 SG and Clinitek Status system that effectively demonstrated calibration verification and reportable range for the test instruments. Similar linearity standard sets could be developed for other urinalysis systems in the future.

B-274

An RNA Aptamer that Specifically Binds to Mycolactone and Serves as a Diagnostic Tool for Buruli Ulcer

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Background: Buruli Ulcer Disease (BUD) is a neglected tropical disease caused by *Mycobacterium ulcerans*. It is the third most common mycobacterial disease after tuberculosis and leprosy. It is characterized by extensive destruction of skin and soft tissues with the formation of large ulcers usually on body extremities. BUD is often diagnosed late and molecular methods for confirmation are unavailable outside of major reference laboratories. Mycolactone, the virulence factor responsible for the pathology of BU is present in BUD infected human tissue. It is widely distributed within a lesion. It can also be found at every stage of the disease and thus, a useful marker for diagnosis. However, the chemical nature of mycolactone as a poor immunogenic lipid molecule has impeded efforts to produce a diagnostic based on an anti-mycolactone. With the advent of antibiotic therapy it is even more imperative for early diagnosis of BUD for effective treatment to prevent the morbid effects of

the disease. Aptamers, a novel sensitive and specific class of detection molecules, has hitherto not been raised against mycolactone.

Methods: The ribozyme hammerhead was used as a template for aptamer selection using the systemic evolution of ligands by exponential enrichment (SELEX) process. Aptamers were selected against target mycolactone over counter-target comprised of cellular protein extract. A given round of selection began with incubating library members in the buffer alone (negative selection), then collecting the portion of the library that did not respond (i.e. cleave). Each round finished with a positive selection against mycolactone in buffer, where the non-cleaving material collected from the prior step was incubated with the target and the responsive (i.e. cleaved) material was then collected to regenerate the library for the next round. A counter-selection step was included between the negative and positive selections. Each selection round was followed by reverse transcription to generate cDNA, library amplification through PCR, and regeneration of the RNA library by transcription. After subjecting the initial library of diverse random sequences to 7 consecutive rounds of selection, the enriched library was divided into three fractions to perform the parallel assessment after which the three clones were sequenced. Bioinformatics analysis of the sequencing data identified candidate aptamers. To assess their affinity and specificity to the mycolactone, aptamers were screened using enzyme linked oligonucleotide assay (ELONA). The most promising aptamer was evaluated using samples obtained from 20 BUD patients and healthy volunteers. Cut-points were evaluated using ROC curve analysis.

Results: 197007 sequences were analyzed from the positive target-exposed library. From this set of data, 6859 sequence families were constructed containing between 10 and 583 members each. Five RNA aptamers specific to mycolactone were identified, their structure predicted by the M-fold program, based on Zuker algorithm. Their dissociation constant (kd) values were in the nano-molar range. One aptamer, designated NM209D was evaluated using swab and needle aspirate samples. NM209D had sensitivity and specificity of 100% and 65.9%.

Conclusion: Ribozyme based RNA aptamers specifically binds to mycolactone and can serve as a diagnostic tool for early diagnosis of BUD

B-275

Inaccurate Point-of-Care Glucose in a Critically Ill Patient due to Norepinephrine Administration

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Background: In light of the recent CMS warning that use of waived blood glucose measurement systems (BGMS) on critically ill patients is "off label" and the follow-up CAP requirement for hospitals to define "critically ill" and the use of BGMS in these patients, we evaluated the possible causes of undetectable capillary point-of-care glucose (POC) in a 29 y/o African American male who was admitted in the surgical Intensive Care Unit (SICU) following a traumatic brain injury. **Method:** Capillary whole blood POC glucose was measured with the NOVA StatStrip®, now FDA-cleared for use on Intensive Care patients. The StatStrip uses an amperometric, plasma calibrated glucose oxidase method, but results are independent of sample PO_2. Laboratory glucose was measured in venous plasma samples on the Vitros® 5600 by the glucose oxidase method. The bias in glucose results (POC – Lab) was calculated for samples with collection times within 60 minutes of each other. Glucose bias was plotted against arterial PO_2 and pH, systolic blood pressure (SBP), hematocrit and platelet count, and the administration of drugs, including a neuromuscular blocker (cisatracurium), a vasoconstrictor (norepinephrine), and an antiarrhythmic (amiodarone). These graphs were evaluated visually for temporal correlation of changes in glucose bias with the listed potential factors. **Results:** Clinically significant negative bias (-25 to -134 mg/dL) occurred on hospital days 3 and 4. During this time arterial PO_2 ranged from 53 to 230 mmHg without correlation between PO_2 and glucose bias. The arterial pH was low (7.14-7.30) during the period of maximum negative bias, but pH was also low at times when there was no significant bias. SBP was very low (60-100 mmHg) throughout the hospital stay and did not correlate with glucose bias. Hematocrit and platelet count did not correlate with glucose bias. In addition, during the time when all of the discrepancies occurred, 3 different meters were used by 6 different operators. Thus, it appears unlikely that a meter or operator is responsible for the discrepancies. Of the drugs listed above, the only one for which the administration correlated with the glucose bias was norepinephrine. **Conclusion:** Norepinephrine administration accounts for the marked negative glucose bias. Norepinephrine, among other actions, functions as a peripheral vasoconstrictor to raise the blood pressure. This results in markedly reduced capillary blood flow, blood stasis in the capillaries and metabolism of the capillary glucose. Patients who are in shock or otherwise have poor peripheral circulation should not have POC glucose measured on capillary blood.

B-276

Discrepancies in Hematocrit and Hemoglobin Measurements by Point-of-Care (POC) Devices and Laboratory Instruments in Patients with Hemodilution

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Background:

Hematocrit (HCT) and Hemoglobin (HGB) can be measured with multiple systems based on different principles. i-STAT is one of the frequently used POC devices to measure HCT and HBG in hospital settings. For patients undergoing surgeries, HCT and HBG can be skewed due to hemodilution, if the modes for calibration and compensation of hemodilution are not selected on i-STAT. However, bias may still exist when compared with other laboratory systems, even if the right mode is selected for i-STAT. The objective of the study is to determine the accuracy of i-STAT for HCT and HGB measurements in patients undergoing surgeries when K3EDTA/CPB (Cardio Pulmonary Bypass) mode is selected by comparing i-STAT with other laboratory instruments.

Methods:

In i-STAT, HCT was measured based on conductivity of red blood cells and HGB was calculated according to HCT values. Similarly, HCT was measured by Sysmex according to voltage changes produced by cells, but HGB was measured spectrophotometrically by sodium lauryl sulfate reaction with HGB. HCT was calculated based on HGB, which was measured by oximetry in Radiometer. The study was performed using 25 samples from patients undergoing surgeries in an operating room. HCT and HGB were analyzed in duplicate on all systems. Deming linear regression analysis was performed for HCT and HGB results for i-STAT vs Radiometer, i-STAT vs Sysmex, and Radiometer vs Sysmex.

Results:

The Deming linear regression analysis for HCT and HGB between different systems was shown below.

HCT

i-STAT (Y) vs Radiometer (X): Y = 0.84 X + 4.39 (r = 0.92); i-STAT (Y) vs Sysmex (X): Y = 0.99 X + 0.09 (r = 0.92); Radiometer (Y) vs Sysmex (X): Y = 1.14 X - 3.70 (r = 0.98).

HGB

i-STAT (Y) vs Radiometer (X): Y = 0.86 X + 1.68 (r = 0.91); i-STAT (Y) vs Sysmex (X): Y = 0.89 X + 1.29 (r = 0.90); Radiometer (Y) vs Sysmex (X): Y = 1.04 X - 0.44 (r = 0.98).

Conclusions:

i-STAT is in a better agreement with Sysmex than with Radiometer for HCT with a slope of 0.99 and 0.84, respectively. i-STAT has a similar negative bias in HGB values compared to Radiometer and Sysmex with a slope of 0.86 and 0.89, respectively. Radiometer and Sysmex compare well with each other for HGB with a slope 0.98, but Radiometer has a positive bias with a slope of 1.14 for HCT. The discrepancy in HCT and HGB results between i-STAT and other laboratory instruments still exists, even when a correct mode is selected. The bias among multiple systems is most likely due to the difference in methodology, calibration, and calculated versus directly measured parameters. Therefore, it is recommended to use a single method to trend the HCT and HGB in patients undergoing procedures that causes hemodilution.

B-277

Method comparison and bias estimation at clinical decision levels for lactate measurements with ABL80 and ABL90 blood gas analyzers

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Background: Lactate measurement provides relevant information for diagnostic and prognostic assessment of critical patients and is often used as a marker of adverse outcomes. Modern Point-of-Care Testing (POCT) constitutes a useful tool for real-time monitoring of lactate at the patient's bedside. Similarly to ABL90 blood gas analyzer, a new electrode-based biosensors cassette that incorporates lactate measurement was developed for ABL80. The aim of this study was to estimate bias at the medical decision levels in order to establish whether lactate results measured with ABL80 and ABL90 (Radiometer Medical Aps®) are interchangeable.

Material and Methods: Two ABL80 and one ABL90 blood gas analyzers were utilized in the study. For method comparison, ABL90 was considered as the reference

measurement procedure. According to Clinical and Laboratory Standards Institute (CLSI) protocol EP09-A2-IR, 40 whole blood heparinized samples from adult patients were analyzed in replicates. Linear regression including slope and intercept calculation was used in order to estimate bias at the medical decision levels. The allowable bias was established according to desirable total error based on biological variation criteria. Statistical analysis was performed using StatisPro™ software (CLSI).

Results:

	Medical decision levels (mmol/L)	Estimated bias (mmol/L)	95% Confidence interval	Allowable bias (mmol/L)	Allowable bias (%)
ABL80 -1 vs ABL90	1	0.06	-0.14 to 1.48	0.30	30.4
	2	0.31	0.19 to 0.43	0.61	30.4
	4	0.81	0.71 to 0.91	1.22	30.4
	6	1.31	0.89 to 1.25	1.82	30.4
ABL80 - 2 vs ABL90	1	0.10	-0.10 to 0.29	0.30	30.4
	2	0.29	0.16 to 0.43	0.61	30.4
	4	0.68	0.60 to 0.77	1.22	30.4
	6	1.07	0.89 to 1.25	1.82	30.4

Conclusions: The estimated bias for lactate measurements was lower than the allowable bias at different medical decision levels. Patient's results for lactate are interchangeable between ABL80 and ABL90 and both could be used alternatively with no impact on patient care. This could be specially useful at institutions with POCT projects were both analyzers are installed.

B-278

Pre-analytical Implications of Sample Mixing

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Pre-analytical errors make up the vast majority laboratory errors resulting in questionable patient results. (Bonini *et al.,* 2002) Questionable results can result in improper medical intervention or suspicion of analyzer performance. One of the common forms of pre-analytical errors is improper or inadequate sample mixing prior to sampling. Inadequate sample mixing will yield erroneous hematocrit and total hemoglobin patient results and improper mixing may cause hemolysis adversely affecting a patients electrolyte results, mainly potassium.

Common forms of sample mixing include the Rock and Roll method, 8-3 Axis method, and vortex shaking. In order to assess the effects of each of the above methods, preliminary testing was conducted in order to quantify which type of sample mixing, or lack thereof, would cause a clinical significant shift in potassium (±0.25 mmol/L), hematocrit (2%), and/or total hemoglobin (0.35 g/dL). Each one of these methods of mixing, as well as no mixing, was evaluated using GEM Premier® 4000 blood gas analyzers (Instrumentation Laboratory, Bedford, MA). Each of the four methods was evaluated using a 3 cc syringe filled to the final graduation with adult whole blood and allowed to sit on bench for >30 minutes, the results are summarized in Table 1. Per this study, a two sample t-test (α=0.05) on the correlation between the mean bias of each method when compared to initial concentrations. In this experiment, no sample mixing had a clinically significant shift in both hematocrit and total hemoglobin results (Table 1).

Table 1: Summary of the Effectivity of Various Sample Mixing Methods on Method Bias

Analyte	No Sample Mixing	Rock and Roll Mixing	Vortex Shaking	8-3 Axis Mixing
K^+	0.02 p = 0.551	0.00 p = 1.000	0.03 p = 0.263	0.00 p = 1.000
Hct	**6.00** p = 0.000	-0.33 p = 0.343	-0.17 p = 0.667	0.83 p = 0.070
tHb	**1.57** p = 0.000	-0.10 p = 0.403	-0.02 p = 0.895	0.03 p = 0.652

Based on the results, we recommend the Rock and Roll, or 8-3 axis, methods of sample mixing when considering potassium, hematocrit, and/or total hemoglobin as indicators of either inadequate or improper sample mixing.

B-279

Comparison of the Alere i to the BD Veritor in the rapid detection of Influenza A and B viruses

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Background:The use of point-of care-testing (POCT) in patient management decisions is becoming increasingly more common. This trend requires POCT devices to generate accurate and reliable test results and makes the comparison of available testing devices a necessity when deciding which testing platform to adopt. Our goal is to evaluate the diagnostic performance of two commercially available rapid Influenza A+B virus POCT devices, the Alere™ i Instrument (isothermal nucleic acid amplification, Alere, Inc., Scarborough, ME) and the BD Veritor™ System (chromatographic immunoassay, BD Diagnostics, Sparks, MD).

Methods:This ongoing, prospective, multi-site study is a comparison to determine how well each of these testing devices detects Influenza A+B in symptomatic adult patients. A swab is collected from each nostril of symptomatic patients visiting outpatient clinics during the 2014-2015 Influenza season. The Veritor™ is performed onsite in real time, while the additional swab is stored frozen and transported to the central laboratory to be batch-tested using the Alere™ i. Discordant results between the Alere™ i and Veritor™ are resolved using the Simplexa™ Flu A/B and RSV Direct real-time reverse transcription polymerase chain reaction (RT-PCR) assay (Focus Diagnostics, Cypress, CA).

Results:Preliminary results have revealed that there is 85.7% agreement between the Alere™ i and the BD Veritor™ in the detection of Influenza A, while the detection of Influenza B showed 100% agreement between the two assays.

In the case of the discordant result between the two assays, the Simplexa™ assay was used to determine that the result obtained from the Alere™ i was correct and that the Veritor™ result was a false negative.

Conclusion:While the preliminary data suggest that the two assays showed similar performance in the detection of Influenza B, the Alere™ i may be more sensitive in its detection of Influenza A as compared to the BD Veritor™.

Agreement between the Alere™ i and the BD Veritor™			
Categories	Alere™ i	BD Veritor™	% Agreement
Influenza A	7	6	85.7
Influenza B	3	3	100.0
Negative	13	14	92.9
Total	23	23	

B-280

Evaluation of Siemens Immulite and CLINITEST hCG Assays for Recognition of hCG Variants in Urine

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Background: The Siemens IMMULITE® 2000 HCG assay has been well-documented in current literature for its excellent specificity to multiple hCG variants in urine. The Siemens CLINITEST® hCG pregnancy test is a qualitative, analyzer-read assay performed on the CLINITEK Status®+ analyzer which is most commonly utilized in point-of-care (POC) sites such as emergency departments, clinics, and physician offices. Siemens R&D and/or Support groups are often asked for data indicating how well our CLINITEST hCG assay is able to identify variant forms of hCG. We were also interested to find a respected laboratory-based test to use as a reference assay in the resolution of suspected discrepant results received in POC clinical settings. The objective of the study was to address both of these needs.

Methods: Although it has been known for a long time that human chorionic gonadotropin (hCG) exists in several different molecular forms throughout pregnancy, in blood and urine, until recently these hCG variants have not been available to evaluate their impact on immunoassay performance. Utilizing WHO hCG variants reference materials (5th IS hCG, intact hCG, hCGn, hCGα, hCGß, hCGßn and hCGßcf) and externally sourced variants (hCG-h, hCG-hn, hCG-hnct and asialo hCG), we evaluated the Siemens IMMULITE 2000 assay and CLINITEST hCG assay for reactivity and variant concordance.

All 11 hCG variant stock solutions and dilutions were prepared using an hCG negative urine pool and stored at ≤ -20°C. Frozen samples were utilized during testing at two Siemens sites participating in the study. Prior to testing on all immunoassay platforms, samples were thawed and mixed thoroughly and run in quadruplicate.

Results: The data was compiled and analyzed yielding two major observations: 1) neither assay recognized the hCGα variant; 2) both assays recognized hCGßcf. The first observation on the negative recognition of the hCGα subunit was expected as the hCGα subunit is shared by other protein hormones (LH, FSH and TSH) and is not specific to hCG. The second observation is important as assay developed for serum or plasma detection do not typically detect hCGßcf as serum and plasma do not contain hCGßcf.

Conclusions: In this hCG variant research study, the IMMULITE 2000 hCG and CLINITEST hCG assays recognized all of the variants tested with the exception of hCGα which was expected. Based on the similar hCG variant recognition profiles observed, the IMMULITE hCG assay was selected as the laboratory reference assay to assist in the resolution of suspected discrepant results received in the POC clinical settings. Having POC and laboratory-based assays with similar hCG variant recognition profiles also allows for stronger concordance across different testing sites in a Siemens end-to-end solution.

B-281

High Sensitivity Assays for Cardiovascular Risk Stratification using a Comprehensive Bioelectronic POC System

D. Georganopoulou, J. N. Fawver, F. Lai, E. Van Groll, R. S. Hoo, T. J. Meade, Y. P. Bao. *Ohmx Corporation, Evanston, IL*

Background

Novel applications for a comprehensive bioelectronic platform are described, which demonstrate high-sensitivity levels for hs -Troponin I, hs -Troponin T and hs -CRP. The eDx platform is designed for multiple Point-of-Care (POC) diagnostic applications, including protein, DNA and small molecule detections. An application for high-sensitivity testing for primary care in routine clinical practice has recently emerged from multiple cardiovascular KOLs. At least 5 recent randomized clinical trials (RCT) support that elevation of basal levels of Troponin in asymptomatic individuals, represents occult disease and can be used as a risk stratification tool. Patients considered at low or intermediate risk could be reclassified and receive therapy of known benefit, like Statins and ACE inhibitors in addition to other behavioral modifications. Observational data for a Statin RCTs shows cholesterol reduction associated with a lowering of cTn that predicts outcomes. Ohmx presents herein data for high-sensitivity Troponin I and T as well as hs-CRP as part of their menu for a comprehensive POC platform offered for routine clinical practice.

Methods

Assays have been developed based on bioassay procedures where a mediator substrate specifically reacts with nanolayers on micro-electrodes. An adaptable redox nanolayer technology is presented that demonstrates quantitative, ultra-sensitive, precise and accurate measurement of numerous clinical analytes in various sample matrices (e.g. whole blood, urine, semen, prostatic fluid, saliva etc). Electrochemical signal processing techniques produce a self calibrating signal allowing for a rapid, fully quantitative dose response over a broad, 1000-fold range of analyte concentration. The eDx automated system is fully developed as a programmable automated system for sample to results for all assays. Clinical data presented here for hsTnT, hsTnI and hsCRP use both whole blood and serum clinical samples.

Results

Using dilution series in analyte-free serum, all assays developed to date demonstrate a dose response that spans the analytes' clinical relevant range (hsTnT, hsTnI and hsCRP). Results for the high sensitivity hs-Troponin I, hs-Troponin T and hs-CRP tests are presented. The automated test is presented with a TAT for hsCRP of 10 minutes. The Ohmx test LOD for hs-TnI is 0.8 ng/L , hs-TnT is 1 ng/L and hsCRP is 1 pg/mL. Correlations by Passing-Bablok regression yielded correlations with predicate devices with R2 values from 0.9 to 0.95. The CV% at the 99th percentile for Troponin I (estimated at 10 ng/L) is 6.3%.

Conclusions

An alpha system, utilizing a versatile bioelectronic platform, is presented with validated tests for various clinical targets that is amenable to a high sensitivity applications, including hs-TnI, hs-TnT and hs CRP for use as cardiovascular risk stratification tests in routine clinical practice.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Proteins/Enzymes

B-282

The three dimentional structures of human and microbial transglutasminase complexes to gliadin are similar

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Introduction: Due to their ability to cross-link proteins, there has been great interest in transglutaminases and they have multiple applications. In the food industry, microbial transglutaminase (mTg) is used to modulate texture and improve the properties of food products, acting as a universal food glue. Due to their common enzymatic functions, the question has arisen whether complexes of mTg formed by transamidation reactions could be relevant for celiac patients.

Aim: To compare the linear and three dimensional structure of the complexes formed by tTg or mTg and gliadin, and look for potential immunopotency.

Material and Methods: Complexes of mTg or tTg and gliadin were formed, resulting in mTg neo-epitopes or tTg neo-epitopes. These complexes were separated by asymmetric field flow field fractionation (AF4) and confirmed by SDS-PAGE and multi angle light scattering (MALS). For the structural alignment and docking experiment, the molecular-graphics -modelling and -simulation program YASARA was used. Structural alignment was performed with the primary structures of mTg (PDB-ID: 1IU4) and tTg (PDB-ID: 2Q3Z) by using the MUSTANG-algorithm in a way such that the amino acids of the catalytical triade have maximum alignment.

Results: No alignment on the α-C atoms of the protein backbones and the 3D structure between mTg and tTg were observed. Glutamine or other positive residues are directed to the active centre due to a mainly negatively charged surface. After docking of the gliadin peptide (PDB-ID: 1NNA) to mTg and tTg, both enzymes show adjacent partial-positive charges. Moreover, both neo-epitope complexes show a superimposed epitope similarity, even though the homology at the entrance to the active centre is still low.

Discussion: Analogous to the neo-epitope described in the literature, it is hypothesized that mTg neo-epitopes are formed by stochastic cross-linking between mTg and gliadin peptides. It is only after docking of the gliadin peptides to both Tgs that charges and structure of the epitopes' similarities appear. If molecular mimicry leads from mTg neo-epitopes to tTg-neo-epitopes there must exist a similarity between these enzymes. If an antibody-paratope exists, there is a possibility that an anti-mTg neo-epitope antibody shows cross reactivity and binds to a tTg neo-epitope.

B-283

A Comprehensive Database of Pediatric and Adult Reference Intervals for Biochemical Markers based on the Canadian Health Measures Survey (CHMS)

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Background:

The lack of appropriately partitioned age- and gender-specific reference intervals can compromise the ability of physicians to correctly interpret laboratory test results. The Canadian Health Measures Survey (CHMS; a program of Statistics Canada) collected comprehensive nationwide health information and biological specimens from the Canadian household population. The Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) has collaborated with Statistics Canada to gain access to the CHMS data and develop a robust national database of reference intervals for 24 routine chemistry biomarkers for pediatric, adult, and geriatric populations.

Methods:

Between 2007-2011, the CHMS collected health information, physical measurements, urine, and serum blood samples from approximately 12,000 Canadians aged 3-79 years. The Ortho Vitros 5600 analyzer was used to measure 23 chemistry based analytes and a manual microplate analysis was used for measurement of urine iodine. Exclusion criteria were applied to remove subjects that were pregnant, had serious medical or chronic illness, or that used prescription medication. Reference intervals were calculated using SAS and R software in accordance with CLSI C28-A3 guidelines. Extreme outliers were removed by visual inspection of scatter and distribution plots, and suspected partitions were statistically verified using the Harris and Boyd method. The normality of the data was assessed and data was transformed using the Box-Cox method. The Tukey or adjusted Tukey tests were used to remove outliers from normal or skewed partitions, respectively, Reference intervals were then calculated for partitions with >120 samples using the nonparametric method, and for partitions with >40 but <120 samples using robust methods. Finally, 90% confidence intervals were calculated for the upper and lower limits of each reference interval.

Results:

All analytes were grouped into four categories based on the trends in concentration. (1) Sodium, chloride, potassium, bicarbonate, albumin, total calcium, total protein, and total bilirubin remained relatively constant throughout the age range. (2) Phosphate, ALKP, AST, LDH, and urine iodine all had higher concentrations in childhood that declined with age. (3) Creatinine (both serum and urine), total cholesterol, HDL, LDL, triglycerides, uric acid, and urea all had higher concentrations in adulthood compared to childhood. (4) GGT, ALT, and glucose had substantial fluctuations in concentration throughout the age range. Gender partitions were required for most biomarkers, except bicarbonate, total cholesterol, total protein, urine iodine, and potassium.

Conclusion:

This is the largest population study to simultaneously determine pediatric, adult and geriatric reference intervals for most commonly used biochemical markers from a large cohort of apparently healthy subjects. The data is particularly useful for clinical laboratories using Ortho chemistry assays, but should be further validated by each laboratory on their analytical platform and local population as recommended by CLSI. Clinical implementation of the robust CHMS database will also significantly improve biochemical test interpretation across pediatric, adult, and geriatric age groups and contribute to better clinical decision making and healthcare delivery.

B-284

Assessing Analytical Quality of Hb A1c Assays Using Accuracy Based Grading and Sigma Metrics

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Background: Hb A1c assays are used to diagnose Type 2 Diabetes and require minimal bias and high precision. Four common commercial Hb A1c assays were evaluated using accuracy based grading and Six Sigma metrics.

Methods: Eight frozen whole blood samples from the European Reference Laboratory (ERL) for Glycohemoglobin were tested. Sample target values were assigned by the IFCC Hb A1c reference method. Assays evaluated: Abbott ARCHITECT Next Generation enzymatic; Roche Tina-quant A1c-2; Tosoh G8 HPLC; and the Bio-Rad Variant II Turbo HPLC. Reference samples were tested in two separate trials, five replicates/trial, n = 10 results per sample per assay. The mean values and % CV were calculated for each sample and assay and Sigma metrics were estimated [Sigma = (TEa – bias)/% CV], bias = absolute difference between the target values and the observed values, TEa (total error allowable) = 6%.

Results: The table lists mean observed bias and precision for all assays. Results for each assay expressed as number of samples with a Sigma value of $\geq$ 6 are as follows: Abbott ARCHITECT 6/8 (range of 3.5 - 30 Sigma); Roche Tina-quant 2/8 (range of 0 -7.2 Sigma); Tosoh G8 0/8 (0 – 4.2 Sigma); and Bio-Rad Variant 5/8 (range of 0.4 – 21 Sigma).

Target Value (% A1c)	Architect Bias (%)	Architect Precision (%CV)	Roche Bias (%)	Roche Precision (%CV)	Tosoh Bias (%)	Tosoh Precision (%CV)	Bio-Rad Bias (%)	Bio-Rad Precision (%CV)
4.99	1.4	1.0	5.0	4.6	1.8	3.4	3.8	3.3
5.70	0.9	0.9	6.8	1.8	1.6	2.9	1.6	0.5
6.72	0.3	0.8	3.7	2.9	4.0	3.4	2.4	0.9
7.55	1.1	0.6	0.8	1.9	6.5	1.2	2.0	0.0
8.44	1.7	0.5	0.8	1.1	5.6	1.3	2.4	0.6
9.33	2.9	0.0	0.9	1.5	6.5	1.2	2.9	0.0
10.36	1.4	0.3	2.0	1.2	3.9	4.9	0.5	0.5
11.26	1.9	0.4	2.5	1.5	4.4	1.6	1.4	2.4

Conclusion: Hb A1c is a critical assay because of the world-wide diabetes epidemic and analytical quality is imperative for early detection of patients at risk. The IFCC reference method for Hb A1c is internationally accepted and commutable whole blood samples with reference method target values are available so "true bias" of assays, instead of "relative bias," can be measured. A TEa of 6% based on clinical needs for diagnosis is established. Sigma metrics allows assay quality to be objectively assessed. Comparison of analytical quality of common Hb A1c field methods demonstrated some marked differences.

B-285

Metrological Traceability of ARCHITECT Amylase and Alkaline Phosphatase Assays to IFCC Reference Methods

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Background: ISO 18153, In vitro diagnostic medical devices — Measurement of quantities in biological samples — Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials, describes traceability of enzyme assays. The IFCC has established reference measurement procedures (RMPs) to standardize the measurement of enzyme catalytic concentrations. This study validated the metrological traceability of optimized enzyme calibration factors for the ARCHITECT alkaline phosphatase (ALP) and amylase (AMY) assays to ensure agreement with the RMPs. It's necessary for the three ARCHITECT systems (c4000, c8000, and c16000) to not only to provide comparable test results but accurate results as determined by comparison to results from the "gold standard" reference procedures.

Methods: Human serum samples were assigned enzyme activity target values for ALP and AMY using the primary RMPs maintained at the IFCC reference laboratory in Hannover, Germany. Sample aliquots were stored at −75 C before testing using the field assays on two ARCHITECT c8000 systems using different reagent lots.

Results: The mean results from both ARCHITECT systems using different reagent lots were compared to the results from the RMPs for ALP and AMY. The mean % bias was 1% (95% limits of agreement; range -8.8 to +10.8%) for ALP and 0.1% (95% limits of agreement; range -8.5 to +8.2%). The Passing-Bablok regression data is presented in the table below.

Assay	N	Correlation Coefficient (r)	Slope (95% CI)	Intercept (95% CI)
Alkaline Phosphatase	57	0.9965	1.02 (1.01, 1.04)	-1.09 (-6.33, 2.25)
Amylase	51	0.9973	0.99 (0.96, 1.01)	2.35 (-0.65, 4.24)

Conclusions: The metrological traceability of the ARCHITECT enzyme assays for ALP and AMY to the internationally recognized reference procedures of the highest metrological order, RMPs listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database, was established. The optimized enzyme calibration factors determined allow ARCHITECT results to be in excellent agreement with the IFCC reference methods. Metrological traceability is necessary for the global standardization of clinical laboratory practice and direct comparability of patient test results.

B-286

Development of a New ELISA Kit for the Specific Detection of Monoamine Oxidase B (MAO-B) in Gel Filtered Platelet Preparations

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Background: Monoamine oxidase B (MAO-B) is a dimeric, integral outer mitochondrial membrane flavoenzyme that catalyzes the oxidative deamination of arylalkylamine neurotransmitters such as dopamine and serotonin. MAO-B activity increases with age in humans. It has been reported that Alzheimer's plaque-associated astrocytes presented elevated MAO-B levels when compared with age-matched controls. The changes in

MAO-B activity in the prefrontal cortex seem to occur very early in Alzheimer's disease (AD) patients and remain relatively constant as the disease progresses. Furthermore, it was shown that MAO-B activity in platelets is increased in Alzheimer's, but not in Parkinson's disease patients. The availability of convenient immunoassays for the detection of this enzyme is relevant for research applications. This study reports the development of a new ELISA kit incorporating lyophilised calibrators containing recombinant MAO-B for the specific detection of this protein in platelet preparations. Platelets express MAO-B and are a model system for neuronal cells. This kit will facilitate research into the potential

use of patient stratification for treatment strategies in Alzheimer's disease. Methods:Sheep were immunized with recombinant MAO-B expressed in *E. coli*. Lymphocytes were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of specific MAO-B antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by direct binding ELISA to determine their specificity MAO-B. Initial screening results indicated optimal antibody combinations that were likely to meet assay requirements. These antibody combinations were then assessed and an antibody pair was selected for the sandwich ELISA. The ELISA experimental procedure involved an initial incubation of 60 minutes at +25°C upon addition of assay diluent and sample, followed by a wash step. Conjugate was then added and after a second incubation of 60 minutes at +25°C and wash step, signal reagent was added. After 20 minutes at room temperature +19°C - +25°C the reaction was stopped. The absorbance was measured at 450 nm. 50 µl of sample were required. A total protein determination of the sample was also required to allow for normalization of samples following lysis.

Results: The assay was specific for MAO-B (cross-reactivity <0.8% for MAO-A). The assay sensitivity was 3.1 ng/mL (calibration range: 0-75 ng/mL). The spiked recovery of recombinant protein from platelet lysate ranged from 100.4 to 122.8%. The intra-assay precision expressed as %CV (n=12) was <10% for different concentration levels.

Conclusion: The results show applicability of this new ELISA kit to the specificdetection of MAO-B from gel filtered platelet lysates. Platelets express MAO-B and are a model system for neuronal cells. Limited alternatives using other matrices are currently available. Furthermore, this ELISA kit incorporates calibrators containing recombinant MAO-B, hence facilitating the assay procedure. This represents a useful analytical tool in research studies into stratification of patients for treatment strategies for Alzheimer's disease, as well as the monitoring of changes in expression levels.

B-287

Development of New Antibodies for the Specific Detection of Cleaved SLPI to Facilitate More Rapid Testing of Bacterial Infection Status in Chronic Lung Disease

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Background: Cystic Fibrosis (CF) is a debilitating hereditary disease in which the lungs produce thick sputum difficult to clear and is classically

associated with chronic bacterial infection resulting in an increased,

detrimental inflammatory response. One such effect of this chronic infection is disruption of the body's natural defence to proteases; the antiprotease screen. One antiprotease that is affected is human Secretory Leucocyte Protease Inhibitor (SLPI), which in general, protects the body's tissues from the detrimental effects of different proteases such as human neutrophil elastase (HNE). Previous research has shown that, when CF patients are chronically infected with *Pseudomonas aeruginosa*, SLPI is cleaved due to the excess level of HNE resulting from the recruitment of activated neutrophils to the sites of infection. HNE cleavage of SLPI results in the generation of a C-terminal polypeptide fragment, cleaved SLPI (C-SLPI). The current methodology of testing for bacterial infection during an exacerbation in CF patients involves classical culture-driven microbiological techniques. The availability of rapid, less laborious tests will facilitate a more efficient diagnosis leading to quicker treatment strategies to reduce the pathological burden of infection within CF patients. The aim of this study was to generate monoclonal antibodies for the development of efficient immunoassays based on the detection of C-SLPI as a potential biomarker for bacterial infection.

Methods: An amino acid sequence within C-SLPI was used as an immunogen to produce hybridoma clones expressing monoclonal antibodies to C-SLPI. Basic Local Alignment Search Tool (BLAST) analysis of the immunogen against the Pfam protein families database was performed as was *in silico* modelling of the predicted C-SLPI epitope. Monoclonal antibodies purified from the supernatants of these hybridoma clones via affinity chromatography were assessed by ELISA for activity against both

C-SLPI and full-length SLPI and antibodies recognising specifically C-SLPI were selected. Additionally, 26 CF patient sputum samples were assayed.

Results: Respectively, BLAST analysis and *in silico* modelling showed the immunogen to be specific for C-SLPI and that the epitope is exposed on the C-SLPI peptide but not on full length SLPI. ELISAs using recombinant C-SLPI demonstrated that monoclonal antibodies purified from supernatants of the hybridomas clone lines were able to detect C-SLPI ranging in concentration from 2.70 ng/mL to 2000 ng/mL. Using a C-SLPI monoclonal antibody paired with a conjugated detector antibody the cross-reactivity with full-length SLPI was <0.5% indicating high specificity for C-SLPI. Initial analysis of sputum samples from CF patients experiencing an exacerbation showed higher levels of C-SLPI antigen when compared to those patients who were not experiencing an exacerbation.

Conclusion: The results indicate the successful development of antibodies applicable to the development of efficient immunoassays for the specific detection of C-SLPI. This is relevant in clinical settings to facilitate a more rapid testing of bacterial infection status. These novel antibodies could be employed on various diagnostic platforms to detect bacterial infection or exacerbation in patients with chronic infective lung disease.

B-288

Molecular Defects in Proexosites of Prothrombin Molecule Result in Abnormal Thrombin Generation

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Uncontrollable bleeding at the site of vascular injury is the inception of diseases like stroke, cardiac arrest and other CVD. Upon vascular injury, proteolytic conversion of prothrombin (pro) to thrombin occur in presence of prothrombinase complex. Prothrombinase is an enzymatic complex between factor Va (fVa) and factor Xa (fXa) assembled on a membrane surface in the presence of divalent metal ions. Although fXa is capable of activating Pro through the initial cleavage at Arg^{271} followed by the cleavage at Arg^{320} (pre2 pathway), it would take approximately six months to form a clot, which is not physiologically compatible with life. However, the incorporation of fVa into prothrombinase results in a 5-fold increase in the catalytic efficiency of fXa for thrombin generation and the order of cleavages reversed by initial cleavage at Arg^{320} followed by Arg^{271}(meizo pathway),which is physiologically compatible with life. Factor V dictates the pathway of prothrombin activation and previous studies in our lab, have suggested that fXa interacts with Pro within amino acid region 478-482 in a fVa-dependent manner and it has been also been reported that basic exosites on prothrombin are involved in recognizing fxa in prothrombinase complex in Va depended manner. The exact role of fVa and prothrombin interaction which leads to the exposure of cryptic exosites in the fxa during the prothombinase complex is yet to be known .The catalytic function of basic amino acid region have been studied extensively further we investigated the structural and functional analysis of these basic exosites. In this study, using site directed mutagenesis we generated recombinant mutant by mutation two amino acids Arg^{382} →Ala (R382A), Lys^{385} →Ala (K385A) **($rpro^{W2}$)** and second recombinant prothrombin mutant by deleting 5 amino acid $rPro^{\Delta 478-482}$ along with $rpro^{W2}$(**$rPro^{\Delta 478-482}$/$rpro^{W2}$**).The mutants and wild type Pro ($rPro^{WT}$) were stably transfected in BHK-21 cells. All the recombinant molecules were purified to homogeneity, according to an established protocol and, at the last step, a FPLC equipped with a strong anionic Mono-Q 5/50 column and a calcium gradient were used to isolate fully carboxylated recombinant molecules. The recombinant molecules were characterized with respect to their ability to function as zymogens for both factor Xa and the prothrombinase complex using SDS-page analysis. Gel electrophoresis revealed fXa alone exhibited slightly impaired catalytic activity toward the mutant substrates compared to wild type; however, its activity in the prothrombinase complex towards the recombinant mutants was impaired. The functional activity of the recombinant mutants was assessed by clotting assay and by prothrombinase assays using a chromogenic substrate to assess for thrombin formation. In addition, clotting assays revealed $rPro^{\Delta 478-482/W2}$ and $rpro^{W2}$ was devoid of clotting activity, while $rPro^{WT}$ had clotting and chromogenic activities comparable to human plasma-derived Pro. In conclusion, our data suggest that the proexosites are directly involved in the interaction of fV. There has been a natural mutations reported in proexosite region Arg382 which may lead to congenital abnormalities like hypoprothrombinemia resulting in severe bleeding disorders associated with dysfunctional prothrombin which is in preposition with our data which is devoid of clotting and normal amydolytic activity.

B-289

Evaluation of a new Urinary and CSF Albumin Assay on the Beckman Coulter AU5800® Clinical Chemistry System

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Background: Mildly increased urinary albumin excretion (3-30 mg/dL) is considered a clinically important indicator of progressive renal disease, atherosclerotic disease and cardiovascular mortality. It is used to predict the development of diabetic nephropathy as this protein tends to appear ahead of other serum proteins in urine during the course of renal glomerular damage. Screening for urinary albumin is therefore recommended by the American Diabetes Association and other guidelines for all diabetic patients.

Beckman Coulter has developed a new sensitive albumin assay for the quantitative measurement of albumin in urine and CSF. The performance of this assay was evaluated on the Beckman Coulter AU5800® Clinical Chemistry System.

Methods: This method was compared with existing Beckman Coulter Urine and CSF albumin assays and the Siemens BN ProSpec following CLSI guideline EP09-A3. Precision was assessed following CLSI guideline EP05-A2. The linear range was assessed following CLSI EP06-A and the high dose hook effect was evaluated. Interferences for endogenous analytes and common drugs were assessed following CLSI guideline EP07-A2.

Results: The new assay demonstrated a good correlation with other Beckman Coulter and competitor assays with Deming regression results vs.: Beckman Coulter AU Microalbumin of y = 1.12x + 0.28 mg/dL; Beckman Coulter DxC of Microalbumin y = 1.09x + 0.03 mg/dL; Beckman Coulter Immage CSF Albumin of y = 1.05x -0.77 mg/dL; Siemens BN ProSpec urine of y = 0.96x - 0.16 mg/dL; Siemens BN ProSpec CSF of y = 0.97x - 0.03 mg/dL.

Estimates of precision were obtained using three urine pools with repeatability of 0.9% CV 0.6% CV & 1.4% CV, and within laboratory precision of 4.3% CV 2.5% CV, 2.1% CV at albumin concentrations of 1.68, 3.28 & 20.0 mg/dL respectively.

The assay was shown to be linear up to 450 mg/L and there was no high dose hook effect up to 20,000 mg/L.

No significant interference was observed up to: 300 mg/dL Creatinine; 3000 mg/dL Glucose; 5000 mg/dL Urea; 500 mg/dL Ascorbate; 50 mg/dL Citrate; 400 mg/dL Magnesium; 30 mg/dL Oxalate; 40 mg/dL Conjugated Bilirubin; 500 mg/dL Hemoglobin; 350 mg/dL Acetone; 10 mg/dL Uric Acid; 2.25 mg/dL Urobilinogen; 300 mg/dL Acetaminophen; 400 mg/dL Ibuprofen; 600 mg/dL Metronidazole; 150 mg/dL 5-Aminosalicylate; 78 mg/dL Calcium.

Conclusion: The results of our study demonstrate excellent performance for the new Beckman Coulter AU Urine/CSF assay demonstrating that the assay is accurate, precise and reliable. Method comparison results show good agreement with existing assays.

B-290

Determination of storage conditions for candidate SRM2924 C-reactive protein in solution.

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Background: The objective of this study was to determine the effects of a freeze/thaw cycle on the concentration and structure of candidate reference material SRM2924 C-reactive protein (CRP) in solution, currently stored at 4 °C. Stability of this material in long-term storage would be enhanced if the material were kept in a frozen state (-80 °C). This material is intended to provide a "pure substance" calibrant material for the concentration assignment of future CRP reference material in biological matrices relevant to clinical chemistry such as plasma, serum, urine, etc. SRM2924 was generated in a lot size projected to be sufficient for 3-5 years of demand, therefore, determination of optimum storage conditions over this period is critical to providing a stable reference material.

Methods: Vials of SRM2924 (n=24) were randomly selected and assigned to one of two groups (n=12) which were either frozen (-80 °C) or kept at 4 °C. Six vials were removed from each group after one week for analysis. Concentration was determined by amino acid analysis (AAA) and pentameric structure by size exclusion chromatography (SEC). The remaining vials were kept in storage for an additional twenty-one weeks prior to SEC analysis. AAA was conducted by isotope dilution mass spectrometry using leucine, isoleucine, proline, valine and phenylalanine amino acids liberated by vapor phase acid hydrolysis (6 M HCl, 120 °C, 48 hr) of the sample in two

separate reactions. Concentration was determined using an external calibration curve generated using ratios of pure substance amino acids in both natural and isotopically enriched forms using triple quadrupole mass spectrometry (Agilent 6490). SEC was conducted by HPLC (Thermo Scientific 3000 UltiMate) using isocratic flow through column (TSKgel) and detected by fluorescence (excitation- 295 nm, emission- 350 nm). AAA and SEC were validated by using reference material NMIJ CRM6201b C-reactive protein in solution. This material is certified for both concentration and structure and was included in all runs of AAA and SEC. The slopes (range = 0.93 to 1.12) and y-intercepts (range = 0.002 to 0.020) of the individual amino acid linear regressions were acceptable and all had r2 values greater than 0.999.

Results: The concentration of CRP in the first hydrolysis were 20.7 (%cv=2.7) µmole/kg and 20.4 (%cv=0.6) µmole/kg for the -80 °C and 4 °C group, respectively, while the values were 20.4 (%cv=1.1) µmole/kg and 20.4 (%cv=2.6) µmole/kg, respectively, in the second. ANOVA (t-test) did not indicate a difference between the either the hydrolysis or storage temperature groups. The concentration for CRM6201b was 39.5 µmole/kg and 39.3 µmole/kg for each of the respective hydrolysis groups which compares well with the certified concentration value of 40.0 (+/- 1.6) µmole/kg. SEC results were consistent through all groups, conditions, time points and validation material in that only a single peak was present in the fluorescent detection of proteins indicating retention of pentameric structure.

Conclusion: This study indicates that there are no adverse effects of storage of SRM2924 at -80 °C for the time studied. Periodic checks should be performed to monitor stability throughout the lifecycle of the current batch.

B-291

Performance of random albumin creatinine ratio for detection of micro and macro-albuminuria in patients with type 2 diabetes mellitus.

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Abstract

Background: Twenty four hour urine albumin excretion (24 hour UAE) considered as gold standard is traditionally used to assess renal damage. This study was performed to evaluate the performance of a less cumbersome spot or random albumin creatinine ratio (RACR) for screening of renal damage in patients with type 2 diabetes mellitus (T2DM).

Methods: This was a cross-sectional study of 122 patients with T2DM (mean age 54±15) comprising of 104 female and 18 male patients performed at King Khalid University Hospital, Riyadh between March 2011 and June 2012. Urine samples for both 24 hour UAE and RACR were collected for assessment of albuminuria. Urine albumin levels of <30, from 30-300 and over 300 mg/g were considered as normo-albuminuria, micro-abluminuria and macro-ablbuminuria respectively.

Results: Concordance between the two assays was observed in 114 (93.4%) samples including 36 (29.5%) with microalbuminuria and 46 (37.7%) with macro-ablbuminuria. Whereas the mean urinary albumin among normo-albuminuria samples assessed in 24 hour UAE (7±5.4 mg/g) was lower (p=0.03) than that of RACR (9.6±7.1 mg/g), samples with micro-albuminuria (80±41vs115±73) and macro-albuminuria (1615±10vs1416±85) were no different. The sensitivity of RACR against 24 hour UAE assay was 100% and specificity was 91.3% with a positive predictive value (PPV) of 95% and a negative predictive value (NPV) of 100% in micro-abluminura range. For macroalbuminuria RACR had a sensitivity of 100%, specificity of 76% with PPV of 94.1% and NPV 100%.

Conclusion: RACR was comparable to 24 hour UAE assay particularly in excluding renal impairment among patients with T2DM.

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Development of a New Rapid Enzyme-Linked Immunosorbent Assay Kit to Detect Glial Fibrillary Acidic Protein in Human Serum

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Background: Glial fibrillary acidic protein (GFAP) is the major intermediate filament and cytoskeletal protein expressed predominantly in astrocytes. As a cytoskeletal protein, it is thought to regulate astrocyte structural stability and mobility. GFAP is primarily known for its requirement in the central nervous system (CNS), where its deregulation or loss has been linked to degenerative conditions. In healthy individuals, GFAP is maintained within astrocytes and as such is generally not detectable in the plasma or serum. It has been reported however, that GFAP can be used to distinguish ischemic stroke (IS) from acute intracerebral haemorrhage (ICH), based on the speed of its release from astrocyctes upon stroke. In cases of IS a delayed release of GFAP is observed with levels below the detectable range for the first 24 hours. Conversely, GFAP is released rapidly into the blood within 2- 6 hours of an ICH event. The availability of rapid tests enabling the detection of this protein represents an advantage in clinical settings. This study aimed to develop a new rapid enzyme-linked immunosorbent assay (ELISA) for the specific and rapid detection of GFAP in human serum.

Methods:A colorimetric 2-step sandwich immunoassay was employed. The capture antibody is immobilised and stabilised on a 96-well microtitre plate surface. The analyte, if present in the sample, is bound to the capture antibody and then a second antibody labelled with horseradish peroxidase is bound to the analyte. Absorbances are read at 450nm. The signal is proportional to the concentration of the analyte in the sample. All assay kit reagents are ready to use and serum samples do not require dilution. Recognition of native GFAP was confirmed with analysis of serum samples from ICH patients (n=3) compared with controls (n=3). Difference was assessed with Kruskal Wallis Test (Medcalc version 12.7.8.0).

Results: The ELISA was specific for GFAP, with cross-reactivity of <0.1% for vimentin, desmin and peripherin. The assay exhibited a functional sensitivity of 0.29 ng/mL and a measuring range of 0-100 ng/mL. Median concentration of GFAP from ICH serum samples (Median 8.796 ng/mL) was significantly higher than controls (Median<0.29 ng/mL), p<0.01. Inter- assay and intra-assay precision values (n=10) were expressed as CV and the values were 7.2% and 3.8% respectively.

Conclusion: The results show applicability of the developed ELISA for the specific and sensitive detection of GFAP in serum. Median concentration values were significantly higher in ICH serum samples when compared with controls. The assay presents all kit reagents ready to use, the total assay time is less than 3 hours and serum samples do not require dilution. This assay is a useful analytical tool for clinical research studies.

B-293

Molecular Defects in Proexosites of Prothrombin Molecule Results in Abnormal Thrombin Generation

M. Na, J. Wiencek, M. Kalafatis. *Cleveland State University, Cleveland, OH*

Uncontrollable bleeding at the site of vascular injury is the inception of diseases like stroke, cardiac arrest and other cardiovascular diseases. Upon vascular injury, proteolytic conversion of prothrombin (Pro) to thrombin occurs in the presence of the prothrombinase complex. Prothrombinase is an enzymatic complex between factor Va (fVa) and factor Xa (fXa) assembled on a membrane surface in the presence of divalent metal ions. Although fXa is capable of activating Pro through initial cleavage at Arg271 followed by the cleavage at Arg320 (pre2-pathway), it would take approximately six months to form a clot, which is not physiologically compatible with life. However, the incorporation of fVa into prothrombinase results in a 300,000-fold increase in the catalytic efficiency of fXa for thrombin generation and the order of cleavages reversed (initial cleavage at Arg320 followed by Arg271 (meizo-pathway), which is physiologically compatible with life. Recently, we have shown that the concentration of fVa locally at the place of vascular injury dictates the pathway of Pro activation and that fXa has a fVa-dependent interactive site on Pro within amino acid region 478-482. In addition, several specific basic amino acid residues within Pro have been shown to interact with fXa in a fVa-dependent manner (proexosite I). Thus, in order to elucidate the contribution of amino acid residues from both proexosite I and the region 478-482 in Pro activation by fXa-alone or prothrombinase we constructed several recombinant Pro (rPro) molecules. The first rPro was mutated with two point alanine mutations at Arg382 (Arg382→Ala) and Lys385 (Lys385 →Ala) known herein as rProW2. Next, a rPro molecule containing the two previous mutations and the deletion of amino acid residues 478-482 (rProΔ478-482/W2) was also constructed. The two mutant rPro molecules and wild type Pro (rProWT) were stably transfected in BHK-21 cells, and all rPro molecules were purified to homogeneity according to a well established protocol. The last step of the procedure utilized a Fast Performance Liquid Chromatography instrument equipped with a strong anionic exchanger that employed the use of a step-wise calcium gradient to isolate fully carboxylated rPro. The rPro molecules were analyzed for their ability to be activated by both fXa-alone or the prothrombinase complex by SDS-PAGE. Gel electrophoresis revealed fXa-alone exhibited slightly impaired catalytic activity

toward rProW2 and rProΔ478-482/W2 when comparing to rProWT activation, whereas prothrombinase activity towards both rPro molecules was severely impaired. Subsequently, we further analyzed prothrombin times (PTs) for plasma-derived Pro, rProWT and both rPro mutants. While rProWT and plasma-derived Pro had standard PTs, the clotting assay employed revealed that rProΔ478-482/W2 and rProW2 were devoid of functional activity. In conclusion, our data suggest that amino acids Arg382 and Lys385 together with a previously described deletion of amino acids 478-482 of Pro hold combined significance for the fVa-dependent binding of fXa on prothrombin within prothrombinase. Our results also provide further explanation for a natural mutation in proexosite I (Arg382→Cys) that was reported in the literature. Patients harboring this natural mutation have dysfunctional abilities to form a fibrin clot and, thus, are prone to be severe bleeders.

B-294

Evaluation of Seven Commercially Available Clinical Chemistry Assays on the ARCHITECT® c System

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Evaluation of Seven Commercially Available Clinical Chemistry Assays on the ARCHITECT® *c* System

Background: Expansion of instrument analyte menus is often needed to accommodate country specific needs. To address specific needs in China, seven clinical chemistry assays have been modified for evaluation on the ARCHITECT *c* system. These analytes are used in the investigation of several diseases including disseminated intravascular coagulation, liver damage, lung cancer, diabetes, and myocardial damage.

Methods: The following analytes were evaluated: Fibrin/fibrinogen degradation products (FDP), fibronectin (FN), glutamate dehydrogenase (GLDH), glycated albumin (GA), heart fatty acid binding protein (H-FABP), myeloperoxidase (MPO), and sialic acid (SA). Reagents from Beijing Strong Biotechnologies Inc. (BSBE) were evaluated on the ABBOTT ARCHITECT *c* system. Precision, accuracy, LoQ, and linearity range were evaluated with guidance from CLSI documents EP15-A2, EP6-A and EP17-A2. Correlation to other commercial kits was performed using human serum/plasma samples across the measuring range of each analyte.

Results: In the studies, all assays demonstrated good performances. Key performance characteristics are summarized in the following table:

Analytes	Precision: Within-run CV	Accuracy: Bias from Target Value	Linearity (correlation factor r)	LoQ	Correlation (correlation factor r)
FDP	1.8%	-0.42%	0.9987	0.83 µg/mL	0.9921
FN	0.8%	-2.99%	0.9993	40 mg/L	N/A
GLDH	2.0%	4.7%	0.9991	10.66 U/L	0.9995
GA	0.8%	-4.69%	0.9998	7.13 mg/dL	0.9917
H-FABP	6.9%	5.69%	0.9999	2.5 ng/mL	0.9838
MPO	3.2%	0.75%	0.9975	13.97 ng/mL	0.9937
SA	1.3%	5.4%	0.9999	2.89 mg/dL	0.9918

Conclusions: These initial results are very promising. All the assays tested exhibit good precision, accuracy, linearity, anti-interference, and LoQ performances on ARCHITECT *c* system. In addition, they also have excellent correlation with commercial assay kits on market e.g. GLDH kit from Roche, MPO kit from Diazyme, FDP kit from Leadmanbio, GA kit from Asahi Kasei, SA kit from Dongou, and H-FABP kit from Randox.

B-295

Evaluation of an advanced Cystatin C assay on DiaSys automated analyzer respons®920

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Background:

Cystatin C is an endogenously expressed, non-glycosylated protein that represents an excellent biomarker for moderate impairment of kidney function. Increased Cystatin C levels indicate an even slightly reduced glomerular filtration rate (GFR) compared to conventional parameters like e.g. Creatinine. Since kidney diseases develop slowly and at least in the beginning painless, the majority of individuals with early stages of chronic kidney disease remain undiagnosed. Therefore, early detection of renal insufficiency by a sensitive marker as Cystatin C is of increasing importance to avoid the irreversible condition of renal failure.

The aim of this study was to establish an advanced particle-enhanced Cystatin C assay for DiaSys respons®920, a bench top random access clinical chemistry analyzer. The requirements for this test were superior performance and traceability to IFCC reference material for reliable detection of impaired GFR.

Methods:

Assay adaption as well as performance verification have been carried out on DiaSys respons®920. All reagents, calibrators and controls were provided by DiaSys Diagnostic Systems GmbH. Calibration stability was optimized by the use of an aqueous 5-level calibrator set containing recombinant Cystatin C, reflecting various conformations of native Cystatin C in different sample material. Method comparisons were performed against nephelometric and immunoturbidimetric competitor assays. Data have been evaluated by using regression analysis according to Passing and Bablok. Inter- and intra-assay imprecision were performed according to the CLSI protocol (EP5-A2). Traceability was investigated by using IFCC reference material ERM-DA417/IFCC.

Results:

Comparative studies of Cystatin C FS on respons®920 were carried out with 104 native serum and heparin-plasma samples against Hitachi as a common laboratory analyzer [r=0.999; Passing/Bablok: y=0.977 x + 0.006 mg/L] confirming equivalent performance. Good correlation of Cystatin C FS against latest immunoturbidimetric [r=0.9975; Passing/Bablok: y=0.984 X + 0.032 mg/L] as well as a current nephelometric competitor assays [r=0.9970; Passing/Bablok: y=0.974 X + 0.017 mg/L] was demonstrated. Moreover, DiaSys Cystatin C FS is highly precise with an intra-assay precision of a CV≤ 2.53% and an inter-assay precision of CV ≤ 3.71% on respons®920. Based on an advanced calibration approach high calibration stabilities of up to 6 weeks were achieved. Due to good correlation of DiaSys calibrator to IFCC reference material traceability was demonstrated [r=0.999; Passing/Bablok: y=1.0 X + 0.02 mg/L].

Conclusion:

Here we present a Cystatin C assay with outstanding performance especially for specificity and precision.

This test performs very well on common analyzers as Hitachi systems, but was also proven for equivalent performance on DiaSys respons®920 systems. The advantages of combining Cystatin C FS with this flexible and convenient system are reliable results, optimized workflow and high efficiency (achieved by the perfect match of analyzer, system reagents and applications). Moreover, Cystatin C FS highly correlates to nephelometric and immunoturbidimetric tests and is traceable to ERM-DA471/IFCC reference material. In summary, DiaSys Cystatin C assay represents an excellent tool for early and reliable detection of even slightly impaired kidney function.

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Development of a latex-enhanced immunoturbidimetric assay for the measurement of L-FABP levels on automated clinical chemistry analyzers

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Liver-type fatty acid binding protein(L-FABP) is a 14kDa protein found in the cytoplasm of human renal proximal tubules. It has been demonstrated that measuring

urinary L-FABP levels is useful for early diagnosis of renal disease accompanying tubular dysfunction.

We have developed a new method for measurement of urinary L-FABP levels for use in clinical chemistry analyzers. This method is based on latex-enhanced immunoturbidimetry, using anti-human L-FABP mouse monoclonal antibodies. The concentration is determined by measuring the change in absorbance that results from agglutination of latex particles.

The reagents are supplied ready-to-use, and the assay can be completed within 10 min. Using a Roche/Hitachi 917 auto analyzer, 3.0 µL of human urine was mixed with 150 µL of the first buffer solution and incubated for 5 min at 37ºC. Subsequently, 50 µL of the second reagent, which contains the monoclonal antibody-coated latex particles, was added and the absorbance was monitored at 570 nm/800 nm (main/sub wavelengths) for 5 min.

The lower detection limit for L-FABP was 1.0 ng/mL, and the upper quantitation limit was 200 ng/mL. No prozone effect was observed in L-FABP samples of concentrations from 200 to 2000 ng/mL. The within run C.V. (n=20) at 10 ng/mL, 50 ng/mL, and 100 ng/mL was 2.3 %, 1.1 %, and 1.0 %, respectively. The between run C.V. (n=10) at 10 ng/mL, 50 ng/mL, and 100 ng/mL was 1.9 %, 2.3 %, and 2.2%, respectively. Interference studies showed no effect from bilirubin, hemoglobin, glucose, ascorbate, rheumatoid factor, or chyle at concentrations of 50 mg/dL, 500 mg/dL, 4000mg/dL, 100mg/dL, 500 IU/dL, and 2000 formazin turbidity units, respectively.

Comparison of our assay kit with a commercially available kit , the principle of which is enzyme immunoassay(EIA), yielded a correlation coefficient of 0.984 and an equation of Y (present method) = 0.98X (the EIA kit) - 0.16 (n = 321 urine samples).

We concluded that this assay reagent provides an accurate, precise, and simple method for routine measurement of L-FABP levels in urine samples.

B-297

Extending Capillary Zone Electrophoresis (CZE) of Serum Proteins

C. Wunsch. *University of Miami School of Medicine, Miami, FL*

The objective of this study is to extend the clinical utility of CZE. The CZE system used is CapillaryS(tm). The system's server software can provide data downloads to a PC client. Our lab uses data downloads and our middleware to transform and analyze the serum protein electrophoresis (SPE). The SPE zones are: albumin, alpha-1, alpha-2, beta-1, beta-2 and gamma. Reference ranges for the protein in these zones assist the SPE interpretation. The peaks of these zones consist mainly of albumin, alpha-1-antitrypsin, alpha-2 globulins, transferrin, C3+C4 complement and immunoglobulins, respectively, and the zone protein is often used as a surrogate measure of the main zone protein. The higher resolution of CZE allows better estimates of a peak's area, shape, center and height, and the UV absorbance of the eluate provides a better estimate of SPE protein than the scanning of stained gels. These features, for example, allow our middleware to find a quantifiable prealbumin peak in more than 90% of specimens, but this peak is usually not seen in routine gel electrophoresis. This study emphasizes the beta-1 and beta-2 peaks. Several algorithms for quantifying the area of these peaks, which translates directly to protein mass, were tested. The areas of the better algorithms were found to agree well with routine clinical assays of transferrin and C3+C4 complement. Subtraction of these peaks from the ELP curve also allowed an estimate of other beta zone proteins, particularly immunoglobulins (IGs), which can be added to the gamma zone IGs to form an estimate of the total IGs. In specimens with hypergammaglobulinemia, the estimates of total IG agreed well with the sum of IgG, IgA, and IgM IGs. For a systematic comparison, SPE specimens that also had a clinical result for transferrin or C3+C4 complement or quantitative IgA, IgM and IgG, were chosen at random until at least 20 specimens for each analyte or analyte group were found. The values of the clinical analytes were then paired with the corresponding beta peak protein and with the beta zone protein. The quantitative clinical immunoglobulins were paired with the total IG as decribed above, and with the gamma zone protein. Bland-Altman plots of the pairs were made, and the variances of the peak values versus zone values were calculated and compared. In each case the peak protein pairs showed a significantly smaller *F*-test (variance ratio), $p<0.01$, than the zone protein pairs.

This study shows the measurement of peak areas helps in the detection of abnormal results. Further benefits of our middleware are the assessment of monoclonal gammopathy peaks and assistance in the interpretation of results. A similar system for immunotyping analysis and interpretation has been developed.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

TDM/Toxicology/DAU

B-298

Analytical Performance of an Assay on ARCHITECT i System for Measurement of Methotrexate in Human Serum or Plasma

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BACKGROUND: Cancer therapeutic drug methotrexate (MTX) is often monitored in patients to ensure appropriate therapy. An assay on the ARCHITECT *i* System (ARCHITECT Methotrexate) was analytically evaluated for measuring MTX in human serum and plasma. The ARCHITECT Methotrexate assay is under development and has not been cleared by the FDA.

METHODS: The ARCHITECT Methotrexate is a one-step immunoassay. The instrument mixes and incubates a sample (calibrators, controls, sera or plasma) with anti-MTX antibody-coated paramagnetic microparticles and acridinium-conjugated MTX followed by washing and chemiluminescent reaction triggering. Signals obtained as Relative Light Units (RLU) are inversely proportional to the amount of MTX in the sample.

RESULTS: The MTX assay showed a maximal LoQ (Limit of Quantitation) of 0.020 µmol/L. The measuring range was from 0.040 to 1.500 µmol/L, and up to 2500 µmol/L with specimen dilution. The 20-day imprecision study showed a total CV ≤ 7.5% for samples in which MTX levels ranged 0.040 - 12.500 µmol/L and a total CV ≤ 10% for samples in which MTX level > 12.500 µmol/L (n = 80). Deviations from linearity were ± 10% within the range of 0.040 to 1.500 µmol/L MTX. In the therapeutic interference studies, the cross reactions of 20 individual therapeutics at ≥1000 µmol/L were 0%. The cross reactions of Aminopterin and DAMPA, respectively, at 5 µmol/L ranged 43% - 83%. Aminopterin and DAMPA were also tested individually in human serum at 1000 µmol/L in the absence of MTX, and The cross reaction was 61% and 46%, respectively. Each of 11 Endogenous interfering substances, including human anti-mouse antibody and rheumatoid factor, was individually spiked into human serum or plasma samples, and yielded a difference of -7% to 8% away from the control samples. Method comparison of ARCHITECT Methotrexate to tDx/TDxFLx MTX II (TDx) generated a Passing Bablok correlation as [ARCHITECT] = 0.005 + 0.946x [TDx] for samples ranging 0.040 - 0.993 µmol/L (n = 86) and [ARCHITECT] = -0.004 + 1.016x [TDx] for the samples ranging 0.040 - 888.000 µmol/L (n = 119), respectively. Method comparison ARCHITECT Methotrexate to LC/MS/MS ACUITY TQD generated a Passing Bablok correlation as [ARCHITECT] = 0.016 + 0.923x [LC/MS/MS] for the samples ranging 0.040 - 1.438 µmol/L (n = 101). MTX values in specimen stored at room temperature for 24 hours or at 2-8°C for 48 hours were within ± 10% deviation from that of the baseline control ones. The mean concentrations of MTX in specimens collected in K2-EDTA, sodium heparin, and lithium heparin tubes were within ± 10% deviation from that collected in serum tubes (Red Top only) within the Range of 0.040 - 1.500 µmol/L MTX. Reagent on-board stability showed that the ARCHITECT Methotrexate reagents could remain on the analyzer for a minimum of 30 days with no more than 10% shift from baseline.

CONCLUSION: The ARCHITECT Methotrexate assay is an analytically accurate, precise, sensitive and robust assay for the measurement of methotrexate in human serum and plasma.

B-299

Use of Urine Drug Screening Positivity Rates to Evaluate the Clinical Impact of Replacing Immunoassay and Gas Chromatography Mass Spectrometry Testing with Liquid Chromatography Tandem Mass Spectrometry

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Background: Urine drug testing (UDT) is often performed to identify potential aberrant drug behaviors (e.g., drug addiction, abuse, misuse, diversion and/or non-compliance). Urine specimens referred to our regional reference laboratory by community-based independent physicians traditionally received UDT using a combination of immunoassay (IA) and gas chromatography mass spectrometry (GC-MS) testing. This tandem IA and GC-MS approach was replaced by a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) testing protocol in September 2013. Using defined positive/negative cut-off concentrations, this LC-MS/MS method tests for the urinary presence of 63 different licit and illicit drugs and was primarily designed to identify specific opioid and benzodiazepine use. As a quality measure, the efficacy of this new approach to UDT was reviewed post-implementation. UDT positivity rates were used to characterize the prevalence of licit and illicit drugs respectively detected by IA+ GC-MS and LC-MS/MS testing and evaluate the clinical impact of the testing methodology change.

Methods: Urine drug testing results from 134377 and 165209 unique specimens respectively tested by IA+GC-MS (from June 2012 to August 2013) and LC-MS/MS (from September 2013 to December 2014) were retrospectively reviewed. No clinical histories or medication lists were available for either heterogeneous patient cohort. The percent positivity rates for all analytes detected by the IA+GC-MS and LC-MS/MS UDT methods were determined. The relative reported prevalence of analytes contained within both protocols was directly compared. Method specific drug positivity rates were also tabulated to quantify the detection sensitivities for analytes unique to each procedure. IA-based testing included: benzodiazepines; cannabinoids; cocaine; ethanol; opiates; and oxycodone. GC-MS and LC-MS/MS sample pretreatment included: solid-phase extraction and chemical derivatization; and β-glucuronidase incubation and protein precipitation.

Results: Positivity rates for opiate and benzodiazepine IA testing were 22.9 and 8.6%, respectively. LC-MS/MS UDT yielded a relatively higher number of positive test results for the individual opioid and benzodiazepine-related analytes contained in both chromatographic methods. The GC-MS vs. LC-MS/MS positivity rates for selected analytes were: methadone+EDDP, 41.9 vs. 51.4%; oxycodone, 15.9 vs. 16.2%; codeine+morphine+hydromorphone, 11.1 vs. 21.4%; fentanyl+norfentanyl, 3.9 vs. 6.4%; buprenorphine+norbuprenorphine, 0.01 vs. 8.9%; diazepam+nordiazepam+oxazepam+temazepam, 0.01 vs. 7.3%; clonazepam+7-aminoclonazepam, <0.01 vs. 9.0%; and lorazepam, <0.01 vs. 4.9%. Higher LC-MS/MS positivity rates were also generally observed for stimulant, antidepressant and anesthetic drugs common to both methods. The GC-MS testing protocol identified the presence of multiple unique antidepressant, cardiac, antipsychotic and sedative drugs that were not included in the targeted LC-MS/MS method. Their collective positivity rate was ≤4.1%. Evidence of cannabinoid and cocaine-use was respectively detected by IA+GC/MS and LC-MS/MS testing in 30.0 and 41.3% and 7.8 and 10.5% of all tested specimens.

Conclusion: Replacing our combined IA+ GC-MS UDT procedure with a LC-MS/MS protocol increased the detection sensitivity for the targeted licit and illicit drugs but reduced the overall number of drugs that could be detected. Drug positivity rates can be used to identify the impact of a UDT methodology change to patient management. The study's respective drug prevalence can also be used towards the development of LC-MS/MS UDT methods.

B-300

Inappropriate Use of Suboxone® Film to Pass Drug Testing with Cross-Talk to 6-MAM

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Background: Suboxone® is a commercial preparation of buprenorphine and naloxone imbedded in film for administration via sublingual application to achieve better bioavailability than oral administration. Typically, patients receiving Suboxone provide urine specimens with low or undetectable naloxone and buprenorphine. Instead, norbuprenorphine, an active metabolite of buprenorphine, is detectable for

1-2 days. Thus, we were concerned when specimens from three patients each showed the presence of both naloxone and buprenorphine at very high concentrations and no detectable norbuprenorphine. Surprisingly, all three urine specimens also had a low but detectable 6-monacetylmorphine (6-MAM) peak, but no evidence of morphine, which would be expected if heroin had been used. The objective of this study was to investigate suspected specimen adulteration, which was indicated by the presence of the unusual metabolite patterns observed in all three samples.

Methods: All samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a pain management panel capable of detecting and quantifying buprenorphine, naloxone, norbuprenorphine, 6-MAM and morphine, in addition to many additional compounds. Each compound is identified and quantified by two ions using multiple reaction monitoring (MRM) and a unique retention time (RT). The relevant parameters for this study were: buprenorphine, MRM 468.3-396.2 and 468.3-414.3, RT 5.63 min; norbuprenorphine, MRM 414.3-101.1 and 414.3-165.2, RT 4.66 min; naloxone, MRM 328.2-212.0 and 328.1-253.0, RT 3.91 min; and 6-MAM, MRM 328.1-165.1 and 328.1-211.1, RT 3.74 min. Suboxone film was provided by our pharmacy to investigate the possibility of direct addition into the specimens by dipping the film into drug-free urine and assaying the sample by the LC-MS/MS method.

Results: Following immersion of Suboxone film into drug-free urine, we found high concentrations of buprenorphine and naloxone and no evidence of the norbuprenorphine metabolite. Additionally, a small peak associated with 6-MAM was observed, similar to the patient samples as noted above. To confirm cross-talk as a possibility we assayed a naloxone standard (5000 ng/mL) and identified a peak corresponding to 6-MAM. The 6-MAM quantified at a concentration of 85 ng/mL, with appropriate ion ratios; however, the retention time was 3.84 min, shifted from the expected retention time of 3.74 min. It is interesting to note cross-talk the other way was also observed. Injecting 6-MAM (5000 ng/mL) resulted in a detectable signal of naloxone. However, the ion ratios between the two quantifying and qualifying MRMs for naloxone were discrepant: The ion ratio of the ions [MRM 1/MRM 2] for the naloxone control and the cross-talk peak were 1.068 and 0.272 respectively making this non-reportable.

Conclusion: Immersion of Suboxone film into urine samples produces an atypical metabolite pattern of elevated buprenorphine and naloxone with undetectable norbuprenorphine. Naloxone, which is isobaric with 6-MAM, appears to interfere with the MRM's selected for 6-MAM in our assay. This interference is approximately 1.7% of naloxone. Thus, we have identified an unusual metabolite pattern in our assay of buprenorphine, naloxone, and 6-MAM without additional detectable metabolites, which strongly suggests specimen adulteration by directly adding Suboxone to urine.

B-301

Tobramycin Level Monitoring - Discrepancy Between Immunoassays

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Objective and Relevance: Tobramycin, an aminoglycoside, is used primarily to treat gram-negative bacterial infections. Therapeutic drug monitoring is an essential component of clinical therapy. Various immunoassay techniques with applications on many clinical chemistry analyzers have been developed for this purpose. A local, preliminary inter-laboratory comparison demonstrated a bias >30.0% between two such methods. Subsequently, a research plan was devised to investigate performance of tobramycin immunoassays used in Alberta clinical laboratories.

Methodology: Drug-free plasma and serum pools were prepared and spiked with tobramycin stock (100 mg/L) to obtain concentrations of 0.5, 2.0, 5.0 and 10.0 mg/L. Aliquots of each pool (0.5 mL) and aliquots of drug-free serum and plasma were distributed to four Alberta Health Services laboratories. In addition, Validate® linearity testing material (Maine Standards Company, Cumberland Foreside, ME, USA) was purchased, aliquoted and distributed for analysis. Target concentrations (independent of instrumentation) were graciously provided by Maine Standards. The lowest (0.6 mg/L) and highest (11.8 mg/L) concentration Validate linearity samples are prepared gravimetrically and three intermediate concentrations by mixing appropriate proportions of low and high material. Tobramycin analyses were performed as follows: Laboratory A - Beckman Coulter DxC 800 analyzer (Brea, CA, USA) using a particle-enhanced turbidimetric inhibition immunoassay (PETINIA); Laboratories B and C - Siemens Dimension Vista analyzers (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) by a PETINIA assay; Laboratory D - Roche cobas c501 analyzer (Hitachi High-Technologies Corporation, Tokyo, JP) using a homogenous enzyme immunoassay (HEIA) method. Analyses were carried out within 48h of sample preparation.

Results: The Beckman Coulter DxC800 PETINIA assay gave results within 10.0% of target for all spiked samples with one exception. Serum and plasma samples targeted at 0.5 mg/L produced results less than the assay limit of quantitation (0.5 mg/L), subsequently producing bias greater than 10.0%. Biases on the Siemens Vista PETINIA assay ranged from -12.0 to -40.0% (Laboratory B) and -20.0 to -25.0% (Laboratory C). Results from the Roche cobas c501 HEIA assay demonstrated biases of +20.0% and +40.0% at 0.5 mg/L in plasma and serum respectively and biases ranging from -5.0% to -18.0% in the remaining samples. Analysis of the Validate linearity samples produced similar results. The Beckman PETINIA assay gave results within 7.0% of target for all samples with one exception (0.6 mg/L target: +16.7%). Results from the Siemens PETINIA instruments ranged from -11.8 to -31.4% (Laboratory B) and -33.3 to -41.2% (Laboratory C) of target. The Roche HEIA assay gave results from -17.6 to -26.3% of target with one exception (0.6 mg/L: +16.7%). However, 2013-2014 data from the New York State Therapeutic Substance Monitoring Proficiency Testing Program (n=30 samples) demonstrated average biases from gravimetric target of 15%, -9% and -4% for the Beckman Coulter DxC 800, Siemens Dimension Vista and the Roche cobas c501 analyzers respectively.

Conclusions: Different methods for assessing accuracy of tobramycin immunoassays give inconsistent results. Diagnostic companies should use "gold standard" methods (e.g. tandem mass spectrometry) to validate the accuracy of their tobramycin assays. This will prevent misguided dosage adjustments and potential undesirable clinical consequences for patients treated with tobramycin.

B-302

A Fast, Sensitive, and High-Throughput LC-MS/MS Assay for Benzodiazepines/Z-Drugs/Barbiturates

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Background:

Benzodiazepines, Z-drugs, and barbiturates belong to a group of psychotropic drugs that are often prescribed for the treatment of anxiety, depression, and insomnia. However, as controlled substances, these drugs have the potential for overdosage or abuse. The development of fast and accurate methods for the screening and confirmation analysis of these drugs therefore becomes very critical in toxicological, clinical, and forensic laboratories. LC-MS/MS offers superior sensitivity, selectivity, and robustness for simultaneously detecting benzodiazepines and non-benzodiazepines in complex biological matrices. The current work presents a fast, reliable, and accurate LC-MS/MS method on an IONICS 3Q 120 triple quadrupole mass spectrometer with Restek Biphenyl column for the analysis of a total of 43 compounds, using fast polarity switching.

Methods:

The mixed drug standard solutions and analytical LC column were obtained courtesy of Restek. The mixed drug standard solutions were further diluted with 50/50 mobile phase A (100% H2O, 0.1% formic acid) and mobile phase B (100% Methanol, 0.1% formic acid) to make a series of concentrations ranging from 0.024-50 ng/mL for benzodiazepines, Z-drugs and other anxiolytic/sedatives/muscle relaxants, and 0.24-500 ng/mL for barbiturates. An IONICS 3Q 120 mass spectrometer, equipped with a heated coaxial flow ion source and Hot Source-Induce Desolvation (HSIDTM) interface was used for the best ionization and sampling efficiencies. Electrospray ionization was used for this analysis. The time-managed MRM in MolanaTM software was used to optimize the dwell time for each compound based on the retention times and the number of MRM transitions within given experiments. Fast polarity switching allowed for simultaneous analysis of positive and negative ions within a single run. A Shimadzu Prominence LC system was used. Restek Raptor Biphenyl column (50X2.1mm, 2.7um) gave good separation and nice peak shapes in the chromatogram. The injection volume used was 10 µL. A gradient method was created with a flow rate of 0.6 mL/min and a total LC cycle time of 6.5 minutes.

Results:

The Restek Raptor Biphenyl column (50X2.1mm, 2.7um) gave good separation and nice peak shapes in the chromatogram. A gradient method was created with a flow rate of 0.6 mL/min and a total LC cycle time of 6.5 minutes. All 43 compounds eluted within this 6.5 minutes run time. No carryover was detected in the blank injections immediately following the upper level calibration samples. The calibration curves showed good linearity for all the analytes across the full concentration range with coefficients R2>0.996. All calibration curves used a linear weighting regression of 1/x. The LLOQs for all 43 drugs were in the range of 0.024 to 4 ng/mL, which is much lower than the typical confirmation cutoff concentration (50 ng/mL) for most of

the drugs. At the LLOQs, the accuracy was between 83-116%, and CVs were <14% for all analytes.

Conclusion:

These results clearly demonstrate that this LC-MS/MS method using the IONICS 3Q 120 mass spectrometer and Restek Raptor Biphenyl column can provide a fast, accurate and high throughput solution for clinical pain management to monitor patient drug use and program adherence or for drugs of abuse or other workplace drug testing.

B-303

An Evaluation of the Rapid MEDTOXScan Drug Screening Method for Emergency Department Patients Presenting at Satellite Hospitals

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Objective: While rapid drug screening is routinely available in large medical center core laboratories, satellite hospitals must rely on accurate point-of-care (POC) drug screening methods for emergency department (ED) patients. However, with adoption of such drug screening methods, care must be taken to insure that results obtained correlate closely with those observed in the core laboratory. Indeed, due to variations in methodologies, cross-reactivites, and cutoffs, a clear understanding of the degree of agreement between POC testing and core lab drug screening is essential. Hence, we present a comparison study of a rapid POC drug screen method with our existing core laboratory ultraviolet kinetic method.

Method: We examined 71 ED patient samples (351 individual drug assays) with the PROFILE®-V MEDTOXScan® Drugs of Abuse Test System (MEDTOX, Burlington, NC) a one step, rapid, qualitative immunochromatographic test with results in 10 minutes comparing the latter with the ADVIA® 1800 Clinical Chemistry System (Siemens, Malvern, PA). Eleven drug classes were tested including amphetamine(AMP), barbiturate(BAR), benzodiazepines(BZO), cocaine(COC), methamphetamine(MAMP), opiates(OPI) including methadone(MTD) and oxycodone, phencyclidine(PCP), tricyclics(TCA), and cannabinoids(THC). Data were statistically analyzed using chi-square, Fisher's exact test, and McNemar's exact test.

Results: While we observed 17(4.8%) discrepant results in 351 individual drug assays, statistically there were no significant differences ($p>0.05$) between individual and pooled MEDTOX and ADVIA tests (see table). Also, MEDTOX appeared to be more sensitive than ADVIA for BZO, oxycodone, TCA, and MAMP. Discrepancies between methods were largely due to differences in cutoff levels with cross-reactivity differences also playing a role.

Conclusions: Rapid urine testing for drugs of abuse plays an essential role in ED patient care regardless of the hospital setting. We observed that the MEDTOX method for drug screening on ED patients seen at our satellite hospitals compares favorably with results obtained on our presumably more sophisticated ultraviolet core laboratory method.

Statistical Comparison of MEDTOX Versus Advia						
Drug	Total Discrepant	MEDTOX + Advia -	Advia + MED-TOX -	McNemars's Test	Chi-Square	Comment
Benzodiaz-epines	4/71	4	0	P=0.13	P=0.22	MEDTOX cutoff 150 ng/mL; Advia 200 ng/mL
Oxycodone	3/71	3	0	P=0.25	P=0.34	MEDTOX cutoff 100 ng/mL; Advia 300 ng/mL
Tricyclics	2/71	2	0	P=0.50	P=0.69	Unresolved Discrepancy
Metham-phetamine	4/71	3	1	P=0.63	P=0.67	MEDTOX cutoff 500 ng/mL; Advia 1,000 ng/mL
Amphet-amine	1/71	0	1	P=1.0	P=1.0	Unresolved Discrepancy
Opiate	3/71	0	3	P=0.25	P=0.46	Possible crossreactiv-ity differ-ences
Total Dis-crepancies Individual Assays	Total Cases with Discrepan-cies	MEDTOX + Advia -	Advia + MED-TOX -	McNemar's Test	Chi-Square	
17/351 (4.8%)	17/71 (23.9%)	12	5	P=0.14	P=0.27	

B-304

Monitoring two transitions by LC-MS/MS is not always adequate to identify benzoylecgonine in patient urine samples

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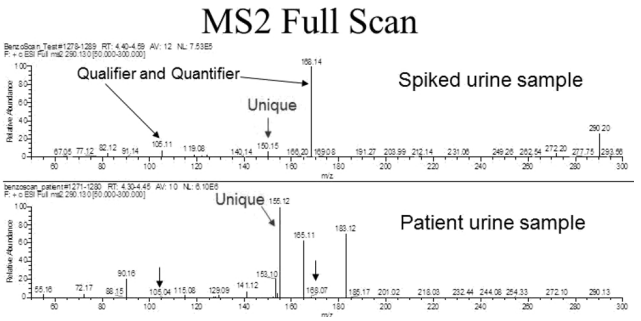
Background: Cocaine has a very short half-life in humans and measuring its major metabolite, benzoylecgonine (BZE) is routinely used to detect cocaine use. LC-MS/MS methods are highly specific and are used to confirm the presence of drugs or metabolites, such as BZE. As such, multiple reaction monitoring (MRM) is frequently used on the MS to monitor fragments resulted from a specific precursor. Typically one precursor ion to two fragment ions is utilized for quantitation and identification. Identification is achieved by calculating the ion ratio of the two ions. In our experience of BZE analysis by LC-MS/MS, some pain management doctors and patients questioned the accuracy of BZE identification. The aim of this study was to explore whether we may improve the accuracy of BZE identification by adding additional transitions. **Design:** Our routine LC-MS/MS method monitors two MRM transitions for BZE, 290→168 as quantifier and 290→105 as qualifier. MS2 Full scan was performed on both a sample spiked with BZE at 45 ng/mL and a patient sample with BZE identified but questioned by the ordering physician. **Results:** The MS2 full scan showed that in the spiked BZE sample, both 290→168 and 290→105 were present. An additional MRM of 290→150 was also present. In the MS2 full scan of the questionable patient sample, both 290→168 and 290→105 were present. However, 290→150 was absent while 290→155 was present. The unique MRMs 290→150 and 290→155 led us to conclude that the patient sample was not BZE. **Conclusion:** Although it is generally believed that monitoring 2 MRM transitions is sufficient for analyte identification it may not be adequate to accurately identify BZE in certain patient samples.



B-305

Reducing excessive inpatient phenytoin level orders - a test utilization review

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Background: Therapeutic drug monitoring (TDM) of phenytoin is critical as it displays non-linear elimination, has a narrow therapeutic index, has many drug interactions, and is 90% protein bound. Phenytoin also has a long half-life (24 ±12 hrs), and will reach steady state only after five to ten days following dose adjustment.

We noticed highly variable ordering patterns for serum phenytoin concentrations at our institution. Some samples were repeatedly drawn within hours and others up to two days following a dose adjustment. We even noted several cases of daily phenytoin (Total and Free) orders continuing for greater than 1 week, despite results indicating therapeutic concentrations or no change in clinical status. Repeated measurements prior to reaching steady state can potentially result in premature phenytoin dose adjustments and unnecessary blood draws.

Objective: Our objective was to develop institutional guidelines for the monitoring of phenytoin concentration in hospitalized patients. Our goal was to decrease unnecessary testing of both total and free phenytoin levels without adversely affecting patient care.

Methods: We began by examining every total and free phenytoin order on inpatients from August to September 2014. Next, we designed and initiated phenytoin monitoring guidelines that were created by a combined effort from the pathology, neurology and pharmacy departments on October 1, 2014. We then assessed the results of the intervention by recording all total and free phenytoin orders on inpatients over a four-month span.

Results: In the eight weeks prior to distributing our guidelines, there were a total of 227 total phenytoin and 139 free phenytoin orders on 42 different patients. After instituting our guidelines, there were a total of 128 total phenytoin and 43 free phenytoin orders on 50 different patients in a four-month span. Using the Mann-Whitney U test, the differences were significant ($p<0.001$) for both total and free phenytoin orders. The number of tests ordered per admission was 4.73 for total phenytoin and 2.90 for free phenytoin before institution of guidelines. Post-implementation, both the average per-admission total and free phenytoin orders decreased significantly to 2.46 and 0.83 ($p<0.05$), respectively. The number of total and free phenytoin results within therapeutic range did not change significantly post-intervention ($p=0.32$, $p=0.28$) nor were there differences in the number of phenytoin dose adjustments per admission ($p=0.078$).

Conclusion: Through a collaborative effort, we were able to successfully decrease the number of unnecessary total and free phenytoin tests ordered. With our data, we estimate that our guidelines will save each patient 12 mL of unnecessary blood loss per admission. We also calculated that our guidelines decreased patient billing by $42,000 over a four-month span. Phenytoin therapy requires monitoring using serum levels, but with a more standardized approach, we can both reduce the cost and improve the quality of care by reducing unnecessary blood draws.

B-306

A Novel Liquid Chromatography Tandem Mass Spectrometry Method for the Simultaneous Quantification of 44 Drugs/Metabolites in Meconium

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Background: Illicit drug use during pregnancy is a major social and medical issue. Meconium is advantageous for detecting prenatal drug exposure. The aim of this study was the development of an LCMSMS procedure for quantifying drugs and metabolites likely to be encountered in meconium.

Method: Patient samples (0.05±0.005 g) were weighed into glass vials. Fifty microliters of a 100 µg/mL internal standard, containing deuterated standards in methanol, was added followed by 1.5 mL of a 0.1M sodium acetate buffer (pH 5.1). The mixture was homogenized for 30 s, transferred to a 1.5 mL tube, and centrifuged at 13,000 rpm for 5 min at room temperature. The supernatant was transferred to a clean glass tube containing 1g of a buffered salts mixture (sodium chloride, sodium carbonate, sodium bicarbonate, 1:1:12 ratio,w/w) and 3 mL of an extraction solvent (methylene chloride, cyclohexane, isopropanol, 9:9:2 ratio,v/v). Tubes were inverted 5 to 10 times. The mixture was centrifuged at 3,500 rpm for 5 min at room emperature. Centrifugation was repeated until the buffy coat wasn't visible. The supernatant was transferred to a clean glass tube and gently evaporated to dryness at 40°C using a gentle stream of air. The extracted samples were reconstituted with 200 µL methanol, vortex mixed, allowed to stand at room temperature for 5 min, and evaporated to dryness at 40°C. Samples were reconstituted with 150 µL of mobile phase A, transferred to autosampler vials, and injected onto a RESTEK Ultra bi-phenyl analytical column (5µm, 50 x 2.1 mm) maintained at ambient temperature. Mobile phase A was 0.1% formic acid in water (1:1, v/v). Mobile phase B was 0.1% formic acid in acetonitrile (1:1, v/v). The acquisition method utilized 10 µL injection volume, 0.6 mL/min flow rate, and a gradient program of 98% A, increased to 95% B over 5.5 min, held for 0.8 min, decreased to 2% B over 0.2 min, and re-equilibrated at 2% B for 1.5 min. Run time was 8 min (injection to injection). The HPLC system consisted of Shimadzu pumps and autosampler. MSMS was performed on a Sciex API 4500 triple quadruple mass spectrometer with an electrospray source monitored in positive and negative ion modes.

Results: Specificity was assessed by retention times and quantifier/qualifier transition peak area ratios. Total imprecision (%CV), assessed at two concentration levels and 80 observations, was <15% for all analytes. Linearities ranged from 0.6 up to 1250 ng/g. Except for m-hydroxybenzoylecgonine, extraction efficiencies/recoveries were all >60%. Matrix effects of native analytes were similar to corresponding deuterated analogues and did not affect quantification. No carryover, endogenous or exogenous interferences were observed. Qualitative correlation between our procedure and a commercial LC-MSMS method showed 100% agreement at cut-offs. Quantitative correlations were also excellent (<20% differences).

Conclusion: We present the development of a LC-MSMS procedure for the simultaneous quantification of 44 drugs/metabolites in meconium employing a small amount of a single sample, deuterated internal standards, and single extraction for all analytes - without derivatization, enzymatic hydrolysis, additional chromatographic resolution, or preliminary immunoassay screening. This novel method is suitable for routine clinical use.

B-307

Improved Sensitivity and Throughput for the Quantification of Buprenorphine, Norbuprenorphine and Naloxone in Human Oral Fluid by LC-MS/MS.

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Background

Buprenorphine belongs to a class of medications called opioid partial agonist-antagonists while naloxone belongs to a class of medications called opioid antagonists. Buprenorphine is primarily metabolized in the liver by dealkylation to form the active metabolite norbuprenorphine. The combination of buprenorphine and naloxone (suboxone) is used to treat opioid dependence by preventing withdrawal symptoms when someone stops taking opioid drugs. However, suboxone produces similar effects to opioid drugs. Consequently, there is an urgent need to develop a sensitive and selective method to simultaneously quantify buprenorphine, norbuprenorphine and naloxone in human oral fluid. Utilizing a highly sensitive triple quadrupole mass spectrometer, our laboratory has developed a quantitative LC-MS/MS method with a limit of quantitation lowered to 20 pg/mL for buprenorphine.

Methods

Oral fluid samples were collected in Quantisal sampling devices from Immunalysis and diluted 2.5 times in a solution containing the internal standards. Considering the 4 times sample dilution in Quantisal, samples were finally diluted 10 times before injection into the mass spectrometer. Samples were analyzed by LC-MS/MS on an ABSciex Triple Quad 6500 System using an Agilent 1260 chromatographic System and a Phenomex Kinetex 2.6 µm Phenyl-Hexyl (50 x 2.1 mm) column. The total run time was 3.5 min and no sample clean-up or extraction was performed. Mass spectral data were obtained in positive electrospray mode. Detection and quantitation were performed by MRM of at least two transitions for each analyte and one transition for each internal standard.

Results

Seven-point calibration curves generated for buprenorphine (0.02-16 ng/mL), norbuprenorphine (0.05-40 ng/mL) and naloxone (0.5-400 ng/mL) with duplicate injections using 1/x2 weighting showed a good linearity (R2 ≥ 0.99). No significant matrix effects were observed and no interferences were observed from common pain medications. This method was applied to the quantification of buprenorphine, norbuprenorphine and naloxone from patient oral fluids. The liquid chromatography Phenyl-Hexyl column showed stable performance throughout the application and the Triple Quad 6500 mass spectrometer showed high sensitivity and reproducibility. This study demonstrates that the application of LC-MS/MS dilute and shoot method to the analysis of oral fluid samples reduces time and labor spent on sample preparation. In addition, this method provides the means to offer a faster, more sensitive and sample-conserving assay to clinicians.

B-308

Determination of Hydrogen cyanide in biological and non-biological matrices by Headspace Gas Chromatograph coupled to Flame-Ionization Detector Technique.

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A simple, rapid and reliable method for the quantitation of cyanide was developed on headspace gas chromatograph coupled to flame ionization detector using HP-Innovax (Polyethylene glycol bonded) column on an Agilent 7890A GC. Cyanide in blood or other matrices was liberated by the conversion of Potassium cyanide to the volatile Hydrogen cyanide (HCN) through the addition of 5N Sulfuric acid in headspace vial and analyzed using Agilent G1888 headspace auto sampler. HCN gas diffuses into the headspace above the specimen in the sealed headspace vial based on Henry's Law of partial pressure. The method showed good linearity in the range of 2.0-8.0ug/mL with correlation (R2 = 0.999) in the calibration curve and the detection limit was 1.0 ug/mL. Accuracy of the method at three different concentrations (2.0, 6.0, 8.0 ug/mL) was in the range of 94.5% to101.9% whereas the day-to-day precision at these three concentrations was in the range of 1.3% - 3.9%. Identical calibration curves with coefficient of correlation above 0.99 were obtained when blood, gastric contents and water were used as the calibration standard matrices. Therefore, single calibration curve is sufficient for diverse matrices and preparation of matrix-matched standards is not required. The method showed successful quantitation of Hydrogen cyanide in gastric contents, which is one of the most variable matrices in clinical and forensic toxicology. The method is well adopted because of its wide linear range, its adaptability to different biological and non biological matrices and its ease and rapidity of use.

B-309

Determination of methyl ethyl ketone (MEK) in urine by headspace gas chromatography autosampler and flame ionization detector - GC / FID-HS.

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The determination and quantification of toxic volatiles are extremely important in clinical analysis, which involves the routine monitoring of industrial workers to check the exposure to potentially toxic components and the detection of endogenous metabolites in the body in certain clinical diagnoses.

The main action of MEK in the human body is depression of the central nervous system, producing narcosis potentiating the toxicity of other solvents, particularly carbon tetrachloride hepatotoxicity and neurotoxicity hexane (inhibition of the biotransformation metabolite 2,5- cyclohexanedione).

The MEK is a volatile substance widely used in the footwear industry, furniture, chemicals, paints, wood processing, among others. It is also used as solvent in paint removers, adhesives and polymer coatings. The MEK excreted in the urine can be used as an indicator for evaluation of occupational exposure.

The aim of this study was to validate a simple, rapid and sensitive method for the determination of MEK in urine by headspace gas chromatography with flame ionization detection.

The method consist in a simple extraction of MEK using 2-propanol as internal standard by evaporationand sampling the vapor above the fluid (blood, urine or others) after reached thermal equilibrium gas in a closed vial. The volatilized components present in headspace are aspirated by a syringe and injected into chromatograph.

The extraction involves the addition of 5 ml diluent solution containing the internal standard and 1 mL of the sample, standard, or control into a vial. The vial is shaken for 30 seconds on vortex. Chromatographic separation was performed on PerkinEkmer BAC 1: 450°C: 30m x 320µm x 1.8 µm column and mobile phase Nitrogen 99.999%. The chromatographic running time is approximately 6.0 minutes.

The parameters evaluated in the validation were selectivity, linearity, accuracy, precision, repeatability and reproducibility, detection limit, quantification limit and matrix effect. The calibration curves for all compounds were linear with $r^2 > 0.9993$, The linear analytical range of the procedure was between 1.0 and 6.0 mg/L. Accuracy (99.7-101.9%), intra-assay precision (0.8-5.17%) and inter-assay precision (2.8-4.3%) were acceptable. The determination limit was 0.1mg/L and the quantification limit was 0.2 mg/L. The method was applied to the measurement of MEK in urine of a pool contaminated with MEK. In conclusion, the GC / FID-HS method has been developed successfully for monitoring occupational exposure ofindustrial workers and the quantitative analysis of MEK.

B-310

Triazole Antifungal Drug Monitoring in Serum using LC-MS/MS

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Background: Invasive fungal infections are increasing in both number and scope with immunocompromised and critically ill patients. Therapeutic drug monitoring (TDM) of some antifungals can assist clinicians with dose optimization and improve outcomes. While not all antifungals benefit from TDM, those with concentration-dependent toxicity or variations in absorption, metabolism, or other pharmacokinetic factors are candidates for TDM. The University of Wisconsin Hospital and Clinics Toxicology group has developed an assay which will monitor three triazole antifungals (posaconazole, voriconazole, and itraconazole) and one active metabolite of itraconazole (hydroxyitraconazole) in serum using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods: Patient serum is mixed 1:3 with acetonitrile containing 350 ng/mL of all four deuterium labeled compounds as internal standards. The sample is centrifuged and an aliquot is passed through a Supelco Hypersil phospholipid column. This sample is then diluted with 400 µL of acetonitrile and 5 µL is injected onto the LC-MS/MS. Separation occurs using a Kinetex C18 column (100x3.0 cm) with 10 mM ammonium formate/0.1% formic acid as Buffer A and 0.1% formic acid in acetonitrile as Buffer B. The gradient is 70% to 0% Buffer A over seven minutes. The drugs are quantitated on an API4000 tandem mass spectrometer with twelve different ion transitions monitored using multiple reaction monitoring (MRM) corresponding to two MRMs per drug or metabolite and one for each internal standard. All stock standards were purchased from Toronto Research Company (Toronto, Ontario Canada) and Cerilliant (Round Rock, Texas).

Results:

The analytical measurement range (AMR) for posaconazole is 0.1-4 µg/mL with a limit of detection (LOD) of less than 0.025 µg/mL. The intra and inter-assay precision was less than 2.2% and 2.8% respectively. Correlation of this method with a reference lab had a regression of y=1.0642x +0.0827, and correlation coefficient (R^2)=0.991.

The AMR for voriconazole is 0.2-8 µg/mL with a LOD of less than 0.05 µg/mL. The intra and inter-assay precision was less than 0.6% and 1.6% respectively. Correlation of this method with a reference lab had a regression of y=1.003x +0.131, and R^2=0.997.

The AMR for itraconazole is 0.2-8 µg/mL with a LOD of less than 0.05 µg/mL. The intra and inter-assay precision was less than 0.8% and 3.5% respectively.

Correlation of this method with a reference lab had a regression of y=1.0054x +0.0451, and R^2=0.992.

The AMR for hydroxyitraconazole is 0.2-8 µg/mL with a LOD of less than 0.05 µg/mL. The intra and inter-assay precision was less than 1.8% and 3.0% respectively. Correlation of this method with a reference lab had a regression of y=0.9948x -0.0338, and R^2=0.987.

Standard addition experiments with high hemolysis and bilirubin showed no interference. However, grossly lipemic samples showed a bias to under report the antifungal concentrations by as much as 15% which could be due to under pipetting serum.

Conclusion: A new antifungal assay was developed to better monitor drug therapy in immunosuppressed and critically ill patients. This allows faster turnaround time and quicker response to invasive fungal infections.

B-311

The Application of QuEChERS, a Novel Sample Preparation Technique for the Quantitative Determination of Benzodiazepines and Anabolic Steroids in Whole Blood

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Background: In clinical laboratories, the commonly used sample preparation techniques include immunoassay, liquid-liquid extraction (LLE) and solid phase extraction (SPE). QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) is a novel sample preparation technique that was originally reported by the scientists at the USDA in 2003 for the determination of multi-class pesticide residues in fruits and vegetables. Since then, QuEChERS has been adopted quickly and widely to determine various analytes of interest in different matrices, such as veterinary drugs in animal tissues, mycotoxins in grains, polycyclic aromatic hydrocarbons in seafood, bisphenol A in canned foods and beverages, and cannabinoids in medical marijuana and cannabis foods. In this study, QuEChERS methodology will be applied for the analysis of clinical therapeutic drugs, such as benzodiazepines and anabolic steroids in whole blood.

Method: Add 2 mL extraction solvent (e.g. acetonitrile) with internal standards (optional) to a 15-mL centrifuge tube with pre-packed extraction salts (e.g. magnesium sulfate and sodium chloride), add 1 mL whole blood to the centrifuge tube, shake and centrifuge. After centrifugation, the proteins, blood cells and some un-dissolved extraction salts remain in the bottom of the centrifuge tube, while the target analytes are extracted into the upper, clear solvent layer. For sample cleanup, transfer 1 mL of the blood extract to a 2-mL centrifuge tube containing SPE sorbents (e.g. PSA and C18). The matrix co-extractives, such as organic acids and lipids, are retained on the sorbents which results in a purified extract for instrumental analysis.

Results: Matrix matched calibration curves were constructed for analyte quantification. The responses for 10 representative benzodiazepines and 12 steroids were linear with R2 ranged from 0.9963 to 1.0000 over the concentration range of 10 - 500 ng/mL. The matrix effect was evaluated by comparing the slopes of the matrix matched calibration curves to those of the calibration curves of solvent standards. The matrix effect was found to be insignificant, from -22 to 18%, which indicated that the QuEChERS method effectively removed the matrix interferences that may cause significant ion suppression or enhancement. Excellent analyte recoveries (81.4- 105%) and relative standard deviations (RSD% ≤ 10%) were obtained. This method was applied to 6 real whole blood samples, no target analytes were detected above the limit of quantitation of 10 ng/mL.

Conclusion: A novel sample preparation technique, QuEChERS, has been demonstrated to be simple, fast, and effective for the quantitative determination of benzodiazepines and anabolic steroids in whole blood. This provides clinical laboratories a successful alternative sample preparation method for drug assays in whole blood samples.

B-312

Measurement of synthetic cannabinoids in human urine by liquid chromatography-tandem mass spectrometry

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Background:

Synthetic cannabinoids (i.e., K2, Spice) were originally designed as research tools to aid in the investigation of the endocannabinoid system due to their ability to bind to cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2) receptors. Often referred to as herbal incense or potpourri, synthetic cannabinoids are gaining in popularity among recreational drug users as an inexpensive and "legal" alternative to marijuana. Synthetic cannabinoids do have some effects common to that of marijuana and its primary psychoactive compound, Δ^9-tetrahydrocannabinol (THC), with a higher degree of intoxication associated with their consumption. Users of synthetic cannabinoids however may demonstrate more serious side effects such as hypertension, hallucinations, tachycardia, sinus bradycardia, chest pain, dysrhythmias, seizures and even death. The metabolism of synthetic cannabinoids occurs via cytochrome P450 enzymes and generally includes either hydroxylation and/or dehalogenation with excretion in the urine as glucuronide conjugates.

Methods:

Deuterated stable isotopes were added to 500µL of urine as internal standards. Ammonium acetate buffer (0.5M, pH = 5.0) and beta-glucuronidase were then added and this mixture was incubated at 50ºC for 30 minutes. The synthetic cannabinoids and internal standards were extracted by solid phase extraction using Bound Elut SPEC C18 3mL (15mg) columns (Agilent Technologies, Santa Clara, CA.). The samples underwent separation via liquid chromatography using a Kinetex™ 5µm C18 50x4.6mm column (Phenomenex, Torrance, CA) on a TLX4 high-throughput liquid chromatography system (Thermo Fisher Scientific, Waltham, MA), followed by analysis on a tandem mass spectrometer (6500 QTRAP, AB SCIEX, Foster City, CA) equipped with an electrospray ionization source in positive mode. Ion transitions were monitored by multiple reaction monitoring (MRM) mode.

With the exception of JWH-073, metabolites that are structural isomers were not chromatographically separated. Chromatographic separation was deemed unnecessary as the clinical interpretation is identical and independent of which isomer is detected. The above method includes detection of JWH-018 N-(4/5-hydroxypentyl), JWH-073 N-(3-hydroxybutyl), JWH-073 N-(4-hydroxybutyl), JWH-122 N-(4/5-hydroxypentyl), JWH-210 N-(4/5-hydroxypentyl), JWH-250 N-(4/5-hydroxypentyl), AM2201 N-(4-hydroxypentyl), RCS-4 N-(4/5-hydroxypentyl), UR-144 N-(4/5-hydroxypentyl) and XLR11 N-(4-hydroxypentyl).

Results:

Baseline separation of all 10 analytes and their internal standards was achieved within 7 minutes. Method performance was demonstrated using precision, linearity, recovery and analytical sensitivity and specificity studies. Precision and linearity studies were performed using urine fortified with synthetic cannabinoid standard solutions. Intra-assay precision coefficients of variation (CVs) ranged from 2.0% to 4.7%. Inter-assay precision CVs ranged from 5.3% to 16.2%. Linearity was demonstrated for each analyte with the slopes of the equations ranging from 0.9842 to 1.0328 and correlation coefficients (R^2) ranging from 0.9989 to 0.9997. Recovery studies demonstrated averages of 96% to 102% from expected values. The assay is reported qualitatively with cutoffs ranging from 0.1 to 0.8ng/mL depending upon the analyte. To assess accuracy, comparison to an external reference laboratory was performed and demonstrated an overall agreement of 99%. Additionally specimens were shown to be stable under ambient, refrigerate and frozen conditions for up to 35 days.

Conclusion: This method provides for the simultaneous and reliable analysis of multiple synthetic cannabinoids in urine.

B-313

Performance Characterization of ARK Gabapentin Immunoassay on an Advia 1200 Analyzer

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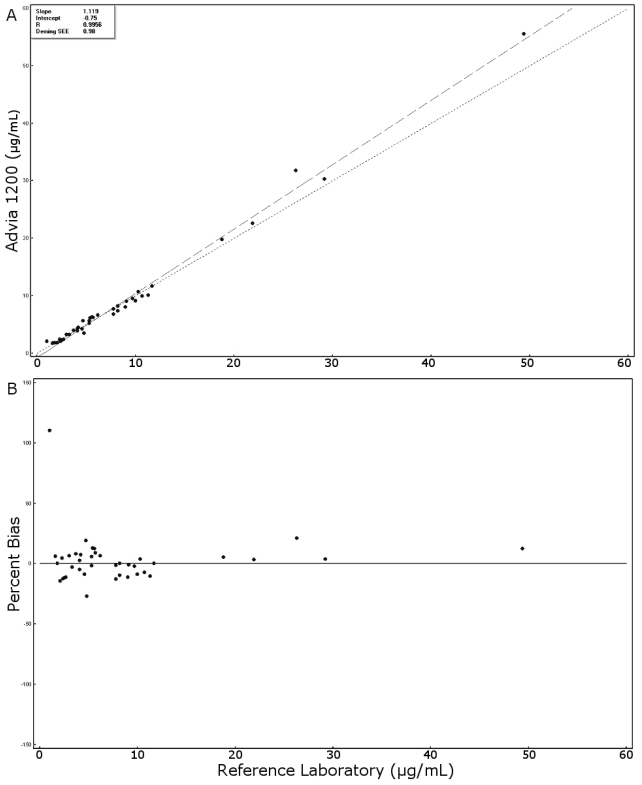
Background: Gabapentin is an anticonvulsant used for the treatment of epilepsy and neuropathic pain, and various off-label applications such as anxiety disorders and restless leg syndrome. Therapeutic drug monitoring of gabapentin is helpful for optimizing individual therapy, managing comedications, and assessing compliance. Our objective was to evaluate a gabapentin immunoassay (ARK Diagnostics, Sunnyvale, CA) on a Siemens ADVIA 1200 (Siemens Healthcare Diagnostics, Deerfield, IL). **Methods:** Linearity was assessed by spiking a left-over patient serum sample to a gabapentin level of 40 µg/mL followed by serial dilution with saline and analyzing the resulting specimens in triplicate. Intra-day precision was evaluated by analyzing three quality control materials included in the ARK reagent package for 10 replicates a day while inter-day precision was assessed by analyzing the three quality control materials once a day for 35 days. Accuracy was assessed by comparing results of split patient samples by the ARK immunoassay to a liquid chromatography tandem mass spectrometric method offered by an independent clinical laboratory (n=40). **Results:** The assay was linear from 0.8 to 40 µg/mL with recoveries ranging from 80.4% to 113.1%. No carryover was observed up to 101.0 µg/mL. Intra- and inter-day precision were less than 14.6% for all concentrations tested. The assay compared

favorably with the reference laboratory method with Deming regression parameters of slope 1.119 (95%CI 1.084 to 1.153), intercept -0.75 (-1.18 to -0.31) µg/mL, standard error of estimate 0.98 (Figure 1A), and mean bias

3.2% (Figure 1B). **Conclusion:** The gabapentin ARK Diagnostics immunoassay on Advia 1200 was validated for clinical use.



B-314

The Use of Biochip Array Technology for the Multi-Analytical Detection of Drugs of Abuse in Oral Fluid to Significantly Expand Current Test Menus

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Background: Drug detection involves initial screening of samples for drugs, and in medical applications the screening tests results are directly used for medical evaluation. For legal purposes, the screening procedure eliminates all negatives, and positive results are regarded as presumptive and require

confirmation using confirmatory methods such as high performance liquid

chromatography and mass spectrometry. Laboratory tests of oral fluid for drugs of abuse have continued to expand in the workplace as well as in legal and medical settings. The collection of oral fluid is simple and non-invasive and can be easily observed. Immunoassays are highly selective antibody-based tests that provide high throughput screening of a range of drugs and their metabolites in different matrices. To expand the screening capacity even further, biochip array technology has been used to provide multiplex screening of different drug classes from a single sample.This is relevant when the volume of sample available for analysis is limited. Biochip array technology employs competitive chemiluminescent immunoassays for drug testing. The immunoassays can be applied to several dedicated analysers.The automation facilitates the integrity, reliability and accuracy of the drug testing process. The aim of this study was to expand the test menu of drugs of abuse in oral fluid through the development of biochip arrays enabling the detection of 23 classes of drugs of abuse at a selected cut-off concentration relevant for an oral fluid testing laboratory.

Methods: Competitive chemiluminescent immunoassays were employed. The capture antibodies are immobilised and stabilised on the biochip surface defining microarrays of discrete test sites. The immunoassays were applied to the Evidence analyser. Two biochips were used for the semi- quantitative detection of 23 drug classes including: amphetamines, barbiturates, benzodiazepines, methadone, opiates, PCP, cocaine, oxycodone, propoxyphene, cannabinoids, fentanyl, buprenorphine, tramadol, meprobamate, synthetic cannabinoids, dextromethorphan, tricyclic antidepressants, meperidine, methylphenidate, mitragynine and ketamine. The Quantisal Oral Fluid Collection Device was used following manufacturer's instructions.

Results: The limit of detection (LOD) for all the drugs of abuse studied was determined by assessing 20 negative sample replicates and was calculated as the mean +3 standard deviations. For all drugs tested the calculated LOD was below the selected cut-off i.e. methamphetamine 0.59ng/mL (cut-off:20ng/mL) benzodiazepines 0.18ng/mL (cut-off:10ng/mL) fentanyl 0.07ng/mL (cut-off:1ng/mL) buprenorphine 0.05ng/ml (cut-off:1ng/mL). The % agreement with LC/MS was assessed for five drugs of abuse in authentic oral fluid samples and it was found to be 100% for buprenorphine (n=18), cocaine (n=33) and oxycodone (n=18), 94.4% for tramadol (n=18) and 91.7% for cannabinoids (n=60). In addition, samples containing all 23 drugs spiked below at and above the required cut-off were assessed and all samples were correctly classified as positive and negative. Samples collected with other collection devices (OraSure and Oral-Eze) were also assessed and the drugs studied were detected.

Conclusion: This study indicates applicability of biochip array technology to increase the screening capacity of drugs of abuse in oral fluid. Two biochip arrays utilising 25 µl sample volume each allowed the simultaneous detection of 23 classes of drugs of abuse present in oral fluid samples in 3 types of collection buffer.

B-315

Detection of cocaethylene and levamisole in cocaine users by ultra performance liquid chromatography-tandem mass spectrometry

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Background: Cocaine, a psychostimulant, is highly abused and accounts for one of the main reasons for drug related ER visits. To enhance its simulative properties, cocaine is often co-abused with alcohol. Cocaethyelene (CE), a metabolite of cocaine and alcohol, is a psychostimulant as well and leads to prolonged euphoria because of its longer half-life compared to cocaine. CE has well documented cardiotoxic effects, and thus, the risk of death is 18 times greater when cocaine and alcohol are abused together than each drug on its own. To enhance cocaine-induced euphoria, several adulterants are used, one such adulterant is levamisole (LEV). It is historically a veterinary antihelminthic and has been recently determined as cocaine adulterant and to cause severe adverse reactions in cocaine users. Because of the highly toxic effects of CE and LEV, our objective was to determine their prevalence in cocaine-positive patient samples at the University of Texas Medical Branch – Galveston.

Method: Cocaine positive urine samples from drug screens performed between December, 2014 and February, 2015 at the University of Texas Medical Branch – Galveston, TX were tested for LEV, CE, cocaine (COC), benzoylecgonine (BE), and alcohol metabolites (ethyl glucuronide (EtG) and ethyl sulfate (EtS)) using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). All standards and internal standards were purchased from Cerilliant (Round Rock, TX). Each standard analyte had its respective internal standard, except for LEV, which had BE internal standard. Six calibrators ranging from 25-2000 ng/mL were used for LEV, BE, CE, and COC, along with a negative control and 2 levels of positive controls prepared in drug free urine. Five calibrators ranging from 250-3000 ng/mL were used for EtS and EtG along with a negative control, 2 levels of positive controls (UTAK laboratory), and an additional positive control prepared in drug free urine. Controls and samples were diluted 1:4 with 0.1% formic acid/water spiked with internal standard and analyzed.

Result: Linearity for LEV, COC, BE, and CE ranged from 10-40,000 ng/mL with R^2>0.998 and cut off value of 50 ng/mL. Linearity for EtS and EtG ranged from 200-50,000 ng/mL with R^2>0.998 and cut off value of 500 ng/mL. Within the 11 week period, 1280 samples were screened for cocaine at our institute and only 7.4% were positive for cocaine. Further analysis of the BE positive samples showed that only 50% were positive for COC and 30% were positive for CE, the metabolite of COC and alcohol. In addition, 52.5% and were positive for EtG and 50% were positive for EtS. LEV was positive in 80% of the BE positive samples. The average age for cocaine positive patients was 45 yr. 61% were male and 43% were Caucasians.

Conclusion: This study highlights the prevalence of LEV and CE in the study population and indicates need for LEV/CE screen in suspected cases of drug abuse due to the associated adverse effect of both substances.

B-316

ARK™ Oxcarbazepine Metabolite Assay for the Roche/Hitachi Modular P Automated Clinical Chemistry Analyzer

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Background: Epilepsy is a medical condition that produces seizures affecting a variety of mental and physical functions. Oxcarbazepine (OXC) and eslicarbazepine acetate are the second and third generation antiepileptic drugs (AED) respectively. These prodrugs are metabolized to 10-monohydroxy derivative (MHD), the active agent. Serum levels usually range 3 to 35 µg/mL. Here an ARK enzyme immunoassay for therapeutic drug monitoring (TDM) of MHD is described.

Methods: The ARK™ Oxcarbazepine Metabolite Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 6 calibrators (0.00, 2.00, 5.00, 12.00, 25.00 and 50.00 µg/mL) and 3 controls (3.00, 10.00 and 30.00 µg/mL). The performance of the ARK assay was evaluated on the Roche/Hitachi Modular P analyzer. Precision, limit of quantitation, recovery, specificity and method comparison were studied.

Results: Total precision ranged 5.4% to 6.9%CV and within-run precision ranged 6.5% to 8.6%CV in a 5-day study using quality controls and spiked serum samples. Acceptable quantitation and recovery was observed from 0.70 to 35.00 µg/mL. The assay crossreacted 20% to 30 % with structurally similar oxcarbazepine and carbamazepine and its metabolites (cis-10, 11-dihydroxy carbamazepine, dihydro-carbamazepine, and carbamazepine epoxide). The assay did not crossreact with other AEDs tested (gabapentin, lamotrigine, levetiracetam, topiramate, and zonisamide). Thirty specimens (5.90 to 26.10 µg/mL) were assayed and gave the following Passing Bablock regression results when compared to UPLC values: ARK = 1.05 UPLC - 0.78 (r2=0.94).

Conclusion: The ARK Oxcarbazepine Metabolite Assay measures oxcarbazepine MHD in human serum with excellent precision and recovery. Ability to measure trough levels of oxcarbazepine MHD with high accuracy and fast turn-around time makes this method clinically useful for TDM.

B-317

ARK™ Lacosamide Assay for the Beckman AU480 Automated Clinical Chemistry Analyzer

K. Pham, M. Yim, S. Oh, B. Moon, J. Valdez. *ARK Diagnostics, inc, Fremont, CA*

Background: Epilepsy is a medical condition that produces seizures affecting a variety of mental and physical functions. Lacosamide (LCM) is a new generation antiepileptic drug (AED). Typical serum levels are 7.9 ± 4.9 µg/mL (31.4 ± 19.5 µmol/L). Here an ARK enzyme immunoassay for therapeutic drug monitoring (TDM) of lacosamide is described.

Methods: The ARK™ Lacosamide Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 6 calibrators (0.0, 1.0, 2.0, 5.0, 10.0 and 25.0 µg/mL) and 3 controls (1.50, 7.0 and 15.0 µg/mL). The performance of the ARK assay was evaluated on the Beckman AU480 Automated Clinical Chemistry Analyzer. Precision, limit of quantitation, recovery, specificity and method comparison were studied.

Results: Total precision ranged 4.4% to 7.7%CV and within-run precision ranged 4.2% to 5.7%CV in a 5-day study for quality controls and spiked serum samples. Acceptable quantitation and recovery was observed from 0.6 to 20.0 µg/mL. The assay crossreacted 1.3% with o-desmethyl lacosamide (10.0 ug/mL in the presence of 5.0 µg/mL LCM) and 2.7% with o -desmethyl lacosamide (20.0 µg/mL in the presence of 10.0 µg/mL LCM). Forty nine specimens (1.1 to 14.4 µg/mL) were assayed and gave the following Passing Bablock regression results when compared to UPLC values: ARK = 0.99 UPLC - 0.02 (r2=0.93).

Conclusion: The ARK Lacosamide Assay measures lacosamide in human serum with excellent precision and recovery. Ability to measure trough levels of lacosamide with high accuracy and fast turn-around time makes this method clinically useful for TDM.

B-318

Evaluation of a Reagent Kit Under Development for the Therapeutic Drug Monitoring of Cyclosporine and Tacrolimus in Whole Blood by LC-MS/MS

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Background: Immunosuppressive agents cyclosporine and tacrolimus are calcineurin inhibitors which play a critical role in interleukin-2 promoter induction after T-cell activation. An assay for the monitoring of cyclosporine and tacrolimus in human whole blood samples as an aid in the management of kidney, liver and heart allograft transplant therapy is described using the Waters® MassTrak™ TDM Immunosuppressants Kit*. This kit is currently under development and not available for sale.

Methods: The MassTrak TDM Immunosuppressants Kit uses a LC-MS/MS system to perform the analysis. The samples (50µL of calibrators, controls or human whole blood) are manually extracted using a simple protein precipitation technique. Following centrifugation the supernatant was injected onto the ACQUITY TQD LC-MS/MS system for multiple reaction monitoring detection and quantification. Evaluation of the reagent kit was performed at Fujirebio Diagnostics Inc.

Results: The measuring range was 25 to 1500ng/mL (up to 3000ng/mL with specimen dilution) for cyclosporine and 1 to 30ng/mL (up to 60ng/mL with specimen dilution) for tacrolimus. The 20 day precision study demonstrated a 2.9-5.5% total CV over the range 33.6-1432.8ng/mL for cyclosporine and a 3.4-5.8% total CV over the range 3.0-20.7ng/mL for tacrolimus using three controls, four panels on one system and one reagent lot. Tacrolimus was shown to be linear over the range 0.0 to 36.6ng/mL and cyclosporine deviation from linearity ranged from -6% to 5% over the range 10.3-2267.7ng/mL. The limit of quantification was determined to be 6.9ng/mL for cyclosporine and 0.2ng/mL for tacrolimus, using three systems and two reagent lots.

Interference studies demonstrated mean levels of cyclosporine and tacrolimus were within 90-110% of that in unspiked control samples when endogenous (including; bilirubin, creatinine, uric acid, hematocrit) and exogenous (including; insulin, intralipid, K_2EDTA, Vitamin B12 and other immunosuppressant agents) potential interfering compounds were tested. Cyclosporine spiked recovery ranged from 91-99% for samples over the range 49.3-1137.1ng/mL. Tacrolimus spiked recovery ranged from 94-101% for samples over the range 3.0-25.1ng/mL.

Accuracy of the assay was assessed by analysing International Proficiency Testing (IPT) samples for cyclosporine and tacrolimus from Bioanalytics, UK (ASI Ltd). Cyclosporine and tacrolimus MassTrak TDM Immunosuppressants Kit determinations (n=40) were compared with the schemes target concentrations. Linear ordinary fit analysis for cyclosporine demonstrated a correlation coefficient of r = 0.999 and the Deming fit was described by the equation MassTrak = 1.06 IPT + 0.68 with no significant constant bias, however, proportional bias was detected ($p<0.05$) although this was within the allowable bias goals of the assay (10%). Linear ordinary fit analysis for tacrolimus demonstrated a correlation coefficient of r = 0.997 and the Deming fit was described by the equation MassTrak = 1.00 IPT + 0.09 with no significant constant or proportional bias ($p>0.05$).

Conclusions: Initial studies indicate the MassTrak TDM Immunosuppressants Kit, currently under development, will provide an accurate, precise, sensitive and robust assay for the measurement of cyclosporine and tacrolimus in human whole blood by LC-MS/MS analysis.

** The MassTrak TDM Immunosuppressants Kit is under development and not available for sale*

B-319

Performance comparison of Six Different Rapid Screening Drug Test Cards / Cups

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Although the use of mass spectrometry for urine drug testing provides the most definitive and accurate answer, urine drug test cup is still of interest because results are readily available in a few minutes and testing can be done with minimal training. We evaluated the performance of six commercially available Rapid Urine Drug Testing kits, and compared their screening results to the drug screens performed on the Chemistry analyzer, and with confirmatory results from Gas Chromatography - Mass Spectrometry (GC-MS) if available.

We concluded that Alere iScreen Dx, Discover™ Multi-Panel Drug Test Cup, MEDTOX® urine drug test cassette, BioRad TOX/See™ Rapid Urine drug test all have acceptable drug screen performance (as compared to our screening performed on the Chemistry analyzer and / or confirmatory results from GC-MS). Unexpectedly, we found that the non-waived products (MEDTOX® and BioRad TOX/See™) do not necessarily outperform waived products (Discover™). While all products we tested utilize the same testing principles, certain product (e.g. ABMC Rapid Drug Screen cassette) has more frequent internal QC failure as well as issues where urine fails to migrate to the test strip. QuikScreen© 12 urine drug test kit also had more discrepancy when compared to screening performed on the Chemistry analyzer and / or confirmatory results from GC-MS.

Table 1 represents concordance of four rapid urine drug testing kits with screening results on chemistry analyzer / confirmatory result from GC/MS if available.

MEDTOX®, Discover™ and TOX/See™ showed the best performance. The advantage of MEDTOX® is that it uses a reader to read the results, thereby remove subjectivity from individual. Discover™ cup is a waive product, therefore is subjected to less regulatory requirement. This makes it simpler to implement as an alternative to performing immunoassay screen on the Chemistry analyzer.

Table 1: Performance of four rapid urine drug testing kits

Drug Class	Alere iScreen Dx	Discover™	MEDTOX®	BioRad TOX/See™
Amphetamines	88%	100%	97%	97%
Benzodiazepines	75%	96%	88%	75%
Cannabinoids	100%	96%	97%	94%
Cocaine Metabolite	94%	100%	97%	100%
Methadone	88%	96%	100%	100%
Opiates	81%	100%	94%	81%
Oxycodone	81%	82%	90%	91%
Overall	87%	96%	95%	91%

B-320

Should mass spectrometry be used for urine drug screens in peri-partum patients?

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Background: Immunoassay methods have been used for urine opiates screens in all patients including those from the labor and delivery (L & D) department at the University of Texas Medical Branch - Galveston. However, due to the high cut off value (300 ng/mL), false negative results were observed in pregnant women from the L & D who used opiates. The babies delivered by this group of mothers have increased risk for some medical problems, including fetal growth restriction, abruptio placentae, fetal death, preterm labor, and intrauterine passage of meconium, just to name a few. The purpose of this study was to compare the current enzyme immunoassay method with the tandem mass spectrometry method to determine the appropriateness of its use in peripartum women as a screening method.

Methods: This is an ongoing prospective study. 405 urine samples were tested for opiates by the enzyme immunoassay method from Jan 21 to Feb 21, 2015 at our institute. 39 urine samples were randomly selected to test for 9 opiate drugs and metabolites (morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, norhydrocodone, noroxycodone, and 6-monoacetylmorphine) by the UPLC-MS/MS quantitative method. For the UPLC-MS/MS quantitative method, the calibrators were prepared in drug free urine using certified reference materials (Cerilliant) at 6 levels ranging from 25 - 2000 ng/mL for 8 high concentration analytes and 2.5 - 200 ng/mL for 1 low concentration analyte. Two levels of control samples were used. Controls and samples were prepared via beta-glucuronidase hydrolysis.

Results: Preliminary results are as follows: the mean age was 42.5 years (range: 1 day-93.2 years). Out of 405 samples, 52.4% were female, 31.4% were African Americans, and 66.3% were Caucasians. The enzyme immunoassay result distribution was as follows: 67.4% (<= 50 ng/mL), 6.7% (50 to 300 ng/mL), 25.9% (>= 300 ng/mL). 39 patients (10 patients with test result <=50 ng/mL, 9 patients with result between 50 to 300 ng/mL, and 20 patients with test result >=300 ng/mL) were randomly selected for UPLC-MS/MS analyses. Among the 39 selected samples, 25 tested positive by the UPLC-MS/MS method, and of that 20 samples were tested positive by immunoassay and 5 were tested negative. The remaining 14 samples were negative from both methods. The sensitivity of the enzyme immunoassay method is 80%, specificity is 100%, positive predictive value is 100%, and negative predictive value is 73.7%.

Conclusion: For pregnant women, UPLC-MS/MS method should be used for urine drug screen/quantification because of its superior analytic sensitivity that will allow us to identify more individual who use the drugs when compared to results obtained by enzyme immunoassay. Thus, the use of UPLC-MS/MS for screening will enhance the identification of mothers using the drugs and therefore, lead to prompt management of associated conditions.

B-321

One-step extraction and quantitation of toxic alcohols and ethylene glycol in plasma by capillary gas chromatography (GC) with flame ionization detection (FID)

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Background: Clinical analysis of volatile alcohols (i.e. methanol, ethanol, isopropanol, and metabolite acetone) and ethylene glycol (EG) generally employs separate gas chromatography (GC) methods for analysis. The added turn-around time to analyze patient samples using separate methods on separate columns can impact patient care in the emergency department. Here, a single robust method for combined analysis of volatile alcohols and EG is described.

Methods: Volatile alcohols and EG were extracted from 200 µL of patient, calibrator, and QC samples with 2:1 (v:v) acetonitrile containing internal standards (IS) 1,2 butanediol (for EG) and n-propanol (for alcohols). Samples were vortexed for ~5 - 10 seconds and centrifuged for 2 minutes at 10,000 rpm on a benchtop centrifuge. 1 µL of the supernatant was injected (with 2:1 split ratio) onto a Restek-200 bonded stationary phase capillary column (30m × 530µm) with a fused silica guard column (10m × 520µm). Chromatographic separation was conducted on an Agilent 6890 GC FID and required modulation of both the gas flow (2 mL/min for 1 minute up to 15 ml/min for elution) as well as the temperature (45°C for 3 minutes to elute alcohols, up to 250°C at 70°C/min to elute EG). The total run time was 7.9 minutes. Six-point calibration curves were fitted with a quadratic regression curve to allow for non-linear recovery of EG at the low concentrations. QC material included Biorad Liquicheck level 1 and 2 serum volatile alcohols and an in-house prepared EG stock.

The method was evaluated for precision, accuracy, reproducibility, linearity, selectivity and limit of quantitation (LOQ), followed by correlation to existing GC methods, using patient samples, Bio-Rad QC, and in-house prepared QC material.

Results: The method yielded inter-day precision values ranged from 6.5 - 8.8% CV (L2 Biorad QC) and 6.8 - 11.3% CV (L1 Biorad QC). Linearity was verified in the range ~0.5 - 50 mmol/L for each analyte and the LOQ was calculated to be between 0.25 and 0.44 mmol/L for each analyte by calculating the noise in the lowest calibrator ($10 \times \sigma$) divided by the slope of the line. Correlation of the new method against current GC methods showed good agreement between them (slopes ranging from 1.03 - 1.12, and y-intercepts ranging from 0 - 0.85 mmol/L; $R2 > 0.98$; $N = 35$). Recovery was shown to be ~100% at all levels tested (low-mid range-high) for all analytes, although EG was more variable at low concentrations. Carryover was assessed to be negligible for volatile alcohols in the measuring range; however EG showed clinically significant carryover at >10 mmol/L; therefore injection of CLRW was required to eliminate contamination. Potential interfering substances include toluene (elutes with EG) and benzene (elutes with methanol); however the method is able to resolve 2,3 butanediol, diethylene glycol, and propylene glycol in addition to the peaks quantified.

Conclusion: Here we describe a simple procedure for simultaneous analysis of EG and volatile alcohols that comes at low cost and with a simple liquid-liquid extraction requiring no derivitization to obtain adequate sensitivity for clinical specimens.

B-322

LCMSMS quantitative analysis of Anti-Depressants and Tricyclic Anti-Depressants in urine, oral fluids and serum and evaluation and comparison of sample preparation techniques

R. M. Doyle. *Agilent Technologies, Wilmington, DE*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. Antidepressants and Tricyclic Anti-Depressants are drugs used for the treatment of major depressive disorder and other conditions including dysthymia, anxiety disorders, obsessive compulsive disorder, eating disorders, chronic pain, neuropathic pain and, in some cases, dysmenorrhoea, snoring, migraines, attention-deficit hyperactivity disorder (ADHD), substance abuse and sleep disorders A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of these Anti-Depressants by QQQ. Various sample preparation techniques that includes dilute

and shoot in urine and oral fluid, protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D) and two (2D) dimensional chromatographic configurations are evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness as well as different mass spectrometer platforms. The described method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure Anti-Depressants.

Methods:

An Agilent 6460 tandem mass spectrometer with Jet Stream technology in negative Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 ul of urine, oral fluid and serum were used for the analysis of Anti-Depressants. Various columns were evaluated and an Agilent Poroshell 120 SB-C18 100 x 2.1 mm, 2.7 um with a water:methanol mixture containing 0.01% formic acid and 5 mM Ammonium Formate gradient achieved baseline chromatographic separation in less than 6 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and urine, oral fluids and serum samples.

Results: Good linearity and reproducibility were obtained with the concentration range from 1 ng/ml to 1000 ng/ml for the respective Anti-Depressants with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.1 to 0.5 ng/ml. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations.

Conclusion:

A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of Anti-Depressants. The sample preparation techniques are quick and easily applied for high throughput analysis.

B-323

LCMSMS quantitative analysis of Benzodizepines in urine, oral fluids and serum and evaluation and comparison of sample preparation techniques

R. M. Doyle. *Agilent Technologies, Wilmington, DE*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. Benzodiazepines possess sedative, hypnotic, anxiolytic, anticonvulsant, muscle relaxant, and amnesic actions which are useful in a variety of indications such as alcohol dependence, seizures, anxiety, panic, agitation, and insomnia. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of these Benzodiazepines by QQQ. Various sample preparation techniques that includes dilute and shoot in urine and oral fluid, protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D) and two (2D) dimensional chromatographic configurations are evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness as well as different mass spectrometer platforms. The described method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure Benzodiazepines.

Methods:

An Agilent 6460 tandem mass spectrometer with Jet Stream technology in negative Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 ul of urine, oral fluid and serum were used for the analysis of Benzodiazepines. Various columns were evaluated and an Agilent Poroshell 120 SB-C18 100 x 2.1 mm, 2.7 um with a water:methanol mixture containing 0.01% formic acid and 5 mM Ammonium Formate gradient achieved baseline chromatographic separation in less than 6 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and urine, oral fluid and serum samples.

Results: Good linearity and reproducibility were obtained with the concentration range from 1 ng/ml to 1000 ng/ml for the respective Benzodiazepines with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.1 to 0.5 ng/ml. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations.

Conclusion:

A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of Benzodiazepines. The sample preparation techniques are quick and easily applied for high throughput analysis.

B-324

LCMSMS quantitative analysis of Anticonvulsants and Antiepileptic's in urine, oral fluids and serum and evaluation and comparison of sample preparation techniques

R. M. Doyle. *Agilent Technologies, Wilmington, DE*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. Anticonvulsants and antiepileptic drugs are a diverse group of pharmacological agents used in the treatment of epileptic seizures and are also used in the treatment of bipolar disorders, act as mood stabilizers, and for the treatment of neuropathic pain. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of these Anticonvulsants/Antiepileptic's by QQQ. Various sample preparation techniques that includes dilute and shoot in urine and oral fluid, protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D) and two (2D) dimensional chromatographic configurations are evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness as well as different mass spectrometer platforms. The described method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure Anticonvulsants/Antiepileptic's.

Methods: An Agilent 6460 tandem mass spectrometer with Jet Stream technology in negative Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 ul of urine, oral fluid and serum were used for the analysis of Anticonvulsants/Antiepileptic's. Various columns were evaluated and an Agilent Poroshell 120 SB-C18 100 x 2.1 mm, 2.7 um with a water:methanol mixture containing 0.01% formic acid and 5 mM Ammonium Formate gradient achieved baseline chromatographic separation in less than 6 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and urine, oral fluid and serum samples.

Results: Good linearity and reproducibility were obtained with the concentration range from 1 ng/ml to 1000 ng/ml for the respective Anticonvulsants/Antiepileptic's with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.1 to 0.5 ng/ml. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations.

Conclusion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of nticonvulsants/Antiepileptic's. The sample preparation techniques are quick and easily applied for high throughput analysis.

B-325

Evaluation and Application of a Commercial Ethylene Glycol Enzymatic Assay in the Clinical Management of Ethylene Glycol Poisoning

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Background: Ethylene glycol toxicity remains an important toxicological problem in medical practice. Ethylene glycol is the most frequently encountered glycol toxin in the United States. Early diagnosis and treatment can prevent significant morbidity and mortality. Gas Chromatorgraphy-Flame Ionization Detection (GC-FID) is the preferred method for ethylene glycol detection. However, these instruments and the needed expertise are not readily available in most clinical laboratories.

Objective: Validate the Catachem rapid enzymatic assay for the quantitative determination of ethylene glycol levels in plasma. Use this method in combination with an in house GC-FID in the clinical management of ethylene glycol poisoning.

Methods: The Catachem Ethylene Glycol procedure is based on the affinity of the bacterial enzyme Glycerol Dehydrogenase (EC 1.1.1.6.) to catalyze the oxidation-reduction reaction of Ethylene Glycol in the presence of NAD. This two point kinetic procedure is read at 340nm, which was detected spectrophotometrically by using the Beckman AU 400 automated chemistry analyzers. Validation of this method was performed against a previously validated GC-FID method. Performance evaluation included accuracy, linearity, analytical sensitivity, imprecision, carryover, endogenous and exogenous interference studies, and parallel studies.

Results: The enzymatic assay showed excellent correlation to an in house GC-FID method (y = 0.964x + 0.3; r2=0.9966; N=40), with an analytical measurement range of 10-150 mg/dL. The analytical sensitivity was 10mg/dL. Both within-run (1.6%-3.1%) and between-run (4.1%-5.2%) imprecision were within acceptable limits. The assay showed cross reactivity with propylene glycol and various butanediols at elevated concentrations. Interference from hemolysis and lipemia was within acceptable limits (±5%). The assay is affected by icterus.

Conclusion: This method was successfully used for rapid rule out of ethylene glycol toxicity. It is successfully used to monitor response to therapy following hemodialysis of patients confirmed of having ethylene glycol toxicity. This assay has contributed to a significant decrease in turnaround times as well as a decrease in labor costs. The assay can be adapted in clinical laboratories with no GC-FID capabilities.

B-326

LCMSMS quantitative analysis of Antipsychotics and Neuroleptic's in urine, oral fluids and serum and evaluation and comparison of sample preparation techniques

R. M. Doyle. *Agilent Technologies, Wilmington, DE*

Background: Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. Antipsychotics and neuroleptics are used to manage psychosis such as delusions, hallucinations, disordered thought, schizophrenia and bipolar disorder, and are increasingly being used in the management of non-psychotic disorders A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of these Antipsychotics/Neuroleptic's by QQQ. Various sample preparation techniques that includes dilute and shoot in urine and oral fluid, protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D) and two (2D) dimensional chromatographic configurations are evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness as well as different mass spectrometer platforms. The described method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure Antipsychotics/Neuroleptic's.

Methods: An Agilent 6460 tandem mass spectrometer with Jet Stream technology in negative Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 ul of urine, oral fluid and serum were used for the analysis of Antipsychotics/Neuroleptic's. Various columns were evaluated and an Agilent Poroshell 120 SB-C18 100 x 2.1 mm, 2.7 um with a water:methanol mixture containing 0.01% formic acid and 5 mM Ammonium Formate gradient achieved baseline chromatographic separation in less than 6 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and urine, oral fluid and serum samples.

Results: Good linearity and reproducibility were obtained with the concentration range from 1 ng/ml to 1000 ng/ml for the respective Antipsychotics/Neuroleptic's with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.1 to 0.5 ng/ml. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations. Conclusion: A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of Antipsychotics/Neuroleptic's. The sample preparation techniques are quick and easily applied for high throughput analysis.

B-327

Monitoring of micafungin use in clinical practice using high-performance liquid chromatography with fluorescence detection

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Background:

Micafungin is an antifungal drug. It has a unique mechanism of action that works by way of concentration-dependent inhibition of 1,3-beta-D-glucan synthase resulting in reduced formation of 1,3-beta-D-glucan in the fungal cell walls. The decreased production of 1,3-beta-D-glucan leads to osmotic instability and thus cellular lysis. Micafungin has been approved for the treatment of candidemia, acute disseminated candidiasis, Candida peritonitis, abscesses and esophageal candidiasis. Previous reports of micafungin pharmacokinetics showed substantial variability in blood and plasma concentrations. Clarification of the effective blood concentration of micafungin by evaluating its clinical effect enables rapid and accurate treatment. Therapeutic drug monitoring (TDM) of micafungin in plasma can be useful for the evaluation and diagnosis of the drug inhibitory concentration, so subsequent medication could be adjusted according to the TDM results.

Methods:

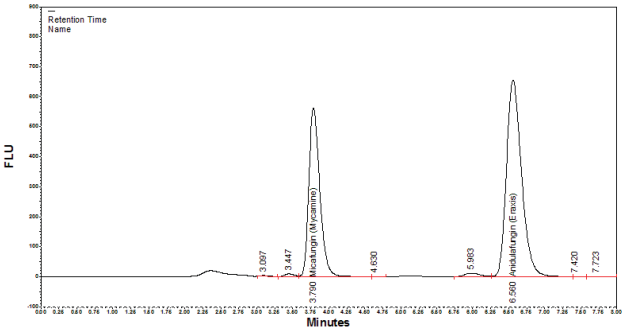
The high-performance liquid chromatography with fluorescence detection system was used for the separation and quantitation of micafungin from plasma samples. The separation of micafungin and internal standard anidulafungin was achieved through the use of reversed-phase high-performance liquid chromatography. Sample preparation involved a single dilution step, solvent extraction, protein precipitation, and ultra-centrifugation. Micafungin and anidulafungin were extracted from plasma samples using methanol and analyzed on a Zorbax Eclipse Plus C18 column with fluorescence detection set at excitation and emission wavelengths of 273 and 464 nm, respectively.

Results:

The standard curve was linear through the range of 0.1-80 µg/mL using a 0.1-mL sample volume. The intra- and inter-day precisions were all less than 5% and accuracies ranged from 94.8 to 105.1%. Average recoveries were 94.1 ± 0.7% and 92.8 ± 1.2% for micafungin and anidulafungin, respectively. Figure 1 represents a typical chromatogram of plasma sample containing 23.3 µg/mL of micafungin.

Conclusion:

The method is very applicable to monitor and optimize micafungin therapy in order to ascertain clinical efficacy and minimize adverse effects.



B-328

Detection of everolimus from dried blood spots using liquid chromatography tandem mass spectrometry

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Background: Monitoring immunosuppressants levels in blood is critical to ensuring proper drug dosage; however, this can be difficult for transplant recipients who live far away from the central laboratory. A potential solution to this problem is the use of dried blood spots (DBS), which can be collected by the patient and mailed to the lab for analysis. Thus, removing the requirement for a phlebotomist and transport of a liquid blood sample. Everolimus is a new immunosuppressant that has been shown to be useful in liver and heart transplantation and has less severe side effects than other immunosuppresants currently in use.

Objective: Establish a method to measure everolimus from dried blood spots using liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Methods: Fifteen microlitres of whole blood spiked with everolimus (2.5, 5, 10 and 35 ng/mL) was spotted onto Whatman 903 protein saver cards. Several extraction solvents were evaluated (water, acetonitrile, methanol:acetonitrile, methanol). Everolimus-d4 was added along with the extraction solvent as an internal standard. The extracts were vortexed for 10 min after which the supernatant was removed and evaporated to dryness under air. It was then reconstituted in 50:50 methanol:water and injected into the mass spectrometer.

Extracts were analyzed using an Agilent 6410 triple quadrupole mass spectrometer coupled with an Agilent 1100 HPLC. Separation was achieved in a 2.8 min run using a valco valve to facilitate switching between a precolumn (POROS R1/20 2.1 mm x 20 mm) and analytical column (Phenomenex Luna C18 (2) 5 µm x 10 x 2.00mm). Following injection, the sample was loaded onto the precolumn (for 0.8 min) using mobile phase A (50:50 methanol:water). The valvo valve then switched allowing mobile phase B (2 mM ammonium acetate, 0.1% formic acid in methanol) to elute the analytes off the precolumn and onto the C18 analytical column (0.7-2 min). Flow rate was 0.5 mL/min and the injection volume was 50 µL. Multiple reaction monitoring (MRM) data for everolimus and everolimus-d4 was collected between 0.7 and 2 min. The source parameters were: gas temperature 250°C, gas flow 10 L/min, Nebulizer gas 50 psi, capillary voltage 4000V. MRM transitions for everolimus and everolimus-d4 were optimized using the Agilent Optimizer software.

Results: Four solvents were evaluated for extraction of everolimus from DBS. Our preliminary results indicate that methanol was the best at extracting everolimus from DBS and will be used for future experiments. Recovery of everolimus from four spiked concentrations (range: 2.5-35 ng/mL) ranged from 85-117% with an average of 101.6% ± 13%. Comparison with the results from the DBS extraction with the protein precipitation and dilution method currently in use in our lab gave an R2 of 0.9978.

Conclusion: We have developed a method to use DBS as specimen of choice for measuring everolimus on LC-MS/MS. Our preliminary conclusion indicates that DBS can be used for measurement of everolimus. We are running 40 more specimens to support our initial conclusion. The completed study will be presented at the national meeting.

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High-Throughput Urine Analysis to Detect Buprenorphine and Ethanol Use by Multi-channeling LC-MS/MS

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Background: In order to maximize sample throughput and minimize solvent consumption, two forensic LC-MS/MS methods used to detect buprenorphine and/or ethanol use were developed for a multichannel UHPLC system utilizing positive-displacement pumps interfaced to a tandem mass spectrometer.

Methods: Urine specimens and corresponding calibrators and QCs to be analyzed for buprenorphine (Bup) and norbuprenorphine (Norbup) were hydrolyzed by incubation with β-glucuronidase solution and then mixed with cold methanol containing Bup-D_3 and Norbup-D_4 internal standards. Urine specimens and corresponding calibrators and QCs to be analyzed for ethyl-glucuronide (EtG) and ethyl-sulfate (EtS) were diluted 1:10 with water containing internal standards EtG-D_3 and EtS-D_3. After centrifugation, 10 uL injections of supernatants from each preparation were made.

Results: The desired quantitation range from 5 to 500 ng/mL for Bup/Norbup and the desired range from 100 to 5000 ng/mL for EtG/EtS were consistently linear (r^2 > 0.995 with 1/X weighting) whether the calibrators were injected into one channel or across all channels. For both methods, internal standard peak areas showed less than 25% coefficient of variation (CV) among calibrators, QCs and specimens (n = 20) on any of the four channels. Retention time variations throughout these batches were less than 3% CV. Results were within +/- 15% of those determined on a conventional multichannel system using reciprocating pumps. Comparatively, the multichannel system with positive-displacement pumps reduced solvent consumption by at least 65%. A maximum throughput of 34 urine samples per hour was achieved when batches were submitted across three channels. Since the data windows of both methods were a little more than 1/3rd of the total run times, adding the fourth channel did not increase sample throughput. However, using all four channels provided assurance that the total throughput would not be compromised in the event of one channel shutting down because of a leak or a column reaching its maximum pressure.

Conclusion: The multichannel UHPLC system achieved the desired sample throughput and solvent consumption.

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Milrinone therapeutic drug monitoring in pediatric cardiac surgery

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Background: Milrinone is a potent selective phosphodiesterase type III inhibitor which stimulates myocardial function and improves myocardial relaxation. It is used extensively post-cardiac surgery in both adults and children. Although therapeutic monitoring is crucial to maintain therapeutic outcome, little data is available. A proof-of-principle study has been initiated in our institution to determine desirable milrinone levels (ie. therapeutic index) in children following cardiac surgery. The objective of this study was to develop an LC-MS/MS method to quantify milrinone in serum of pediatric patients and then to determine its pharmacokinetic parameters.

Methods: A liquid-liquid extraction procedure was used to prepare samples for analysis. Milrinone was measured using LC-MS/MS. Calibrants and internal standard were prepared in blank patient serum and charcoal stripped serum, respectively. Performance of the method was assessed by linearity, LoD, dilution recovery, accuracy, and precision. To determined the pharmacokinetic profile of the drug, patient samples were acquired post-surgery; the first sample was drawn within 2 hours post surgery and then every 6-8 hours for 24-36 hours. Pharmacokinetic analysis will be conducted using Non-Linear Mixed Effects Modeling (Non-MEM).

Results: Calibration curves followed a regression in the linear range of 50 - 800 ug/L. The lower limit of quantification is 5.9 ug/L based on triplicate runs of a low concentration sample that did not exceed 20% coefficient of variance (CV). Drug dilution recovery and accuracy was <120% and 150 ug/L. Within day CV was 5.7% at 95, 4.4% at 339, and 8.3% at 679 ug/L. Between day CV was 11.7% at 77, 8.8% at 318, and 8.2% at 587 ug/L. Data from the pharmacokinetic analysis will be presented.

Conclusion: This simple and quick method proved to be sensitive, specific, and precise for therapeutic monitoring of milrinone in patients post-cardiac surgery, a population not well studied. Pharmacokinetic profiling will help to determine both the pharmacokinetic parameters of this drug as well as the therapeutic range. These results will help improve dosing and monitoring of pediatric patients post-surgery.

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Quantitation of 78 Compounds in Urine by LC-MS/MS

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Introduction

Forensic toxicologists face an ever-expanding list of compounds for analysis. The need to reliably quantitate large-panel assays is continually increasing. Large panel assays are required in order to speed sample analysis time, lower analytical costs and obtain results quicker while keeping good data quality. Herein we developed a fast, cost efficient liquid chromatography tandem mass spectrometry (LC-MS/MS) method which meets laboratory requirements for limit of quantitation for quantitative analysis of 78 compounds in human urine.

Methods

The 78 compounds consist of opiates, amphetamines, sedatives, drugs of abuse and others. Thirty deuterated analogs were used as internal standards. We developed a very simple sample preparation method in which urine samples were diluted by 50 fold. LC-MS/MS analysis was conducted on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer with a HESI ionization probe in polarity switch mode. Two SRM transitions were collected for analytes and one SRM transition was collected for internal standards. Nine minutes of LC gradients on Ultimate™ 3000 RS LC pump were used to separate compounds, which results in analysis of 6 samples per hour. All data acquisition and quantification for this method was performed using TraceFinder™ software version 3.2.

Results

We evaluated the following method performance:

LOQ: by two sets of calibration standards from 5 ng/mL to 5000 ng/mL. The acceptance criterion was difference within +- 20% and ion ratio within specified range.

Precision: by 10 replicate injections of QC samples

Linearity range: by serial dilutions from 5000 ng/mL

In conclusion, we developed a fast, cost efficient method for quantitative analysis of 78 compounds in human urine. The method meets laboratory requirements for limit

of quantitation. A short 9 minutes LC-MS/MS method allows analysis of 6 samples per hour.

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Utilization of comprehensive urine drug screens in Southern Alberta

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Background: Comprehensive drug screens are ordered for several indications including clinical assessment, employment, addiction, rehabilitation, or pain management monitoring. With the rise of recreational drug use, physicians may be tempted to order full drug screens in order monitor their patients. This can create an environment of unnecessary utilization by health care providers. Calgary Laboratory Services performs all clinical comprehensive urine drug testing for Southern Alberta. The toxicology department performs 50000 tests per year, approximately 12000 of which are comprehensive drug screens.

Objective: To investigation utilization of comprehensive urine drug screens in Southern Alberta by clinical health care providers

Methods: Comprehensive drug screens at Calgary Laboratory Services are performed in two steps. Samples are first screened by immunoassay for opiates, cocaine, amphetamines, barbiturates, methadone, EDDP, oxycodone and benzodiazepines. They then undergo liquid-liquid extraction and are run by gas chromatography-mass spectrometry (GC-MS) in total ion monitoring mode. Separate GC-MS confirmation assays for opiates, cocaine and cannabinoids are available if indicated.

Data was obtained for all comprehensive urine drug screens performed at Calgary Laboratory Services between 2010 and 2014 from our laboratory information system (Cerner Millennium). All data was deidentified according to CLS privacy policies. The data obtained included patient age and sex, sample collection time, ordering location, and comprehensive drug screen results. All data analysis was performed in Microsoft Excel 2007.

Results:A total of 51,866 comprehensive drug screens were reported on 12,228 patients during the 5 year period. This number increased from 9239 in 2010 to 11074 in 2014 (increase of 20%). Over half of the workload was from three ordering locations: two methadone clinics and the addictions clinic at our main tertiary care centre which accounted 28%, 20%, and 18% of total comprehensive drug screen volumes, respectively. The ordering practices were further investigated by looking at how often comprehensive urine drug screens were ordered on the same patient. Of the samples run between 2010 and 2014, 76% had another sample submitted on the same patient at least once. When analyzed by number of weeks until the testing was repeated, we determined that testing was most often repeated on a patient within 1 week of the previous sample being submitted (15%). This declined in a time dependent fashion over the following weeks (2 weeks: 12%, 3 weeks: 7%, 4 weeks 8%). Amazingly, most repeat testing within 1 week was on patients from the addictions clinic at our major tertiary care centre, not from the methadone or other rehabilitation clinics.

Conclusion: Data collected from comprehensive urine drug screens performed in Southern Alberta from 2010-2014 show that 15% of repeated tests were ordered within 1 week of initial testing. This is an important observation as there is usually no clinical indication for a comprehensive drug screen to be repeated within 1 week. We plan to implement a guideline to restrict comprehensive urine drug screens to once per month, which will result in a savings of $425,000 per year.

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Effects of sodium arsenite on the some laboratory signs and therapeutic role of thymoquinone in the rats

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OBJECTIVE: Serious health problems in humans are caused by arsenic (As) exposure, which is wide spread in the environment. Sodium arsenite (SAs), capable of inducing macromolecular damage is evaluated for its damaging effect in the blood vessels, liver and kidneys of Wistar rats. This study was undertaken to investigate the ameliorative effects of thymoquinone on SAs-induced oxidative and inflammatory damages in the serum of male Wistar rats.

MATERIALS AND METHODS: 27 Wistar Albino rats divided into three groups of nine rats each were administered to controls saline (10 mg/kg), SAs (10 mg/kg), and SAs plus thymoquinone (10 mg/kg/day) for two weeks orally. Biochemical parameters (albumin, total protein, alanine amino transferase, aspartate amino transferase, urea, creatinine, uric acid, triglyceride, total cholesterol, HDL-cholesterol) were analyzed by otoanalyzer; nitric oxide levels spectrophotometrically, and cytokines (interleukin-6, monocyte chemoattrectant protein-1, macrophage migration inhibitory factor) were measured by ELISA method in the rat serum samples.

RESULTS: Inflammatory cytokines and some biochemical variables were found to be increased in the SAs group compared to control group. On the other hand, thymoquinone supressed these laboratory signs, which are thought to be the characteristic signs of SAs toxicity, most probably by its ameliorative effects including anti-inflammatory and antioxidant properties.

CONCLUSIONS: In conclusion, As causes tissue damage by disintegrating metabolic responsiveness and regulations, vitiating antioxidant systems, decomposing immune competent cells, and finally inducing DNA damages. Therefore, it may be concluded from the present study that supplementation of TQ significantly protects deleterious effects from SAs-induced toxicity by reducing inflammatory and oxidative damages, as indicated by levels of serum biomarker.

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Therapeutic Drug Profiles in Human Breast Milk: To Feed or Not to Feed?

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BACKGROUND: The last two decades has seen a substantial increase in the rates of women breastfeeding. However, it has been reported that 66 - 80% of nursing women are on medication. While many drugs are safely taken by nursing mothers, there is accumulating evidence of toxicity in some breastfed infants. Information on drug excretion into milk is lacking for most drugs, and early phase drug studies exclude breastfeeding women. This uncertainty in the risk of drug exposure causes maternal non-adherence to therapy or avoidance of breastfeeding. This is a clinical problem in drug safety and is an important women's health issue. The objective of this study is to investigate the risk of drug exposure of three drugs in nursing infants. Here, we present drug profile cases of lithium, methotrexate and tacrolimus in breast milk.

METHODS: We established a drug safety monitoring program, Drugs in Lactation Analysis Consortium (DLAC), to measure several drugs commonly used by women breastfeeding. Breast milk is a complex lipid- and protein- rich matrix, with drugs partitioning to either the aqueous or lipid phases; milk composition changes across the feed, thus suggesting that drug concentration can also be variable based on physicochemical properties of the drug. We worked to create a simplified drug extraction method using organic solvents to facilitate efficient drug extraction from both the lipid and aqueous phases of breast milk. Methods were then developed to measure these drugs using LC-MS/MS.

Breast milk samples were obtained from lactating women receiving lithium, methotrexate or tacrolimus. Samples were obtained pre-dose and then at various time-points throughout the dosing interval. A unique feature of this study is that both foremilk and hindmilk were collected. Breast milk samples were aliquotted and stored at -20oC until sample preparation, extraction and analysis.

RESULTS: Time-concentration profiling of methotrexate and its metabolite in breast milk were determined following a once-weekly subcutaneous dose of 25 mg of methotrexate. Foremilk and hindmilk samples were measured and peak drug concentration was found between 1-12 hours post-dose, with low but detectable levels from 48-96 hours post-dose. Tacrolimus breast milk pharmacokinetics was assessed following an 8 mg dose. Peak milk tacrolimus concentration was found between 12-20 hours post-dose. Higher tacrolimus concentrations were present in the lipid-rich hindmilk samples. Lithium time-concentration profiling was established and it was found that lithium selectively accumulates in the aqueous phase of breast milk with peak concentration found between 1-8 hours post- 900 mg oral dose. Data showing the potential risk to the nursing infant will be presented.

CONCLUSION: These cases highlight the importance of determining drug concentrations in breast milk from nursing mothers given the increasing use of medication in nursing women. These results demonstrate that measurable levels of drugs are observed in breast milk. Combining these results with milk-volume consumption, metabolism and clearance, this data can be used to determine the relative infant dose and hence the risk of adverse effects to the nursing infant. The data generated from this study will help guide clinical decisions for drug use in nursing mothers.

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Determination of Voriconazole Concentrations in Serum by GC-MS

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Background and relevance: Voriconazole is a broad spectrum triazole antifungal drug used to treat systemic fungal infections. Therapeutic drug monitoring of voriconazole is necessary for achieving maximal efficiency without inducing toxic side effects. Other publications have reported methods for measuring voriconazole in serum using high performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Here we report for the first time a method for the measurement of voriconazole in serum samples using gas chromatography-mass spectrometry (GC-MS). Methods: Protein precipitation with methanol was used to extract the antifungal which was derivitized with BSTFA and analyzed by GC-MS. Linearity, sensitivity, precision, accuracy and drug interferences were evaluated for this assay Results: Our method was linear up to 10 µg/mL of voriconazole. The LOQ was determined to be 0.4 µg/mL. CV for between day precision was < 12%. Corelation with an established LC-MS/MS yielded a R^2 of 0.96. Tested drugs did not result in >10% error in measurement. Conclusion: To our knowledge, we report here the first GC-MS for voriconazole measurement with acceptable performance. We hope that this method allows clinical laboratories without HPLC or LC-MS/MS instrumentation to measure voriconazole.

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Determination of methanol in urine and plasma by headspace gas chromatography autosampler and flame ionization detector - GC / FID-HS.

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The determination and quantification of toxic volatiles are extremely important in clinical analysis, which involves the routine monitoring of industrial workers to check the exposure to potentially toxic components and the detection of endogenous metabolites in the body in certain clinical diagnoses.

The main risks of a high exposure to methanol are severe recurrent metabolic acidosis with increased anion gap, caused by accumulation of formic acid and, in late stages, also lactic acid. The acidosis and metabolite formic acid cause to the central nervous system depression/toxicity, and visual disturbances that may be permanent. Complete blindness is possible and the target are central nervous system and retina.

The methanol is widely used as an industrial solvent in the manufacture of other chemicals such as paint and varnish removers and is present in automotive antifreeze.

The aim of this study was to validate a fast, easy and cheap method for the determination of methanol in urine and plasma by headspace gas chromatography with flame ionization detection.

The method consisted in a simple extraction of methanol and the internal standard 2-butanol off the sample by evaporation of this analyte, and sampling the vapor above the fluid (blood, urine or others) after reaching thermal equilibrium gas in a closed vial of 22 mL. The volatilized components present in headspace were aspirated by a syringe and injected into chromatograph for separation.

To improve the extraction a salt of ammonium sulfate was added to turn the solution saturated, a technique called salting-out. A simple dissolution of an inorganic salt in water can decrease the solubility of an organic substance in water and consequently increase its volatility.

Chromatographic separation was performed on a PerkinElmer BAC 1: 450°C: 30m x 320µm x 1.8 µm column and mobile phase Nitrogen 99,999%. The chromatographic running time was approximately 4.1 minutes.

The parameters evaluated in the validation were selectivity, linearity, accuracy, precision, repeatability and reproducibility, detection limit, quantification limit and matrix effect. The calibration curves for all compounds were linear with r^2>0.9993. The linear analytical range of the procedure was between 0.1 and 25 mg/dL. Accuracy (93.5-102.0%), intra-assay precision (0.7-1.2%) and inter-assay precision (4.8-8.35%) were acceptable. The determination limit was 0.06mg/dL and the quantification limit was 0.1 mg/dL. The method was applied to the measurement of methanol in plasma and urine of a pooled contaminated with methanol. In conclusion, the GC / FID-HS method has been developed successfully for monitoring industrial workers and the quantitative analysis of methanol.

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Laboratory Investigations on Falsely Elevated Tacrolimus Concentrations on the Dimension Xpand

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Background: Tacrolimus is the most widely used immunosuppressant. Monitoring of blood tacrolimus levels is essential to achieve therapeutic efficacy and avoid toxicity. We measure tacrolimus on the Dimension Xpand using an antibody-conjugated magnetic immunoassay (ACMIA). We recently encountered three cases with unexpectedly elevated tacrolimus levels and further investigations were conducted.

Methods: Whole EDTA blood samples were collected from patients with unexpectedly elevated tacrolimus results. Plasma samples were also prepared from these patients. Samples were run by ACMIA on Dimension Xpand, chemiluminescent microparticle immunoassay (CMIA) on the Architect analyzer, and by liquid chromatography/tandem

mass spectrometry (LC-MS/MS).

Results: Case 1 was a 58-year-old man who was on tacrolimus 1 mg twice daily after liver transplantation. Tacrolimus levels however were noted to be >30 ng/mL for a period of 3 week hospitalization. There were no notable symptoms and signs of toxicity. Tacrolimus level was 8.5 ng/mL as measured by LCMSMS from the same sample in a reference laboratory. When this patient was off tacrolimus for 1 week, the tacrolimus level on Xpand was still >30.0 ng/mL while it was <1.0 ng/mL by LC-MS/MS. Case 2 was a 63-year-old man with a history of myelofibrosis who received a stem cell transplant. Tacrolimus was given 1 mg twice daily. After tacrolimus had been discontinued for 2 weeks, his blood tacrolimus levels were still high varying from 10.0 ng/ml to greater than 30.0 ng/mL. An aliquot of sample with tacrolimus concentration of 12.5 ng/ml from Dimension Xpand was sent to a reference lab and the tacrolimus concentration was reported as <1.0 ng/mL. Case 3 was a 70-year-old man, with chronic myelogenous leukemia who received allogeneic stem cell transplantation and 1 mg tacrolimus twice daily. Although he has been off tacrolimus for 1 week, his blood tacrolimus levels were still high ranging from 12.0-22.0 ng/mL. Of a sample with tacrolimus value of 15.2 ng/mL on Dimension Xpand, LC-MS/MS and Architect gave the results of 3.8 ng/mL and 4.0 ng/mL, respectively. Interestingly, his plasma tacrolimus level was also high (12.8 ng/mL) although no evident hemolysis was determined, whereas tacrolimus levels were <1.0 ng/mL measured by LC-MS/MS and Architect from the same plasma sample. Measurements of tacrolimus from samples with serial dilutions also indicated the presence of potential interference.

Conclusion: Falsely elevated tacrolimus levels can occur due to immune interference when measured by Dimension Xpand using ACMIA assay. These falsely elevated results can potentially impact patient management and outcome. Unexpectedly elevated tacrolimus results should be investigated for potential interference. Measurement of tacrolimus from plasma is an alternative method to quickly rule out the interference while LC-MS/MS remains the standard of measurement.

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A retrospective study of urine drugs of abuse screening positivity rates by immunoassay at a national reference laboratory

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Background: According to the results from the 2013 National Survey on Drug Use and Health, the rate of illicit drug use among adults aged 18 to 25 years old was 21.5% and 7.3% for adults age 26 and older. The purpose of this retrospective study was to evaluate the positivity rate of one of our in-house urine drug screen panels in comparison to the national average for drug use in adults. The urine drugs of abuse panel studied consists of screening by immunoassay; positive immunoassay results are then confirmed by mass spectrometry. This panel can detect several drugs and drug classes, which include: amphetamine, barbiturates, benzodiazepines, cocaine, ecstasy (MDMA), marijuana (tetrahydrocannabinol; THC), methadone, methamphetamine, opiates (morphine, codeine, dihydrocodeine, hydrocodone, hydromorphone, oxycodone and oxymorphone), phencyclidine (PCP) and propoxyphene. Alcohol screening was also conducted if it was included in the client-selected orderable.

Method: Reagents from Microgenics were used for the oxycodone assay, while Syva EMIT® II Plus reagents were used for the rest of the drugs in the screen. The screen was performed on a Beckman AU5810 random access automated clinical analyzer. Percent positivity for each immunoassay was determined. Agreement with previously validated GC-MS or LC-MS/MS confirmatory methods was also evaluated, in order to

assess the true positivity percent versus the percent of false-positive results (positive by screen but negative by confirmation). False-negative results were not investigated.

Results: There were 8825 de-identified screening results for each of the drugs in the panel, except for alcohol (N = 2296). The gender demographics consisted of 45.4% males; overall mean age was 42 yrs old (± 16 SD); range 0-97 yrs. The percent of samples that were preliminary screen positive was 10.0% for amphetamine/methamphetamine/MDMA, 12.8% for benzodiazepines, 43.7% for opiates (including oxycodone), and 20.3% for THC. The percent of preliminary screen positive samples for the following immunoassays: alcohol, barbiturates, cocaine, methadone, propoxyphene, and PCP were relatively low, < 3%. Overall confirmation results demonstrated that 1222 (14.6%) of the samples that tested positive by screen were false-positives and tested negative by confirmation testing. The false-positive rate for amphetamine/methamphetamine was ~14%, ~34% for opiates (excluding oxycodone), 25% for propoxyphene, and 100% for PCP and MDMA immunoassays. In addition, the percent of false-positive samples for the following immunoassays: alcohol, benzodiazepines, cocaine, methadone and THC were ~0-1%. The false positive rates for barbiturates and oxycodone were < 3.0%.

Conclusions: Based on the results from this retrospective study, the positivity rate for THC and cocaine was near the national average; however, our positivity rate for illicit and hallucinogen drug use was significantly less. The discrepancy is likely due to our patient population for testing and limitations for testing other illicit drugs and hallucinogens in this screening panel. Some immunoassay tests were more prone to false-positives, such as, PCP, opiates, MDMA, and propoxyphene. Definitive testing is necessary, especially for immunoassay tests that are prone to false-positive or false-negative results, when quantitative results are important for interpretation (e.g. opiates), and when results are inconsistent with clinical expectations.

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Improved, simple, accurate Tacrolimus assay* without Manual Extraction for the Dimension RxL and EXL systems

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Background: The new Dimension® Integrated Chemistry Systems Tacrolimus (TAC) assay* provides confidence in patient results with improved productivity for complete transplant patient care.

Methodology: The TAC assay is based on ACMIA technology. The principle and operation of the TAC assay are as follows: a new pretreatment reagent is added to a reaction vessel on the Dimension system. Next a sample of whole blood containing tacrolimus is added. On board the blood and pretreatment reagent react to assure the lysis of the whole blood. A new Anti-tacrolimus antibody-β-galactosidase conjugate is added next and allowed to react with tacrolimus from the patient sample. Finally, pre-decorated chrome particles coated with a tacrolimus analog are added and allowed to bind the unreacted conjugate. The tacrolimus bound conjugate does not bind to the chrome but remains in the supernatant when a magnetic field is applied to the above reaction mixture. The tacrolimus bound conjugate is detected by transferring the supernatant from the reaction vessel to a photometric cuvette, where the enzyme tag is detected using a sensitive chromogenic substrate. Time to first result is 15 minutes.

Results: The new TAC antibody has improved cross-reactivity to the MI metabolite,13-O-Desmethyl tacrolimus (1% cross-reactivity vs. 15%

with TACR). MI was identified as the most abundant metabolites in the

literature. An extremely close relationship was observed between the Dimension TAC assay and the LCMS/MS reference method: TAC = 1.04 (LC/MS)-0.30; r =0.97 (n=315, range = 1.3 to 24.9 ng/mL), as well as to the Abbott Architect predicate assay: TAC = 0.99(ARCH) - 0.42, r = 0.98 (n=308, range = 2.4 to 24.2 ng/mL) A few discordant samples which were observed in the previous generation method TACR versus LCMS show excellent agreement with the new TAC assay. The new antibody and conjugate provide more sensitivity and better precision. From a precision profile the limit of quantification has been determined to be 1.0 ng/mL and the maximum within-lab reproducibility (%CV) has been found 8.8% at 1.8 ng/mL tacrolimus.

Conclusion: The new Dimension tacrolimus assay offers the quality along with the enhanced productivity of a fully automated assay for complete care of transplant patients. It allows confident management of tacrolimus dosing at any therapeutic target level in accordance with clinical practice guidelines. *Under FDA review. Not available for sale in the USA. Product availability varies by country.

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Studies on Profiling of Drugs in the Clinical Urine Samples using Automated ToxPrep Method on ToxPrepTM Workstation

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Background: It is a well known fact that chronic pain affects a large portion of the human population and patients are prescribed drugs as a form of pain relief and for treatment. However, some patients do not follow their prescription which can lead to the under-treatment and over-treatment risks to them. As a result, clinics have started setting up point of care laboratories to provide in office drug profile in patient's blood or urine samples that should provide accurate results in a timely manner. In order to meet the ever growing demand for such clinics, urine testing has been found the most common testing matrix used for monitoring drugs and patient treatment.

Methods: The ToxPrep method involves detection of drug compounds with and without hydrolysing the samples using glucuronidase with subsequent addition of reagents followed by analysis of the prepared sample with LC-MS.

Results: In the present studies, automated ToxPrep protocol on ToxPrep™ Workstation was used by running clinical urine samples on a 96 well plate. Linearity, QC of samples, dilution study, sensitivity, and carry-over were studied for 85 clinical urine samples for 25 drugs including Cocaine, Codeine, Morphine, and Methadone. The linearity for individual compounds performed resulted an average R^2=0.999, and coefficient of variance (CV) for precision and accuracy were below 5%.

Conclusion: The measurable, simple, serial dilution study with this method and sensitivity of the linear curve was clinically acceptable with no carry over from well to well. This automated method for patient sample processing is applicable from medium to high volume patients.

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Broad Spectrum Urine Drug Screening: the challenges of assessing qualitative cut-off performance characteristics in an LCMSMS System.

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BACKGROUND: Methods employing chromatographic separation and identification by tandem mass spectrometry are valuable for front-line or confirmatory testing of urine samples for a broad range of drugs and drug metabolites. The cut-offs adopted by most laboratories for positive screening results are derived from the Substance Abuse and Mental Health Services Administration (SAMHSA) regulations. In a pediatric centre, however, there is additional need to reliably detect many drugs below these cut-offs, as well as to assess the qualitative performance of our method at the cut-offs in use. The objective of this study was to determine how best to assess the linearity and qualitative cut-off performance characteristics in an LCMSMS System used for screening.

METHODS/RESULTS: To address these needs two evaluations were performed: (1) Forty-five drugs or drug metabolites were assessed for linearity below the SAMHSA-derived positive cut-offs in use at the Hospital for Sick Children in Toronto, Ontario. (2) The performance of our method at the cut-off of 50 ng/mL for 3,4-MDA and 3,4-MDMA was assessed by application of the CLSI Guideline for Evaluation of Qualitative Test Performance (EP12-A2).

In the linearity study, analyte pools containing between 8 and 10 spiked analytes were prepared at seven concentrations, ranging from 1.56 ng/mL to 100 ng/mL of each analyte, and were analyzed in duplicate on an LCMSMS QTrap 3200 system. The linearity of the peak area under the curve of each analyte in response to concentration was evaluated by application of CLSI Guideline EP6. Differences in linear performance were observed specific to drug, drug metabolite, and between mass-transitions. Additional analysis carried out queried the influence of normalization of analyte peak area to the area of the internal standard (D5-Diazepam), the concentration at which minimum requirements were met for the library matching algorithm, and the success rate of software peak identification without user intervention.

In the qualitative cut-off study, the capacity of our broad spectrum urine drug screen to correctly partition samples into a qualitative screen Positive or screen Negative designation was evaluated. Using a concentration of 50 ng/mL 3,4-MDA or 3,4-MDMA for the C_{50}, that is, the concentration at which the positive rate would be expected to be approximately 50%, replicates of samples at concentrations at and bracketing the C_{50} (e.g. ±20%) were analyzed. Previously established area under the

curve count cut-offs failed to correctly partition samples as Negative at the -20% bracket and partitioned all samples as Positive at the C_{50}. However, normalization of the data to the internal standard and establishing a new normalized area cut-off at the C_{50} improved the method partitioning performance.

CONCLUSION: As a result of this evaluation, there is confidence that our method correctly partitions Positive versus Negative samples with concentrations ± 20% from the SAMHSA cut-off of 50 ng/mL for 3,4-MDA and 3,4-MDMA. This work highlights the challenges with determining performance characteristics in an LC-MS/MS system used for screening.

B-342

Asparaginase Activity Quantification by application of a third-party assay kit to the Abbot Architect

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BACKGROUND: Asparaginase, an enzyme not endogenous to humans, catalyzes the hydrolysis of asparagine to aspartate. Asparagine synthesis is generally absent in hematopoietic cells, leading to their dependence on circulating asparagine for protein synthesis. The depletion of asparagine from circulation by treatment with drugs products possessing asparaginase activity, such as pegaspargase *E.coli* (PEG) or asparaginase *Erwinia* (Erwinia), is a common treatment for certain hematological malignancies such as pediatric leukemias.

The determination of treatment efficacy by therapeutic drug monitoring is a subject of growing importance due to issues of hypersensitivity, neutralizing antibodies, difference in clearance, and silent inactivation (subclinical hypersensivity) that develop in patients receiving these treatments. Personalized TDM is now recommended to attenuate poor patient outcomes as a result of these factors. Direct measurement of asparagine is challenged by the rapid degradation of asparagine ex vivo. Asparaginase activity assessment is available from AI Biotech, however, for more rapid turn around time we desired to evaluate the feasibility of applying a commercially available research asparaginase activity assay for use on an open channel of the Abbott Architect.

METHODS/RESULTS: The asparaginase activity assay kit from Sigma-Aldrich provides a simple and direct colourimetric method of asparaginase activity determination by a coupled enzyme assay. Briefly, asparaginase present in serum samples converts assay substrate asparagine to aspartate. The resulting aspartate generated is then measured by production of a coloured product such that one unit of asparaginase may be defined as the amount of enzyme that catalyzes the formation of 1.0 µmole of aspartate per minute at 25°C. Following condition optimization for use on the Architect, the assay was evaluated for precision, linearity, interferences (hemolysis, icterus, and lipemia), and patient samples were assayed on both this method and sent to AI Biotech for evaluation to determine total error suitability.

CONCLUSION: This study successfully demonstrated the feasibility of applying the Sigma-Aldrich Asparaginase Assay Kit to the Abbot Architect platform for in-house TDM of asparaginase activity for our pediatric leukemia patients.

B-343

Automated Sample Preparation for the Monitoring of Pharmaceutical Drug Diversion by LC-MS/MS

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Background:

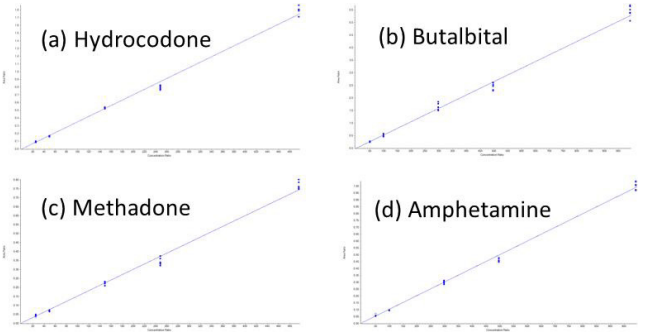
Liquid chromatography-tandem mass spectrometry provides laboratories with a powerful tool for accurate, multiplexed analysis of large panels of compounds. This technology is being increasingly applied to the screening of urine samples, to monitor for the potential diversion of prescription pharmaceutical drugs. As sample numbers increase, the bench time required to process these samples, as well as the opportunity for errors in processing increases. Automating the sample preparation for analysis by LC-MS/MS can increase the possible throughput, reduce the active time required to prepare even low numbers of samples, and reduce the opportunity for human error.

Methods:

In this work, we have utilized a Biomek 4000 Workstation to automate the sample processing for the monitoring of pharmaceutical drugs in human urine by LC-MS/MS. This method enables the analysis of 56 compounds across multiple drug classes, including the opiates, opioids, benzodiazepines, amphetamines, tricyclic antidepressants, barbiturates, THC-COOH, and other illicit drugs. Briefly, precleared urine controls and samples were combined with acetate buffer and beta-glucuronidase. Following automated mixing, the plate was removed from the deck, capped, and heated to 55° C for 2 hours. Following incubation, curve diluent was added to each well and the plate was centrifuged offline to clear any precipitate in the samples. 200 µl of the supernatant was transferred to a new plate and analyzed by LC/MS/MS. Mass spectrometric detection was performed using the AB SCIEX Triple Quad™ 4500 LC/MS/MS system.

Results:

56 target drugs were quantified over a wide range of concentrations from 0.5X to 10X of the cut-off concentration levels. The large majority of the compounds could be analyzed with accuracy over 80% and CV under 15%. This method provides high throughput capabilities for analytical laboratories that currently process samples manually for the monitoring of pharmaceutical compounds in biological fluids.



B-344

The Development of an LC-MS/MS Screening Method for 104 Targeted Compounds in Whole Blood, using Library Searching on a QTRAP Mass Spectrometer

M. J. Y. Jarvis[1], H. Singletary[2]. *[1]SCIEX, Concord, ON, Canada, [2]Metro Nashville Police Department Crime Lab, Madison, TN*

Background:

In order to detect a large variety of drugs in whole blood, many forensic laboratories incorporate multiple screening assays to cover different drug classes. Each assay screens for a single compound class, and frequently the assay cannot distinguish between specific analytes within a class. Our objective was to develop a single LC-MS/MS assay capable of accurately identifying >100 target compounds.

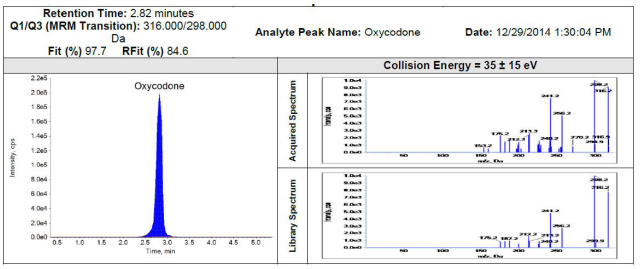
Method:

Our method employs MRM (Multiple Reaction Monitoring) measurements on an AB SCIEX 3200 QTRAP LC/MS/MS system to detect 104 target compounds in less than 10 minutes. The QTRAP enabled simultaneous MRM detection and 'on-the-fly' acquisition of a full-scan MS/MS spectrum for every detected compound. All acquired MS/MS spectra were searched against a spectral reference library, to increase confidence in compound identifications compared to traditional MRM-based methods (see Figure).

500 uL of whole blood containing internal standard was vortex mixed with 3ml of acetone, the sample was centrifuged, and the clean supernatant was collected and dried under nitrogen gas, then reconstituted with MeOH prior to analysis by LC-MS/MS. LC separation was achieved using a Phenomenex Kinetex PFP (50x2.1mm, 2.6um) column.

Results:

A cross-method comparison with an outside laboratory demonstrated that our method (i) provided more specific information about compound identity, (ii) provided superior sensitivity, and (iii) detected more compounds. The external testing only detected the presence of a compound class, for example "opiates", whereas the QTRAP screening method identified specific opiate compounds such as Oxycodone, Noroxycodone, Dihydrocodeine, etc. In certain cases the superior sensitivity of the QTRAP screening method detected the presence of compounds that were missed by the external testing. The established cut-off level was 10 ng/mL for the majority of the basic drugs, 1 ng/ml for the fentanyl group and PCP, 250 ng/mL for Trazodone, Pregabalin, and Gabapentin, and 1000 ng/mL for Carisoprodol and Meprobamate.



	Compound Name	Library Hit	Peak Area	Purity (%)
1	Oxycodone	Oxycodone	1,870,000	83.5

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Technology/Design Development

B-345

Validation of Peritoneal Dialysis Fluid Assays: Does Analytical Performance Meet Clinical Need?

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Background/Objectives

Peritoneal dialysis is an alternative to hemodialysis for patients with kidney failure. Removal of waste products occurs using an osmotic gradient across the peritoneal membrane by instillation and exchange of hyperosmotic solutions (eg. 4.25% dextrose) into the peritoneal cavity. Glucose, creatinine, and urea are measured to derive the patient's membrane transporter category and estimate dialysis adequacy (defined as Kt/V≥1.7). Peritoneal dialysate (PD) fluid composition varies due to manufacturer, addition of antibiotics, and osmotic agent concentration. In vitro diagnostics manufacturers do not list PD fluid as an acceptable specimen type, therefore analytical validation is required. The goal of this work is to evaluate how well the analytical performance of glucose, creatinine, and urea nitrogen assays in PD fluid meets what is required for clinical use of the assays.

Methodology

Validation was performed on the Cobas c701 (Roche Diagnostics) using residual clinical PD specimens. Recovery(n=6) was performed by spiking standard solutions (10% by volume, Sigma or Maine Standards) of creatinine and urea nitrogen, while specimens were serially diluted (n=1 up to x128) and mixed (n=4) to evaluate glucose accuracy. Interference from cefazolin(10mg/mL), vancomycin(31mg/mL), ceftazidime(10mg/mL), and heparin(20 units/mL) was assessed by calculating % difference upon spiking (n=2) for all three analytes. Stability was assessed by calculating average(range) %difference at ambient(20-25°C,n=10), refrigerated(2-8°C,n=10), and frozen(-20to-24°C,n=8) temperatures in plain tubes and NaF/K-oxalate tubes for glucose. Transporter categories were determined using glucose ($PD4hr_{gluc}/PD0hr_{gluc}$) and creatinine ($PD_{creat}/Serum_{creat}$) ratios for a cohort of 25 patients, and Kt/V calculated as ($24hrPD_{urea}/Serum_{urea}$ *24hrDrainVol*7)/(VolUreaDistribution) where $Serum_{urea}$ varied from 40mg/dL to 80mg/dL. A bias in $PD4hr_{gluc}$ and PD_{creat} was simulated by systematically adding -15to+15% glucose, -0.2to+0.2mg/dL and -10to+10% creatinine, and PD_{urea} -15to+15% using Microsoft Excel to determine the tolerance limits defined as changing transporter category for more than 80% of the cohort or interpretation of adequacy defined as >10% decrease in Kt/V near the 1.7 decision limit.

Results

The mean(range)% recovery for creatinine=105.2%(99.6%-108.0%) and urea nitrogen=109.6%(95.0-121.0%). Average(range) %recovery=98.6%(97.1%-99.8%) for serial glucose dilutions and 98.1%(94.2%-99.9%) upon mixing. Drug spiking showed an average %difference for glucose <+/-1%, creatinine <+/-2%, and urea nitrogen <+/-3%. Ambient PD fluid storage for one day had an average(range) %difference for glucose=-3.0(-19.7%-6.6%), creatinine=-1.5(-9.3%-3.6%), and urea nitrogen=-1.2%(-5.9%-3.7%); refrigerated storage for seven days demonstrated glucose=-5.7%(-19.4%-9.1%), creatinine=-0.7%(-9.3%-9.1%) and urea nitrogen=1.5%(-3.5%-5.0%); 30 day frozen storage revealed glucose=-1.7%(-24.6%-1.1%), creatinine=-2.3%(-8.2%-3.1%), and urea nitrogen=-0.5%(-4.5%-2.5%). PD glucose stability assessed in NaF/K-oxalate tubes revealed average(range) difference=-2.8%(-8.8%-2.0%) ambient for one day and refrigerated 1.4%(-4.3%-5.7%) for seven days. The clinically defined tolerance limits for glucose=+/-5%, creatinine=+/-0.1mg/dL or +/-4%, and urea nitrogen=+/-15%.

Conclusions

Average recovery of creatinine in PD fluid did not meet clinical tolerance limits while urea nitrogen and glucose studies did. Drug spiking did not alter results. PD fluid analyte stability was limited to one day ambient, 7 days refrigerated, and 30 days frozen in plain tubes, with the exception of glucose demonstrating a wide range of differences that was mitigated by storage in NaF/K-oxalate tubes one day ambient and 7 days refrigerated. Assessing body fluid assay utility and impact on interpretation is essential to derive meaningful analytical performance criteria.

B-346

Utilization of a Comprehensive Cancer Panel for Somatic Mutation Detection in Ovarian Cancer FFPE Specimens.

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Objective:

Detection of clinically relevant somatic variants in solid tumors.

Relevance:

Solid tumor samples are a complex mixture of normal cells of different types, often containing multiple tumor sub-clones within individual tumors. Such tissue samples are often available only from degraded sources, such as formalin-fixed paraffin embedded tissues (FFPE). Specialized analytical approaches are employed to detect somatic mutations in the background of germline sequence data.

Methodology:

FFPE tumor slides are macro-dissected to exclude surrounding normal tissue, followed by the isolation of DNA. The AltheaDx comprehensive cancer panel (CCP) is comprised of approximately 25 thousand probes targeting exons of 409 genes classified as cancer drivers, drug targets, genes involved in apoptosis, DNA transcription regulators, inflammatory response, and growth factors. Sequencing libraries are generated using a manufacturer-recommended workflow using a custom-made hybridization probe library. Sequencing is performed on the Ion Proton instrument following the manufacturer's recommended protocol. Base calling, sequence alignment, and variant detection is performed using the Torrent Suite 4.2.18 software. Identified somatic variants are further filtered for strand ratio and coverage and then assessed to determine the predicted effect on protein function or clinical significance using SIFT, PolyPhen2 and the COSMIC databases.

Performance Evaluation:

The performance of the assay for detection of minor populations of somatic variants was evaluated using an artificial somatic mutation control sample from Acrometrix™. 88% of targeted bases were covered at >100x, and 60% of targeted bases were covered at >500x. Overall, >80% of the synthetic variants with allelic frequencies in the 15-35% range and >60% of the synthetic variants with allelic frequencies in the 5-15% range that had appropriate coverage were detected in the control DNA.

Data Analysis:

In a performance confirmation study, 86 ovarian cancer FFPE samples with sufficient coverage (>60% of targeted bases covered at >100x) were used in variant analysis. Germline variants that matched the thousand genomes and dbSNP138 databases were excluded from further downstream analyses, and only non-synonymous or splicing-affecting single and multiple nucleotide variants, as well as insertion/deletion variants were retained. Overall, the samples were very heterogeneous, with highly variable number of damaging variants detected, which is consistent with polyclonal composition of these tumors and previously published data. The mean number of functionally damaging variants per sample was 56, varying from 6 to 318. Most of these variants were single nucleotide variants, with insertions, deletions, and multiple nucleotide variants varying between 0 and 6 lesions per sample.

The same common damaging mutations in the *KAT6B, GNAQ, TRIM33*, *SF3B,* and *HSP90AA1* genes were detected in over 20% of all samples. Interestingly, only one of these mutations (*GNAQ*) had been characterized in the COSMIC database.

Results and conclusion:

Starting with 100 ng of genomic DNA, 90% of the FFPE samples produced high coverage libraries suitable for variant analysis. These samples had >75% of targeted bases with coverage >100x, allowing for accurate somatic variant calling. A very large spectrum of damaging mutations was detected, including a number of previously unreported variants.

B-347

Using RFID technology in research laboratories to decrease material inventory and identification time.

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Background: Inventory management is the process of effectively monitoring the flow of products into and out of an area. Companies need to determine their inventory levels to avoid over purchasing since an unlimited amount of items cannot be maintained. Inventory management and identification plays a pivotal role in the operational efficiency of any company. Most companies measure the inventory

turnover of finished on-market products. However, materials utilized in-house for R&D projects are often not inventoried, but have turnover similar to finished goods. This inventory is considerably more critical to control as development materials are produced in smaller volumes and to specific formulations that may not be easily reproduced. Reducing over-stocking of this inventory, as well as the amount of time to find products leads to an increase in productivity with internal research and development groups.

Objective: Utilize passive Ultra High Frequency (UHF) Radio Frequency Identification (RFID) technology to monitor inventory quantities and locations of materials required for research and development activities.

Methods: Using the Abbott Laboratories' Inventory Manager Product, all reagents, calibrators, and controls used by the Diagnostic Division's Assay Development Organization were RFID tagged and placed into room temperature, refrigerated or frozen storage locations. Inventory Manager has the ability to print RFID tags and monitor inventory levels within one or multiple sites. Inventory locations were monitored with fixed RFID read zones. Inventory was additionally monitored on a bi-weekly basis with an RFID handheld reader to ensure the accuracy of the inventory levels.

Results: RFID antennas detected materials stored in room temperature, refrigerated, or frozen environments without any additional work required by laboratory personnel. Inventory was updated immediately after a walk-in refrigerator or freezer door was transitioned. When comparing Inventory Manager's RFID functionality to a manual physical inventory, the monitoring time of that inventory was reduced from two (2) employees for eight (8) hours (16 person-hours) to one employee for 4 minutes when the RFID handheld reader was utilized. This represents a >99% reduction in time spent inventorying materials. The accuracy that was measured over a six-week period of physical inventory actions was on average 97.7% accurate.

The RFID handheld reader was also utilized to find materials using its Product Locator feature. The closer you get to the tagged item, the faster an audible indicator is produced by the handheld reader. The handheld reader also displays how far or close you are to the item you are trying to locate with a visual indicator gauge. The average time to find materials went from

two (2) employees for three (3) hours (6 person-hours) to one employee in less than 10 minutes. The location time of materials was reduced by 97% utilizing the Product Locator functionality.

Conclusions: The level of accuracy and overall productivity in monitoring and managing inventory was significantly improved and provided in real-time by utilizing Inventory Manager's functionality. The time personnel spent in refrigerated and frozen storage locations to perform physical inventories were dramatically reduced as well. Additionally, materials were much easier to find utilizing the Product Locator feature of the RFID handheld reader.

B-348

Study of the correlation between urine test strips and sediment analysis in Urysis®/IQ-200® and Aution Max®/Sedimax®

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Background:

The urinalysis is a valuable tool for the detection and monitoring of kidney and urinary disorders, in addition to systemic or metabolic diseases.

Systematic analysis of the urine is done by test strips, which provide a semi quantitative determination as a screening, and is followed by an analysis of urine sediment in those samples presenting positive values in the parameters determined in the strip.

The aim of the study is to compare the results in test strips with the urine sediment analysis in two automated systems for urinalysis, Urisys® vs IQ-200® and Aution Max® vs Sedimax® to determine the correlation of these results.

Methods:

The measurements were performed with 261 routine urine specimens, which were processed in the Aution Max and Sedimax, after being processed by institution's routine systems (Urysis and IQ-200). Sample processing was performed for three days with prior shaking of the tubes in order to homogenize the sediments. In all of them leukocytes and erythrocytes were determined.

In the statistical analysis we determined the correlation between the results in test strips for leukocytes and blood (hemoglobin) and white blood cells and red blood cells in urinary sediment, calculating sensitivity (SS), specificity (SP), positive predictive rate (PPR), negative predictive rate (NPR), false positive rate (FPR), false negative rate (FNR), accuracy (A), positive and negatives likehood ratios (LR+, LR-) and Cohen's kappa coefficient (k).

Results:

Correlation for Urysis/IQ-200:

SS = 87.50%; SP = 69.48%; PPR = 39.25%; NPR = 96.10%; FPR = 30.50%; FNR = 12.5%; A = 72.80%; LR(+) = 2.87; LR(-)=0.18; k= 0.39.

Correlation for Aution Max/Sedimax:

SS = 100%; SP = 77.29%; PPR = 38.10%; NPR = 100%; FPR = 22.7%; FNR = 0%; A = 80.10%; LR(+) = 4.40; LR(-)=0; k= 0.45.

Conclusion:

Correlation between tests strips and particle analysis in urine sediment is good in both systems, but all the statistical parameters determined in the study give a better result for Aution Max/Sedimax. The rates for specificity are similar, as well as positive predictive rate, because both instruments give a very low number of false positives. The sensitivity, negative predictive rate are clearly better in Aution Max/Sedimax. The most accurate method is also Aution Max/Sedimax.

B-349

Glycation in human finger nail clipping using reflectance IR spectrometry, a new marker for diabetes diagnosis and monitoring.

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Background:

Human fingernail clippings contain ± 85% of keratins, which are prone to glycation. The underlying capillary bed of the distal phalanx of the finger is a source of glucose, which is able to react with the nail keratins. Nail keratin glycation may therefore reflect the average glycemia over the last couple of months. FT-IR spectroscopy allows to assess glycation non - invasively without the use of any reagents. In the present study, we wanted to explore keratin glycation of fingernail clippings as a non-invasive diagnostic tool for assessing long-term glycation in diabetes.

Methods:

Fingernail clippings (± 20 mg) were powdered using a dental drill. After incubation in a solution containing 100g/L glucose (48 h, 37°C), the remaining glucose in the powders was carefully washed out in a ultrasonic bath and then airdried. Subsequently, the powders were analyzed using reflectance infra red spectroscopy in the range from 4500 cm-1 to 450 cm-1 using a Perkin Elmer FT-IR Spectrometer Two (Perkin Elmer, Waltham, MA). Furthermore, incubation of the clippings with fructosamine-3-kinase (F3K) (1 µg/ml, 3h; 37 °) was used to analyse effects of protein deglycation.

Nail clippings of 25 diabetic and 25 healthy subjects were collected and subjected to IR spectroscopy.

Results:

In vitro glycation resulted in an increased absorption at ± 1050 cm-1. After enzymatic deglycation (F3K) this peak greatly diminished, proving this zone of interest represents glycated proteins (lysine-bound glycation). In diabetics ($n = 25$), aborbance at 1050 cm-1 was statistically ($p < 0.01$) higher than in age-matched controls ($n = 25$). The instrument readings were very reproducible (CV < 2%). As nail growth rate of the various fingers is comparable, there is no significant between-finger variability. Storage of the clippings at room temperature for 1 month did not significantly alter the IR spectrum.

Conclusion:

Analysis of protein glycation in human fingernail clippings with FT-IR spectrometry could be an alternative affordable technique for diagnosis and monitoring of diabetes. As the test does not consume reagents, and considering the fact the pre-analytical phase is extremely robust, the proposed test could be particularly useful in developing countries.

B-350

Effective PCR from Clinical Specimens Using an Improved KOD DNA Polymerase with Reduced Carry-over Contamination

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Background: PCR, a powerful technology used in many scientific disciplines, is also important in the analysis of clinical specimens. Recently, novel PCR enzymes have been developed with higher efficiencies than conventional Taq DNA polymerase for use in research and diagnostic fields. Among these, KOD DNA polymerase, especially the 3′-5′ exonuclease-deficient mutant KOD exo(-), is expected to be useful

for diagnostic purposes because of its efficiency at amplifying long DNA and GC-rich DNA targets, and its ability to directly amplify from crude clinical specimens such as blood. However, family B DNA polymerases such as KOD DNA polymerase demonstrate poor incorporation of dUTP used in the uracil-N-glycosylase carry-over prevention system. To circumvent this problem, we developed an improved KOD exo(-) mutant, UKOD exo(-), bearing an enhanced dUTP incorporation ability. Here, we describe the basic performance and application data of UKOD exo(-).

Objective: To determine and evaluate the performance of UKOD exo(-) in the DNA polymorphism analysis of crude clinical specimens.

Methods: The sensitivity and effectiveness of UKOD exo(-) were evaluated using real-time PCR with SYBR Green I. Various probe detection systems (Scorpion, Beacon, FRET, and Q probes) were tested for compatibility with UKOD exo(-), and the latter two systems were used in association with UKOD exo(-) to detect single nucleotide polymorphisms in *CYP2C19* from whole blood samples. All experiments were performed in the presence of dUTP.

Results: Two copies of human *beta-actin* (295 bp) were detectable using the SYBR Green system with UKOD exo(-). Moreover, the quantitative detection of human *CCNI* (179 bp, GC content: 71%) was achieved with the same system, whereas the target could not be amplified using a conventional system based on Taq DNA Polymerase. All four probe detection systems enabled the quantitative detection of all targets with UKOD exo(-). Furthermore, all *CYP2C19* polymorphisms in human whole blood specimens were distinguishable from each other using FRET and Q probe systems and the end point assay, whereas Taq DNA polymerase failed to amplify the targets. The overall performance of UKOD exo(-) was almost identical to that of KOD exo(-).

Conclusion: UKOD exo(-) exhibited excellent PCR performance even in the carry-over prevention system using dUTP. The enzyme therefore shows potential to be a powerful new tool for various high-throughput assays in a diagnostic field with reduced carry-over contamination.

B-351

A Fast Polarity Switching LC-MS/MS Analysis of Benzodiazepines and Barbiturates

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Background:

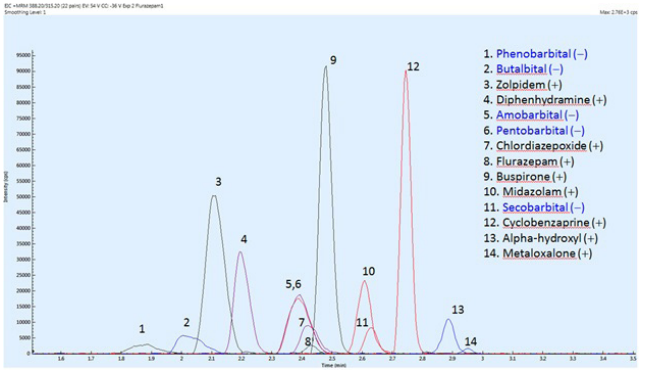
ESI-LC-MS/MS has been widely used to monitor pain management drugs on a routine basis in many labs worldwide. Because certain drugs ionize better in negative mode than that in positive electrospray ionization mode, the panel of interest is usually split into positive mode and negative panels. Recent advances in fast and robust polarity switching technologies allows for these panels to be recombined into a single run. This study seeks to demonstrate a high-throughput, robust polarity switching ESI-LC-MS/MS method on an IONICS 3Q 120 triple quadrupole mass spectrometer.

Methods:

The mixed barbiturates and benzodiazepine drug standards and analytical LC column were provided by Restek. 5 µL of diluted standard at various levels were loaded on a Raptor Biphenyl column (100X2.1mm, 2.7um) and eluted by a gradient method at a flow rate of 0.6 mL/min and a total LC cycle time of 6.5 minutes. The signal is detected by an IONICS 3Q 120 triple quadrupole coupled to a Shimadzu Prominence UPLC system. All solvents are HPLC grade. The 3Q system indicated is able to perform fast polarity switching (<15ms) with high ionization and ion sampling efficiencies. The separation and sensitivity reproducibility is monitored as a function of number of injection.

Results:

All 14 compounds eluted within the 6.5 minutes run time showed good chromatogram separation and excellent peak shape. No sensitivity loss is found for 14 compound panel as compared to the run if split into two panels. The CVs were <5% for all analytes within intra-day run and <9% within 3 series of inter-day run.



Conclusion:

The results indicate that the ESI-LC-MS/MS method with ultra-fast polarity switching using IONICS 3Q 120 mass spectrometer can greatly improve sample throughput in clinical pain management monitoring application. The dwell time and pause time effect for co-eluting positive and negative mode ions will also be explored and discussed.

B-352

A new enzymatic method for determination of serum zinc based on D-aminoacylase activity

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Background: Zinc ions are required for activation of metalloenzymes in vivo and zinc deficiency causes various diseases. Here, we describe an enzymatic assay for measurement of the zinc concentration in serum, based on the zinc-dependent activity of D-aminoacylase 1 (DAA1).

Methods: The assay depends on zinc ion-dependent catalysis of deacetylation of an N-acyl-D-amino acid (Ac-DAA) to a D-amino acid (DAA) by DAA1, followed by D-amino acid oxidase (DAAO) catalysis of oxidation of DAA to hydrogen peroxide and production of a quinone pigment using the Trinder reagent with peroxidase. The rate of product formation is linearly related to the acylase activity of DAA1, which depends on the zinc content of serum, and thus the zinc concentration can be determined from the absorbance of the quinone pigment. Assay principles are shown in Figure 1.

Results: The optimum assay conditions were pH 9.0 with measurements between 2 and 5 minutes after addition of the Ac-DAA (N-acetyl-D-phenylalanine). The within-assay CV was 0.92-1.55%, the between-run CV was 0.96-1.12%, and the day-to-day CV was 1.03-1.80%. The assay gave linear results over zinc concentrations of 0 to 60 µmol/L. The percentage recovery was 95-105%. The results from the new method were correlated with those from the 5-Br-PAPS and ICP-AES methods.

Conclusion: In conclusion, we have developed a novel enzymatic assay for zinc in serum. The method has good recovery, good precision, and a good correlation with the ICP-AES method and other conventional methods. The assay is linear over a concentration range that should be useful for routine zinc measurement in clinical laboratories.

$$\text{Step 1 : Ac-DAA + DAA1} \xrightarrow{\text{Zinc ion}} \text{DAA + } CH_3COOH$$

$$\text{Step 2 : DAA} \xrightarrow{\text{DAAO}} \text{α-keto acid + } NH_3 + H_2O_2$$

$$\text{Step 3 : } H_2O_2 + \text{Trinder + 4AA} \xrightarrow{\text{POD}} \text{Color + } H_2O$$

B-353

Application of Capillarys 2 Flex Piercing HbA1c measurement system for alpha and beta-thalassemia screening

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Background

HbA_{1c} measurement can be affected by hemoglobin (Hb) disorders such as hemoglobinopathies and thalassemia. Recent studies showed that β-thalassemia could interfere with the measurement of HbA_{1c}, similar to other known analytical interferences (e.g., carbamylatedHb, fetal Hb, or labile HbA_{1c}). Beside the analytical interference, Hb variants and β-thalassemia have been reported to be associated with a significant decrease in the lifespan of red blood cells. Thus, the HbA_{1c} value in patients carrying such Hb abnormalities may be misinterpreted if compared to the usual reference cut-off value. Therefore, the assays which are incapable of identifying thalassemia might report misleading HbA_{1c} values for thalassemic patients.

Methods

Whole blood samples from 258 healthy adult patients without Hb disorders, 80 α-thalassemia adult patients with $--^{SEA}/\alpha\alpha$ genotype, and 225 adult patients with β-thalassemia trait from 10 common genotypes were measured using Capillarys2 Flex Piercing HbA_{1c} system and Capillarys2Hemoglobin(e)system (Sebia, Lisses, France). The samples with iron deficiency were excluded.Receiver operating characteristic curve (ROC) were performed to determine the HbA_2 cut-off values for screening α and β thalassemia.

Results

For screening samples with α thalassemia, the optimal HbA_2 cut-off value is 2.35% (area under curve (AUC) 0.969, sensitivity 88.1% and specificity 92.5%), and 2.55% (AUC 0.951, sensitivity 90.9% and specificity 88.6%) for the Capillarys 2 Flex Piercing HbA_{1c} system and the Capillary2Hemoglobin(e) system, respectively. For screening samples with β thalassemia trait, the optimal HbA_2 cut-off value is 3.38% (AUC 0.994, sensitivity 100% and specificity 98.2%), and 3.75% (AUC 0.993, sensitivity 98.2%% and specificity 100%) for theCapillarys 2 Flex Piercing HbA_{1c} system and the Capillary2 Hemoglobin(e) system, respectively.

Conclusion

The Capillarys 2 Flex Piercing HbA_{1c} system can separate and accurately measure HbA_2 values for screening thalassemia besides reporting accurate HbA_{1c} value, which provides valuable information to clinicians for the interpretation of the HbA1c result in patients with thalassemia trait.

B-354

Performance Evaluation of New Biosensing Technologies Based on Functionalized Nanoparticles for in vitro Diagnostics

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Background: Biofunctionalized nanoparticles (NP) are very attractive tools and robust labels for advanced immunoassays and theranostic biorobots (Nikitin et al. Nature Nanotechnology, 2014, 9, 716-722). In this work several types of NPs such as plasmonic (gold, silver) and magnetic (iron oxides) particles have been optimized and used as the nanolabels for two types of biosensing technologies tested for clinical diagnostics.

Methods: Optimization of NP functionalization and bioassay protocols has been carried out by study of affinity constants of antibodies (AB) immobilized on NP based on the spectral correlation interferometry (SCI), which allows recording of thickness changes of a biolayer on a cover slip with a picometer resolution averaged over the sensing area. The sandwich-type immunoassays have been used for development of optical biosensors based on detection of the NP labels by SCI and by localized surface plasmon resonance spectra. The results were compared with electronic biosensors based registration of magnetic nanolabels in 3D solid phase structures by their non-linear magnetization (Nikitin & Vetoshko, EP 1262766, 2001). Recently this detection method was successfully used for toxin detection in complex biological media (Orlov et al. Anal. Chem. 2013, 85, 1154-1163). The sensitivity of the electronic detection method is on the level of the gamma-radioactive technique for counting of magnetic nanoparticles based on the 59-Fe isotope (Nikitin et. al. J. Appl. Phys. 2008, 103, 07A30). Optical and electronic biosensing technologies were tested for quantitative concentration measurements of protein markers of cardio-vascular diseases (cTnI) and cancer (PSA).

Results: It has been shown that the values of kinetic association constants of AB coupled with NP are 4 orders of magnitude higher than those of molecular AB association with antigen due to polyvalence of NP that carry multiple AB. The electronic biosensors demonstrated that the limit of detection (LOD) for total PSA is 25 pg/mL using blood samples of 40 patients. Besides the dynamic range exceeds 3.5 orders of concentration magnitude. CV is less than 10% at low concentrations. Importantly, the developed dry chemistry assay features simplicity and 4 times better LOD for 20 min-long assay as compared with several-hour-long commercially available ELISA kits. It has been shown that developed method combines the advantages of laboratory methods and rapid tests based on dry chemistry. The achieved LOD by optical readout of nanolabels on flat biosensing surface are 90 pg/ml for cTnI and PSA. However the optical technology is well compatible with microarrays for multiparametric diagnostics.

Conclusion: The electronic assay formats based on highly sensitive detection of magnetic nanoparticles in 3D solid phase have demonstrated the LOD of 25 pg/mL for detection of PSA in human blood as well as wide dynamic range of 3.5 orders of PSA concentration. Although the optical readout of nanolabels on a flat 2D solid phase is less sensitive (LOD of 90 pg/ml), it is well compatible with microarrays for multi-analyte detection. The magnetic assay is an attractive diagnostic platform for highly sensitive quantitative detection of proteins in complex biological fluids.

B-355

High-sensitivity immunoassays for IVD applications: Evaluation of the analytical performance characteristics of the Simoa HD-1 automated platform

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Background:

Simoa™ (Single Molecule Array) technology allows the development of ultrasensitive immunoassays by enabling the quantitative detection of protein analytes at very low concentrations. Enzyme-bound immunocomplexes are formed on the surface of paramagnetic microparticles, which are then individually confined into femtoliter-sized wells and imaged with a fluorescent substrate. This digital approach, based on Poisson statistics, allows measurements in the femtomolar range, a sensitivity improvement of up to 1000-fold over traditional ELISA.

Quanterix Corp's HD-1 platform harnesses the benefits of the Simoa technology in a fully-automated package. An RUO version of the system is commercially available for life science research, with a rapidly expanding menu of assays (currently >25) showing up to 1000x higher sensitivity compared to current best-in-class conventional immunoassay systems.

In the prospect of developing an IVD-compliant version of the system, key analytical performance characteristics were evaluated using a prototype assay for the quantification of PSA (prostate-specific antigen) in human serum. An ultrasensitive IVD assay for PSA would have clinical value, as a prognosis marker, to aid in the identification of patients at risk of prostate cancer recurrence following prostatectomy. Other ultrasensitive Simoa IVD assays could contribute to the improvement of standard of care: by providing clinicians with earlier detection of clinically relevant biomarkers, rapid diagnosis and application of appropriate treatment could be achieved. Simoa IVD assays could also be deployed for companion diagnostics.

Methods:

Evaluation of the analytical performance of the system consisted in a set of studies, based on CLSI guidelines, designed to determine precision (CLSI EP5-A2) and sensitivity (CLSI EP17-A2). Three instruments and two distinct lots of assay reagents were used for the evaluation. For precision determination, 10 replicate runs were performed on each instrument. Each run included the measurement of a full calibration curve, from which concentration values for specimens were derived. Calibrators were prepared from WHO standard in a diluted serum matrix. Six human serum samples were measured in triplicate as part of each run.

Results:

Precision levels of 2.6%-9.4% intra-instrument (between-run) CV and 3.6%-7.0% between-instrument CV were obtained for samples ranging from 3 pg/mL to 80 pg/mL in concentration. Intra-instrument precision is maintained when a calibration curve from a different run is used for sample concentration determination. Sensitivity is

characterized by LoB, LoD and LoQ values of 0.019 pg/mL, 0.046 pg/mL and 0.076 pg/mL, respectively.

Conclusions:

Superior analytical performance of the Simoa HD-1 system has been confirmed, with demonstration of ultra-high sensitivity capability and adequate precision levels at low analyte concentrations, in line with IVD requirements. The transitioning of ultrasensitive immunoassays from life science research to IVD is expected to enable successful translation of biomarker discovery into clinical practice, with the potential to address many unmet clinical needs.

B-356

Validation of Ella™, a multiplexed immunoassay analyzer, for the measurement of 4 cytokines.

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Background: Multiplexed immunoassay analyzers provide simultaneous measurement of multiple analytes in biological samples. While these assays offer significant savings in terms of time and sample volume compared to single-analyte assays, there are downsides to measuring multiple analytes at the same time in the same sample. In particular, there can be cross-reactivity between different antibodies and/or analytes, and the dynamic range is often compromised. Simple Plex (Protein-Simple, San Jose, CA) is a novel multiplexed immunoassay platform where a sample is split across different analyte-specific microfluidic channels. Because the individual antibody-antigen reactions are physically separated, this design overcomes many limitations of traditional multiplexed assays and allows the process to be automated. The objectives of this study were to evaluate the performance of the Simple Plex platform and determine the analytical performance characteristics of 4 cytokines: TNFα, IL-10, IL-6 and IL-1β. Methods: Quality control (QC) material, sample diluent, running buffer, Simple Plex cartridges and Ella were obtained from Protein Simple. De-identified patient samples were obtained from the clinical laboratory. Samples were diluted 1:2 with sample diluent and were loaded onto the cartridge. All samples were run in duplicate. Concentrations were calculated using vendor-determined calibration curves. High and low QC material was used to determine assay precision. The limit of detection was established by running blank samples (n=19). Analyte stability was determined by spiking 3 serum and 3 plasma samples with high QC material and incubating at 4 °C or 20 °C for various times. Analytical linearity was determined by serially diluting high quality control material and 3 patient samples. Recovery was tested by spiking normal serum specimens with a fixed amount of each analyte. Reference ranges were determined by measuring each analyte in 35 apparently healthy volunteers. Each analyte was also measured in samples obtained from patients with various disease states including prostate cancer (n=21), breast cancer (n=7), pancreatic cancer (n=7), sepsis (n=7) and non-malignant disease (n=4). Finally, results obtained on Ella were compared to another multiplexed platform (Meso Scale Delivery, Rockville, MD) using patient samples (n=24) that had been previously assayed for the four analytes. Results: Within-run precision ranged from 2.9 - 5.6%; total precision ranged from 6.3 - 13.5%. The precision of replicate sample measurements ranged from 2-3%. The limit of detection was 1.40, 0.31, 0.54 and 0.54 pg/mL for IL-10, IL-1β, IL-6 and TNFα, respectively. Overall, the linearity of the assay was acceptable; slopes ranged from 0.9833 - 0.9955, R2 values ranged from 0.9985 to 0.9998. No significant differences between serum and plasma were observed in terms of analyte concentration or stability. IL-10 and IL-6 were relatively stable over 24 hours. TNFα and IL-1β concentrations decreased 15-30% over 24 hours. Overall, Simple Plex values correlated well with MSD (R2 0.9689 - 0.9876). However, slopes ranged from 1.5 to 2.0. We are currently investigating this discordance. Conclusion: We found Simple Plex to have acceptable precision, limits of detection, linearity, and recovery for measurement of TNFα, IL-10, IL-6 and IL-1β. The instrument is easy to use and is a good alternative to traditional multiplexed immunoassay platforms.

B-357

Paper test card for quantifying iodate in fortified salt

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Background: One-third of the global population is at risk for iodine deficiency disorders, so iodine supplementation programs focus on providing this micronutrient to those who need it the most. The most common delivery platform is table salt fortified with potassium iodate. During production of iodized salt, local regulation usually dictates a level of 30-50 ppm I (expressed as mass of iodine atoms per mass of salt). Because iodine content decreases with time, monitoring agencies performing household and market surveys expect levels of at least 15 ppm. Current portable technologies for accurate measurement of iodate content in iodized salt are relatively expensive, which restricts their use in low resource settings.

Method: A paper millifluidic device was created to quantify iodate in iodized salt samples. A piece of paper the size of a playing card is printed with wax to define reaction zones. All of the reagents needed to perform an iodometric titration are stored dry in the paper. To measure the iodine content in salt, the user mixes 1 part salt and 5 parts water and then applies 125 uL of test solution to 12 regions on the test card. The test is complete in 3 minutes and the response can be assessed either by visual comparison to standard images or by computer analysis of a cell phone image of the card. The response of the card was calibrated to quantify iodate in the range of 0-75 ppm I, detect excessive iodization levels of > 150 ppm, and perform negative and positive controls. Using blinded methodology, 2 analysts ran 110 test cards with standardized iodate solutions to establish the analytical metrics of the test card.

Results: Computer image analysis was more accurate than visual interpretation, particularly for newly trained users. The accuracy and precision for determination of mg iodine/kg salt are both 4.5 ppm for the automated image analysis, and the accuracy is 7.0 ppm and the precision is 4.5 ppm when newly trained users read the test card visually.

Conclusion: The test card can quickly quantify iodate over a range that is useful to both salt manufacturers and monitoring agencies. The test card does not depend upon any specialized glassware or electronic devices and therefore has the potential to be utilized outside of the laboratory. Paper millifluidic devices can provide quantitative information to address quality and regulatory compliance issues in the developing world.

B-358

Analytical evaluation of the 25-OH Vitamin D total assay on the BioPlex® 2200

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Background: Vitamin D plays an essential role in calcium and phosphorus homeostasis. Vitamin D deficiency is linked to numerous diseases and conditions. 25-hydroxy (OH) vitamin D is the major metabolite and therefore is measured to assess the vitamin D status. Testing for 25-OH Vitamin D has increased dramatically over the past decade and several automated immunoassays exist to test vitamin D in serum. Here we evaluate the recently released automated Bio-Rad BioPlex® 2200 25-OH Vitamin D immunoassay, which is claimed to equally detect 25-OH D_2 and 25-OH D_3, against a gold-standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) method and the DiaSorin LIAISON® 25-OH Vitamin D TOTAL immunoassay. **Methods**: Imprecision was determined using third party controls over 21 days. Linearity over the entire measuring range was assessed using low and high patient pools. Correlation between the BioPlex and LC-MS/MS (n=137) or the LIAISON (n=56) was assessed using patient samples with varying amounts of 25-OH D_3 and/or 25-OH D_2. **Results**: The total imprecision was 9.2%, 6.8% and 4.4% at concentrations of 39.3nmol/L, 70.7nmol/L and 242.9nmol/L, respectively. The assay was linear from 18.1-375nmol/L with a R^2 of 0.988. Method comparison revealed a strong correlation between the BioPlex assay and LC-MS/MS for samples containing 25-OH D_2 alone (n=5; R^2= 0.999), 25-OH D_3 alone (n=119; R^2=0.935) and both (n=13; R^2=0.919). A strong correlation with the LIAISON assay was also observed (n=56; R^2=0.853). **Conclusion**: The analytical characteristics of the BioPlex assay make it suitable for the measurement of total serum 25-OH Vitamin D. The assay correlates well with the LC-MS/MS method and to a lesser extent with the LIAISON assay. Importantly, the assay is capable of equivalent detection of both 25-OH D_2 and 25-OH D_3.

B-359

An Imaging Reflectometer for Measuring Dry Slides Technology

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Background: A new reflectometer for reading VITROS® MicroSlide™ Technology was developed using a 2D imaging system and light emitting diode (LED) illumination. Through the use of machine vision, the flexibility of the reflectometer is increased to support the development of new assay formats. Reduction in patient sample volumes is also possible through decreased sensitivity to metering position and incubator alignment.

Methods: A camera containing a full frame, charge coupled device (CCD) image sensor was used to capture images of slides using a VITROS® 4600 Chemistry System. A pulsed LED with bandpass filter provided the necessary illumination. Using time delay and integration, the sensitivity of the camera was increased and images were captured in real-time without any change to normal slide processing. An initial estimate for center was made using criteria of low local pixel standard deviation and intensity. This was refined by locating points along the edge of the spot. These points defined chords, the perpendicular bisectors of which identify the center. Reflectance was computed by averaging pixels contained within a 1mm radius about the center. Performance of the system was tested by measuring the within-run precision of 20 repetitions of the VITROS® Chemistry Products CREA, BUN/UREA, PROT, ACET, Ca and ALKP slides using VITROS® Chemistry Performance Verifier (PV) I and II fluids at two drop volumes: 10µL and 5µL.

Results: The coefficients of variation (CV) were as follows for PVI at 10µL: CREA 2.17%, BUN/UREA 1.17%, PROT 8.25%, ACET 0.75%, Ca 0.55% and ALKP 1.66%. For PVII, CV were: 0.60%, 1.06%, 3.18%, 0.92%, 0.40%, and 0.94%, respectively. Reducing drop volume to 5µL resulted in a decrease in precision due to metering volume sensitivity for both PVI and PVII. Further investigation using CREA revealed LED instability to be a large contributor to imprecision, as improving the stability of the power supply decreased CV from 4.17% to 2.17% for PVI and 1.91% to 0.60% for PVII at 10µL.

Conclusion: We found an imaging system and LED to be a viable method for reflectance measurement of the VITROS® MicroSlide™. Although a decrease in precision was noted due to drop volume sensitivity and light source instability, future work aims to reduce these effects.

B-360

Evaluation of a new chemiluminescence immunoassay for laboratory diagnosis of syphilis

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Background: The resurgence of syphilis in recent years has become a serious threat to the public health worldwide. The serological detection of specific antibodies against Treponema pallidum remains the most reliable method for laboratory diagnosis of syphilis. Despite recent technological advances, there is still a critical demand for simple, rapid, and high-throughput detection of syphilis. This study examines the performance of the recently launched HISCL Anti-TP assay, a new chemiluminescence immunoassay (CLIA) as a screening test for syphilis.

Methods: To evaluate the overall clinical performance of this novel method, 300 syphilis-positive samples, 704 syphilis-negative samples, 48 potentially interfering samples (including hemolytic samples, samples from patients with autoimmune disease, HIV infection, HBV infection, HCV infection and EBV infection) and 30 samples with low levels of antibodies were collected. The HISCL Anti-TP assay was compared head to head with the commercially available and currently used Lumipulse® G TP-N in terms of statistical positive and negative agreement. For samples that yielded discrepant results between the two assays, a third Mikrogen Syphilis Immunoblot assay was performed.

Results: In this study, the HISCL Anti-TP assay was in perfect agreement with the applied testing algorithms. The positive and negative agreement of the HISCL Anti-TP assay were 100% (95%CI 98.53-100) and 100% (95%CI 99.37-100), respectively, comparable to that of Lumipulse® G TP-N. From the distribution of COI in the testing samples, there is clear discrimination between positive and negative results using the HISCL Anti-TP assay without "gray zone".

Conclusion: The automated platform and two-step method of the HISCL Anti-TP assay are well-suited to providing a rapid diagnosis in laboratories with high test volumes, with the first results available within about 17 min and the capability of providing up to 200 results per hour. The HISCL Anti-TP assay utilizes the recombinant treponemal TpN15, TpN17 and TpN47 antigens to simultaneously detect both the anti-treponemal IgG and IgM antibodies. With the innovative bound/free (B/F) separation technology, specific membrane filter and CDP-Star® as the substrate for chemiluminescence, of which the relative sensitivity is 3 times higher than traditional substrates such as AMPPD, the HISCL Anti-TP assay demonstrates advantages to minimize background noise, reduce carryover rate and improve sensitivity. Considering its superior performance, the HISCL Anti-TP assay could represent an outstanding choice for screening of syphilis in high-volume laboratories.

B-361

Evaluation of the Sebia Capillarys 2 FLEX PIERCING Instrument for Determination of HbA1c in a High Volume Laboratory

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Background:

Greenville Memorial Hospital Laboratory is the reference laboratory for a seven hospital system in the upstate region of South Carolina, and performs approximately three million tests per year. The hospital system provides laboratory services to a large number of regional physician practices and health screening services to many regional employers. Hemoglobin A1c (HbA1c) has become a common test performed at the laboratory as a part of the diagnosis and monitoring of diabetes mellitus type 2 and as part of these routine health screens. The laboratory averages 3,500 HbA1c tests per month and requires a reliable high-throughput method to meet this testing volume. This necessity is additionally complicated by the fact that the region's population has a relatively large rate of Hb variants. Empirical evidence has shown that approximately 3% of the region's population carries the HbS variant trait that may interfere with accurate HbA1c determination for some methodologies. Based on these criteria, the laboratory evaluated the Sebia Capillarys 2 FLEX PIERCING system (Sebia, Lisses, France) as a possible alternative to the current Trinity Premier Hb9210 system.

Methods:

The evaluation involved three Sebia instruments, each running different buffer lots, over a three day evaluation period. Reproducibility was evaluated using twenty pre-selected patient samples covering a reportable range of 4-16% HbA1c. Specimens were aliquoted and frozen prior to the evaluation period. Acceptance criteria for precision was a total CV of less than 4% for each of the twenty pre-selected patient samples over the three day evaluation period. Accuracy was evaluated using eight NGSP samples run in duplicate for three days on one of the three instruments. Acceptance criteria for accuracy was +/-5% of the NGSP target values. Additionally, a method comparison was performed versus the current Trinity system using 342 patient samples from daily testing. Where possible, the reproducibility specimens and NGSP samples were also analyzed on the Trinity system.

Results:

The total CV for the twenty pre-selected patient samples ranged from 0.56% to 1.94% for samples run in duplicate on three Capillarys FLEX PIERCING instruments for three days (n=18). All eight NGSP samples passed the accuracy requirement with a total allowable error of less than 5%. The eight NGSP samples were additionally evaluated using six-sigma metrics with an average sigma value of 3.2 using the current standard performance criteria of 6% total allowable error. The method comparison showed a linear relationship between the Sebia and Trinity methods but with average negative bias of approximately 0.3% HbA1c on the Sebia. Six homozygous Hb variant samples were additionally evaluated on the Sebia system producing no quantifiable result as expected (these samples have no Hb A0 present) and verifying that the method is not subject to the variant hemoglobin inaccuracies associated with some HbA1c methods.

Conclusion:

In every instance the Sebia system met or exceeded this laboratory's acceptance criteria and the method is being adopted as our laboratory standard for HbA1c testing.

B-362

Clinical Trial of a Fast, Accurate and Multiplexing Method for CYP2C19 Genotyping

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Accurate genotyping of cytochrome P450 genes is often very challenging due to the extreme similarity of sequences among gene family members and frequently existing pseudo genes. Simultaneous genotyping multiple SNP loci of a cytochrome P450 gene is even more difficult. Here we developed an advanced fragment analysis (AFA)-based multiplexing SNPs detection method that is able to accurately genotyping 3 SNP loci of CYP2C19 gene, CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893) and CYP2C19*17 (rs12248560) in a single tube. In addition to detecting the 3 SNP loci, the multiplex reaction also includes 3 human DNA controls and an internal reaction control. A clinical trial was performed at four major hospitals in different regions of China from April 2014 to October 2014. Total 1,338 of double-blinded human genomic DNA samples were extracted from peripheral blood samples of patients with cardiovascular diseases. Each DNA sample was separately used for genotyping with AFA-based method (trial reagent) and Sanger sequencing (reference method). 10-50 ng of genomic DNA was used for AFA-based PCR reaction. The dye-labeled

PCR fragments were separated, identified and quantified with a genetic analyzer. Genotyping results were determined by the specific fragment lengths and the ratios between alleles. All genotyping results were further compared to the results of Sanger sequencing. The accuracy of the AFA-based genotyping from 1,338 samples was 100% concordant to that from Sanger sequencing. In brief, the present study provides an accurate, fast and cost-effective genotyping method for detection of multiple SNP loci.

B-363

Disposable Dispense Cartridge (DDC) revolutionizing clinical diagnostic automation

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Background:

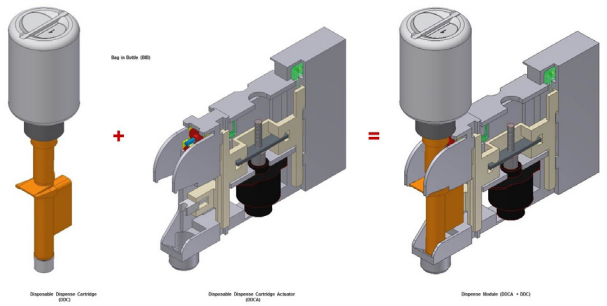
Dispensing of liquids includes typically pumps, tubing, valves and dispense nozzles implicating the known constraints of high dead volume, loss of reagent for priming, the contamination risks between different reagents or lots of reagents as parts of the liquid path are used by more than one liquid. Especially expensive and sensitive (light, gas or temperature sensitivity) reagents require a sophisticated and costly technical effort on the automation side to either reduce the waste of reagent or increase the on board stability of these reagents.

Methods:

STRATEC has developed a Disposable Dispense Cartridge (DDC) which overcomes the above mentioned limitations of the current technologies used for dispensing reducing the hardware cost to a minimum. The solution of STRATEC offers accurate dispensing of liquids while realizing secure identification (RFID) of closed self-contained reagent dispensing containers enabling increased on board stability without the risk of contamination or large priming volumes.

Results:

One of the first implementations of this new dispense technology and reagent concept is realized on the MosaiQ™ a blood typing/grouping automation solution of Quotient Biodiagnostics Ltd. This application requires light and oxygen sensitive reagents containing particles to be dispensed directly in the process consumable. Existing dispense technologies showed to be non-compatible with these reagents or resulting in a not acceptable on board stability; therefore the decision to use of this new dispense technology has been made. The system is still under development but the onboard stability has already been increased from minutes to hours (the goal is to reach multiple days). As the dispensation is depending on the assay type and is highly time sensitive the DDCs are located on axis making it possible to define different points and time of dispense. To realize continuous loading for each reagent two DDCs are loaded onto the automation system.



B-364

Novel drug delivery system based on N-palmitoyl chitosan polymeric micelles encapsulating ellagic acid with addition of cinnamaldehyde for the treatment of triple negative breast cancers

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Background:

Triple-negative breast cancer (TNBC), a highly aggressive malignancy, accounts for about 10%-20% of breast cancer, yet without effective molecularly targeted therapies due to the lack of existing molecular targets of breast cancer, thereby chemotherapy is the major treatment. However, chemotherapy usually causes severe side effects. To minimize the side effects, herbal medicines have drawn lots of attention. For instance, ellagic acid (EA) and cinnamaldehyde (CD) derived from pomegranate seeds and cinnamon, have been exhibited anticancer property in high concentration because of hydrophobic nature and low bioavailability, thus it requires efficient drug delivery platform.

Methods:

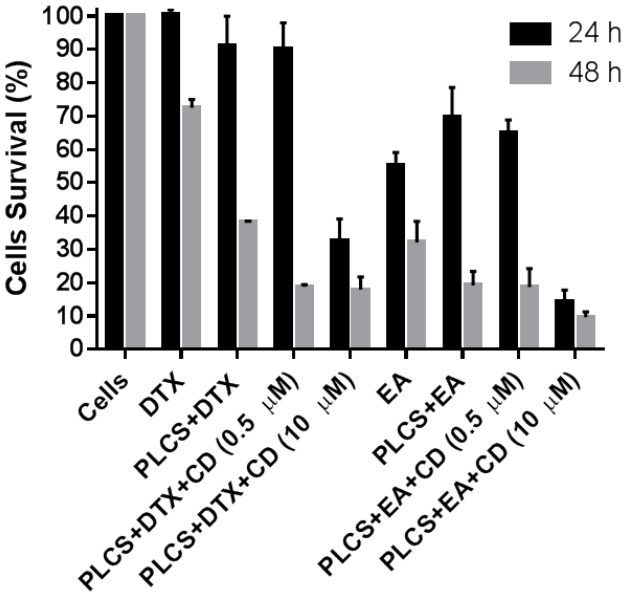
N-palmitoyl chitosan polymeric micelles (PLCS) have demonstrated as an efficient carrier for hydrophobic drugs in our previous study. Herein, we designed a drug delivery system utilizing a very small amount of CD to enhance therapeutic efficiency of PLCS loading with EA.

Results:

As the figure shown, the preliminary results of in vitro experiment indicated that after 48 hours PLCS micelles loading with EA and with addition of CD showed a 2 fold and 3.5 fold enhanced effect on cell growth inhibition of MDA- MB-436 cells, compared to PLCS micelles loading with EA but without the addition of CD, and the free EA. This system might because the CD decrease physical properties of PLCS such as size and drug loading efficiency, resulting in the improving the therapeutic efficiency of EA. To determine this system's suitability, we employed PLCS loading with docetaxel (DTX) and with addition of CD. DTX is a well-studied chemotherapy drug. We found the similar results. This drug system loading with DTX displayed an approximately 2 fold and 4 fold enhanced effect on cell growth inhibition, compared to PLCS loading with EA but without the addition of CD, and the free DTX.

Conclusion:

This drug delivery system can enhance therapeutic efficiency of EA and CD for the treatment of TNBC.



B-365

95% Confidence Intervals for the ELF™ Test Cutoffs

P. W. Dillon. *Siemens Healthcare Diagnostics, Tarrytown, NY*

Background: The ELF™ Test is a composite score calculated from the results of three direct markers of liver fibrosis (HA, PIIINP and TIMP-1).* It correlates well with liver biopsy results (Rosenberg et al, Gastroenterology, 2004, 1704-1713). Using the data from the 921-sample multi-center trial used to establish the ELF equation, two cutoffs (7.7 and 9.8) had been determined; the lower to distinguish Ishak biopsy scores greater than 2 from those 2 or less with 90% sensitivity and those with Ishak 5 or 6 from lower with 90% specificity. A third cutoff (11.3) was proposed (Lichtinghagen et al, J. Hepatology, 2013, 236-242) to distinguish cirrhosis from lesser fibrosis. Finally, other cutoffs, often close to those above, have been proposed (unpublished) for various purposes. The question arises: are these various cutoffs significantly different from each other?

Objective: Estimate 95% confidence intervals for the three cutoffs as defined in terms of Ishak ranges and sensitivities or specificities. These are important to prevent confusing, insignificantly different, cutoff proliferation.

Materials and Methods: ELF scores and Ishak biopsy scores from the original study were used. The ELF score to distinguish high and low biopsy ranges (say, 3-6 vs. 0-2) with a sensitivity or specificity closest to a target was developed. It allows a single ELF score at target, a range of scores each at target, or scores below and above, but not at, target. The 7.7 and 9.8 cutoffs had been established informally from tables of ELF score, sensitivities and specificities. The 11.3 was established from a different data set by Lichtinghagen. A bias-corrected and accelerated 1000-fold bootstrap calculation (Efron and Tibshirani, "An Introduction to the Bootstrap," Monographs on Statistics and Applied Probability 57, Chapman & Hall/CRC, 1993, 1998) was made for three cutoffs: Ishak 3-6 vs. 0-2, 90% specificity; Ishak 5-6 vs. 0-4, 90% specificity; and Ishak 6 vs. 0-5, 97% specificity. A "double-bootstrap" was used. Specifically, an Ishak score was resampled from the observed distribution of Ishak scores. Then an ELF score was resampled from the observed distribution of ELF scores for patients with the selected Ishak score.

Results: The Ishak discriminations, targets, algorithmic cutoffs with the observed data, and 95% confidence intervals are:

Ishak 3-6/0-2, 90% sensitivity, 7.67, 7.48-7.93,

Ishak 5-6/0-4, 90% specificity, 9.81, 9.63-10.02, and

Ishak 6/0-5, 97% specificity, 11.47, 10.99-11.65.

Conclusions: 95% confidence ranges have been established for the three ELF Test cutoffs. Each cutoff's nominal value is near the center of the corresponding range.

* The ELF Test and the HA, PIIINP and TIMP-1 assays have not been submitted to FDA and are not available for sale in the US. This test and these assays are CE marked on the ADVIA Centaur Immunoassay Systems.

B-366

An improved reference method for serum cations measurement by ion chromatography

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Background: In order to improve the accuracy, precision and robustness of the reference method for serum cations based on ion chromatography, an simple sample treatment procedure has been adopted for the determination of serum cations, which can be completely remove the proteins and/or organics in human serum.

Methods: Chromatographic conditions for the separate and simultaneous determination of serum sodium, potassium, magnesium and calcium were investigated. Furthermore, various influencing factors on the mineralization of human serum, such as the selection and amount of oxidant were also examined systematically and optimized. The measurement accuracy and precision was calculated by analyzing IFCC-RELA specimens and serum specimens.

Results: The optimized experimental conditions 1.0 mL of serum specimen digested with 2 mL nitric acid (120°C) followed by 2 mL hydrogen peroxide (80°C). The specimens were then redissolved and determined by ion chromatography under the optimum eluent concentration of 32 mmol/L methanesulfonic acids. The measurement accuracy and precision is less than 1.2% for all the analytes by analyzing IFCC-RELA specimens and serum specimens (listed in Table 1). The results were also comparable with the reference values obtained by ICP-MS, which were found to be in good agreement.

Conclusions: Ion chromatography with a simple sample treatment procedure for the determination of cations in human serum with high sensitivity and specificity. Compared with most of the other reference methods for the determination of serum cations, the advantages of the proposed method can be summarized as follows: (1) simultaneous separation and determination of serum sodium, potassium, magnesium and calcium effectively; (2) this wet digestion method could be completely remove the organics in serum, avoiding the risk of suppressor injury and column contamination which can long-term application with these types of samples; (3) this simple sample treatment procedure could be combined with other methods of analysis, such as ICP-AES and ICP-MS.

Table 1 Inaccuracy of the proposed method for lyophilized and liquid serum specimens (mmol/L)

Cations	Specimen	IC	CV(%)	Reference Value	Bias(%)
Na	Serum	132.79	0.36	133.00	-0.16
Na	2013 RELA-A	126.16	0.32	126.73	-0.45
Na	2013 RELA-B	129.35	0.62	129.88	-0.41
K	Serum	3.444	0.30	3.460	-0.46
K	2013 RELA-A	3.756	0.46	3.749	+0.19
K	2013 RELA-B	6.679	0.59	6.691	-0.18
Mg	Serum	0.732	0.39	0.730	+0.27
Mg	2013 RELA-A	1.439	0.61	1.443	-0.28
Mg	2013 RELA-B	1.370	0.67	1.366	+0.29
Ca	Serum	2.037	0.83	2.030	+0.34
Ca	2013 RELA-A	2.552	0.78	2.582	-1.16
Ca	2013 RELA-B	2.866	0.86	2.879	-0.45

B-367

An extraction-free method for quantification of cell-free plasma DNA in cancer patients

L. Xie, X. Song. *Shandong Cancer Hospital and Institute, Jinan, China*

Background:

Cell-free DNA (cfDNA) in plasma of cancer patients, mainly deriving from apoptosis and necrosis of cancer cells, is able to be served as an important marker for cancer diagnosis and monitoring, which circumvents the difficulties associated with lack of biopsy samples. Currently, extraction of cfDNA from plasma is indispensable for quantification of cfDNA. Nevertheless, the extraction stage is critical in ensuring clinical sensitivity of analytical methods measuring minority nucleic acid fractions. The extraction efficiency and fragment size bias are quite different among DNA purification techniques or commercial reagents.

Methods:

To sweep this obstacle, we developed a cfDNA-extraction-free method and a Super Green quantitative PCR(qPCR) assay based on the 18S rRNA gene for the determination of total plasma cfDNA and DNA integrity. The qPCR assay designed three different assays with comparable efficiency for the amplification of 62, 147 or 297 bp amplicon, respectively. Two integrity index (147/62 and 297/62) were used for the investigation of the circulating cfDNA integrity. We investigated cfDNA yield in plasma samples by comparing our cfDNA-extraction-free method with four specific cfDNA extraction methods [QIAamp circulating nucleic acid Kit (QIA), GenMag Circulating DNA from Plasma (GEN), FitAmp Plasma/Serum DNA Isolation Kit (FIT) and Circulation DNA Kit (CIR)].Then plasma cfDNA in 30 patients with non-small-cell lung cancer and 28 healthy people was analyzed using our extraction-free method.

Results:

We found that the median amount of the same cfDNA quantified by different isolation methods varied from 6.6 to 39.9 ng/mL. The extraction efficiencies among those ranked in the order cfDNA-extraction-free method > QIA kit > GEN kit > CIR kit > FIT kit. The cfDNA from extraction-free method did not effect the specificity and sensitivity of the qPCR assay. Furthermore, our Super Green quantitative PCR assay showed an increase in assay specificity and sensitivity over the conventional qPCR. The cfDNA-extraction-free method gave a better representation of smaller DNA fragments in the extract than the others.

Conclusion:

The cfDNA-extraction-free method gives a more reliable estimate of total cell-free plasma DNA quantity in cancer patients.

B-368

Simultaneous Detection of Multiple Nosocomial Infections and Antibiotic Resistant Mechanisms on the CAS100®- A Novel Molecular-based Microarray Diagnostic Platform.

Z. Grinberg[1], V. Hurgin[1], D. Hodko[2], S. Gross[1], H. Leskes[3]. *[1]Savyon Diagnostics, Ashdod, Israel, [2]Nexogen Inc., San Diego, CA, [3]Gamidor Diagnostics, Petach Tikva, Israel*

Background and Objective. - A major concern in healthcare systems worldwide is to reduce rates of hospital associated infections (HAI), with annual costs now calculated at $23 billion and 115,000 deaths per year only in the US.

To date, the most effective way to reduce and manage HAI is isolation of positively identified carriers of the antibiotic resistance bacteria. Methicillin-resistant Staphylococcus aureus (MRSA), Carbapenem-resistant Enterobacteriaceae (CRE) and Vancomycin-resistant Enterococci (VRE) are the leading causes of HAI, and the conventional turnaround time from sample to their identification is >48h, a period that is long enough to allow further spread of these resistant bacteria in the hospital.

Savyon Diagnostics is developing a novel molecular-based diagnostic test for simultaneous detection of MRSA, VRE and CRE directly from samples. The test utilizes Savyon's proprietary CAS100® molecular electronic microarray technology for rapid, multiplexed and fully automated procedure from sample-to-result. The aim of this work is to present this novel diagnostic approach for management and control of nosocomial MRSA, VRE and CRE infections using the CAS100® technology

Methods. . The platform is composed of two components: (1) the CAS100® analyzer and (2) a self-contained disposable cartridge, which is specific to patient, sample type, and pre-determined gene targets (up to 100). The sample is loaded into the disposable cartridge at the specific sample port. All the required reagents are included within the cartridge. The cartridge is loaded into the CAS100® instrument and the results are presented within 90 minutes.

Results. To mimic infection control patient's swabs, we used spiked samples containing MRSA, CRE and VRE clinical isolates. The microarray was pre-activated with specifically designed capture oligonocleotides, and the generated amplicons following the PCR were electronically directed to reaction loci on the microarray. Detection was achieved through specific fluorescent reporter oligonucleotides, automatically applied on the microarray and then hybridized with respective amplicon sequences. The output analysis of each sample was compared to the original characterization of the respective sample, performed by conventional culture or real-time PCR in the providing laboratory. All results were in complete accordance with the original characterizations of the tested samples including four different carbapememases (KPC, NDM, VIM and OXA) underlying CRE resistance mechanism. The CAS100® demonstrated efficient multiplex analysis and provided rapid results, indicating the presence of the targeted pathogens and antibiotic resistances in the tested samples.

Conclusions. The CAS100® is proposed as an efficient tool for screening nosocomial, MRSA, VRE and CRE infections. The usefulness of this platform is in its capability to perform diagnosis in individual samples when rapid detection of multiple targets is required. The system presents powerful multiplexing capabilities (≤100 plex)a feature which is unique compared to other systems that are intended for single sample tests. All-in-all the CAS100® system presents significant advantages in management of HAI, mainly in terms of minimal sample-to-result duration and hands-on time for detection of multiple targets. These features enable considerable assistance in containing the spread of resistant bacteria in health care settings.

B-369

Ambient stabilization of diagnostic PCR and RT-PCR assays using PCRstable® technology

C. Powers, G. Dodson, J. Romero, H. Balcer, W. Huang, V. Liberal, R. Muller. *Biomatrica, Inc., San Diego, CA*

Molecular assay and diagnostic applications enable early detection of diseases and enable informed treatment decisions. The technology is advancing towards decentralized diagnostics, close to the patient through point-of-care devices and the advancement of lab-on-a-chip technologies. Assay optimization and advanced chip manufacturing are becoming a major bottleneck for scale-up and global assay distribution. Common preservation technologies such as cryopreservation and lyophilization are not suitable for efficient manufacturing and optimized distribution. PCRstable® services enable preservation and optimization of dried PCR and RT-PCR reagents at ambient temperatures. This stabilization technology allows the development of complete assays that can be located in a single spot, are ready-to-use, and can be distributed globally. In this study, we tested the stability and functionality of PCR- and RT-PCR-based diagnostic infectious disease assays preserved with PCRstable® solutions using multiple PCR-based reagents from several major vendors.

PCR and RT-PCR assay performance of the stabilized reagents was assessed following accelerated aging studies at elevated temperatures. Using this technology we show that Superscript III®, GoScript®, GoTaq®, and HawkZ05® enzymes retain full function for at least 1 year at ambient temperature. Furthermore, we show ambient stabilization of these enzymes in a variety of assay formats, including in complete multiplexed diagnostic assays for Influenza, HIV/HCV, C. difficile, and HBV. Dried reagents are rehydrated with purified RNA/DNA sample and PCR-based assays conducted.

These methods can be applied to any PCR-based diagnostic method, including infectious disease, cancer, and genetic screening in a variety of formats to simplify assay design, expand distribution and reduce test complexity. Ambient temperature stabilization through PCRstable technology will expand worldwide accessibility of decentralized, point-of-care, and lab-on-a-chip diagnostic tests.

B-370

Evaluation of General Chemistry Assays on the Mindray™ BS-480 Chemistry Analyzer

B. Medaugh, R. H. Brown. *MedTest, Canton, MI*

Background:

The Mindray™ BS-480 Chemistry Analyzer is a fully automated, discrete, random access chemistry analyzer designed for mid-volume laboratories with a throughput of 400 photometric tests per hour, and up to 560 tests per hour including ISEs. The analyzer is capable of performing general chemistry and urine drugs of abuse screening analysis simultaneously or independently. The sample carousel contains 90 sample positions for barcoded primary collection tubes or sample cups and offers STAT testing capability. The refrigerated reagent carousel contains 80 reagent positions and can accommodate testing methodologies up to 4 reagents. The reaction carousel consists of a dry bath heating system utilizing borosilicate glass cuvettes coupled with an 8-step washing/rinsing/drying process. This analyzer offers many features commonly found on high-volume systems: intuitive software interaction; touch screen monitor; on-board operator's manual with intelligent indexing; intelligent probe management system offering bubble detection, collision protection with auto-recovery, liquid level sensing and clot detection (Sample Probe Only); and remote access diagnostic capability.

Objectives:

This study evaluated the precision, accuracy, linearity, interference and limit of detection of 24 assays on the Mindray BS-480 Analyzer, using the Beckman Coulter AU400 analyzer as a reference testing analyzer. The general chemistry reagents are manufactured at the MedTest corporate headquarters located in Canton Michigan.

Methods:

Analysis was performed based on modified versions of applicable CLSI Protocols. Within Run and Total Precision were determined by running three levels of control material. Within Run Precision was determined by running 20 replicates of controls in a single day. Total Precision was determined by running materials in duplicate across 20 shifts. Accuracy assessment through a correlation of at least 80 patient samples on the Mindray BS-480 and Beckman Coulter AU400 analyzers is in process. Carryover studies using reagents and samples historically observed to cause carryover in cuvettes, probes, and mixers are in process. Interference studies for hemolysis, lipid, and bilirubin will be conducted using a 10% margin of variability and compared to reagent manufacturer's claims. Limit of Detection for calibrated assays will be determined by statistical analysis of response values from five replicates of a low sample and ten replicates of a negative sample. For factored enzymes the limit of detection will be determined by observation of the lowest concentration sample yielding nonzero results.

Results:

All assays yielded within run precision CVs below 6.8% and most assays had CVs ranging between 0.0% to 4.8%. All assays yielded total precision CVs below 8.3%, with the exception of carbon dioxide, and most assays had CVs ranging between 0.7% to 5.0%. Accuracy, carryover, interference, and limit of detection studies are currently in process; but preliminary data suggest comparable performance to the Beckman Coulter AU400.

Conclusion:

The performance characteristics of the general chemistry assays on the Mindray BS-480 Analyzer were comparable to the Beckman Coulter AU400. Based upon data generated to date, it can be concluded that the Mindray BS-480 Analyzer is a suitable instrument for use in mid-volume laboratories based upon throughput capabilities and performance.

B-371

Development of a novel enzymatic assay for determining cardiac troponin I on clinical chemistry analyzer.

Y. Tang, T. Wang, Y. Luo, J. Zhou. *Yujiang Medical College for Nationalities, Baiso,Guangxi, China*

BACKGROUD: Cardiac troponin I (cTnI) is recognized as the most important marker of myocardial injury. Currently, chemiluminescence immunoassay(CLIA) is the most widely used routine method for the determination of cTnI, which have high sensitivity and precision , but the drawback of CLIA is that it can not be implemented on clinical chemistry analyzer. Although Immune enhanced turbidimetry assay for cTnI can be applied to clinical chemistry analyzer, but compared with CLIA, the sensitivity and precision are low. In this paper, We have designated a enzymatic method for measuring cTnI base on the fact that cTnI is able to suppress the activity of ATPase. The assay includes two steps: in first step, the monoclonal antibodies was added to block the ATPase binding sites on the skeletal troponins, while cTnI keep bound with ATPase; in second step, the ATP added was decomposed into ADP and phosphate by ATPase, the phosphate formed reacted subsequently with inosine , and converted to hypoxanthine by purine nucleoside phosphorylase(NP), and further converted to xanthine and H2O2 by xanthine oxidase(XOD). In the presence of H2O2 and peroxidase(POD), N-ethyl-N-(3-sulfonyl)-3,5 dimethoxy aniline(ESPDMA)was oxidized to form a blue compound which has a maximum absorption peak at 585nm. The increase of absorbance at 585nm is inversely proportional to the amount of cTnI in the sample.

METHODS: The method involves use of myosin ATPase(EC 3.6.4.1), ATP, EDTA-Na2, inosine,NP,XOD,ESPDMA,and POD,and measurement of the increase in absorbance at 585nm of the blue compound.

RESULTS: The calibration curve for cTnI was linear (r=0.99) between 0.030ng/ml and 2.550ng/ml. The LoD of the assay was 0.025 ng/ml and the analytical recoveries of exogenous cTnI added to serum was 100-103%. Within-run and between-run coefficient of variation (CV) were 5.8%, 6.2%(0.025ng/ml), 5.5%, 5.8%(0.65ng/ml) and 4.2%, 4.5%%(2.35ng/ml), respectively.

CONCLUSION: An enzymatic method for cTnI determination was successfully developed, the sensitivity and precision of the assay was similar to that of CLIA, and the method can be applied to clinical chemistry analyzer.

Wednesday, July 29, 2015

Poster Session: 9:30 AM - 5:00 PM

Automation/Computer Applications

B-372

Improving Compliance with Practice Guidelines through Changes in Physician Order Sets

S. W. Njoroge, A. Woodworth, J. Field, K. Wolfe, M. M. Zutter, E. Johnson, J. H. Nichols. *Vanderbilt University School of Medicine, Nashville, TN*

Introduction: Unnecessary or inappropriate laboratory testing can lead to delays in diagnosis, treatment and/or other potentially harmful clinical outcomes. Inappropriate test ordering also contributes to increased healthcare costs. Clinical practice guidelines provide physicians with best practice recommendations on the appropriate diagnostic workup for preventive health and disease management. The American Congress of Obstetricians and Gynecologists (ACOG) provides clinical guidelines for prenatal testing, including a CBC, virology screening for HIV, rubella and syphilis, and hemoglobinopathy screening in high risk groups. According to these guidelines, hemoglobin variant screening is recommended for individuals of African, Southeast Asian, and placeMediterranean ancestry. It is not recommended for low risk ethnic groups such as Caucasians. Daily review of hemoglobin variant screening tests at our institution revealed numerous tests ordered on pregnant Caucasians. We investigated restricting this testing to the guideline recommended patient populations.

Objective: The aim of this study was to intervene and improve compliance with ACOG guidelines and to evaluate the effects of our interventions.

Methods: Hemoglobin variant screening tests ordered on pregnant women were tracked over an eight month period (July 2014 to February 2015). Patient race/ethnicity was determined from electronic medical records. Interventions undertaken to improve utilization of hemoglobin variant screening tests in pregnant women included: 1) polling physicians about reasons for test orders and knowledge of ACOG guidelines, 2) reviewing ordering patterns to identify sources of misorders, and 3) changing the electronic order set to remove universal hemoglobinopathy screening for pregnant females (January 18, 2015). Hemoglobin variant screening was performed by BioRad Variant II HPLC and patient cost per test determined using CPT codes.

Results: Discussions with ordering physicians revealed awareness of ACOG guidelines and intent to follow but that test orders were directed by an electronic order set. This order set included universal hemoglobin variant screening, regardless of ethnicity. In keeping with consensus guidelines, we recommended removing the hemoglobin variant test from the order set and requiring physicians to add the test for high risk patients. Prior to our intervention, 245 hemoglobin variant tests were inappropriately ordered on pregnant Caucasians, an average of 35 times per month. One month post-intervention, no hemoglobin variant tests were ordered in this ethnic group. Based on a patient charge of $120 per test, this amounted to a decrease in patient charges of $4,200 per month. At a reagent cost of $5 per test, we estimated a monthly savings of $175 for the laboratory. We also noted significant labor savings for technologists, residents and medical directors who run, report and review the results of hemoglobin variant tests.

Conclusions: Periodic review of the laboratory tests included in order sets, as well as regular communication with ordering physicians, helps achieve compliance with practice guidelines and can contribute to improved patient care and cost-savings. Removal of hemoglobin variant screening tests from the pregnancy order set resulted in a substantial decrease in patient charges and laboratory resources. Evaluation of the clinical impact of these changes is ongoing.

B-373

Towards the Automation of Culture Reading within the Clinical Laboratory: A Multi-site Assessment of an Image Analysis Device

J. Glasson[1], R. Hill[2], M. Summerford[1], A. Liebelt[1]. *[1]LBT Innovations Ltd, Adelaide, Australia, [2]University of Adelaide, Adelaide, Australia*

Objectives

The purpose of the study was to evaluate the diagnostic performance of a culture reading device for screening urine cultures for infection.

Relevance

Few areas within high volume clinical laboratories have not benefited from some form of process automation. One area however, the culture-plate reading workload, has remained a manual process and may not meet the future challenges of increasing workloads, the need for improved turn-around times and requirements for freeing valuable laboratory space and highly trained technologists.

Methodology

The Automated Plate Assessment System (APAS®, Clever Culture Systems AG Switzerland) screens agar plates by using image analysis technology and applying standard rule sets to those findings. It then stratifies cases by the probability of further action such as manual review or additional processes such as isolate identification and antibiotic susceptibility testing.

A total of 4273 clean catch and catheter urines submitted for routine culture to two diagnostic laboratories (Healthscope Pathology, Clayton, Victoria and Healthscope Pathology, Wayville, South Australia) were included in the study.

For processing, a 1µl sample of each urine was inoculated onto tryticase soy agar with sheep blood and MacConkey with CV agar (Remel, Lenexa, USA) and incubated in air at 35oC for 18h. The plates were then read by a reference group of three independent technologists and then a fourth technologist presented the plates to the APAS® instrument.

The results from the reference group and the instrument were then analyzed by an independent statistician and also compared with the final reports produced by the laboratories.

Validation

The manually read plates showed 1258 urines with growth at ≥10^5 CFU/mL, 1058 at 10^4 CFU/mL, 948 at 10^3 CFU/mL and 1009 with no growth. APAS provided the same count as the reference group in 3901/4273 (91%) of cases.

When compared with the consensus result from the reference group, the combined diagnostic sensitivity for all culture reports was 93.6% with a specificity of 92%. When stratified by growth level, the sensitivity for the finding of growth at 10^5, 10^4, 10^3 and 0 CFU/mL was 99, 98, 83 and 4% respectively.

The incidence of the pathogens detected was in line with those reported in several large studies.

For the more rarely encountered isolates, APAS detected 5 cases of infection with viridans streptococci, 7 Candida spp (Including C. albicans) and 2 Aerococcus urinae. No organisms infrequently associated with UTI such as Corynebacterium urealyticum, Gardnerella vaginalis or Haemophilus spp. were detected by the laboratories or APAS during the study.

Conclusions

This study confirmed that automation based on image analysis technology can be applied to the assessment of urine cultures.

At growth levels of 10^4 and 10^5 CFU/mL, the diagnostic sensitivities were 96% and 100% respectively.

The device was able to detect organisms commonly encountered in urinary tract infection as well as a number of species less frequently isolated.

B-374

Development of a Computer Program for Exploring Large Quality Assurance Datasets

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Background: Quality laboratory testing depends on low pre-analytical, analytical, and post-analytical error rates. There is an urgent need for open-source computational solutions that effectively summarize quantitative data related to the quality of laboratory testing. We have developed software that enables the analyst to actively engage large quality assurance datasets through interactive elements and statistical graphics (histograms, bar graphs, heat maps, etc.). As a result, key statistics of the data are rapidly and intuitively queried.

Methods: This software analyzes LIS output and produces an interactive workspace accessed via a web browser. It does this by combining the very powerful statistical, logical, and graphical capabilities of R with the intuitive framework of a web page (generated using a combination of HTML and JavaScript). As a result, the analysis may be leveraged by individuals lacking programming experience.

Results: Prior efforts to analyze LIS output at our institution were done manually using built-in functions within Microsoft Excel. This workflow was time consuming

and only resulted in a summary table. Our software reduces this analysis time to three minutes of data formatting and entry and four minutes of computational time for analysis of ca. 150,000 test order entries. In the figure we demonstrate a heat map analysis of test order cancellations which provides a visual stratification of the frequency of cancellation events, including receipt issues, labeling issues, hemolysis, etc., by ordering location. The actual output of the program would enable interaction by clicking on individual summary statistics. This interaction would display additional statistical graphics for specific cancellations for that location as well as all more details of the associated test orders. Here, the analyst would prioritize investigation of the locations with high QNS and clot frequencies.

Conclusion: We have developed software for generating intuitive workspaces for complex analysis of clinical laboratory quality assurance datasets.

B-375

Initial Implementation of a Risk Based Quality Control Scheme Using Bio-Rad Mission: Control Software

M. A. Cervinski[1], F. A. Polito[1], L. Kuchipudi[2]. *[1]Dartmouth-Hitchcock Medical Center, Lebanon, NH, [2]Bio-Rad Laboratories, Plano, TX*

Background:A robust quality control (QC) program is a balance between detection of critical systematic errors (SEc) and false rejection of an analytical run. Hundreds of patient samples are typically run between QC events and the number of results affected by SEc depends on the likelihood of detecting the error with the next QC event. In the course of converting from a Medically Allowable Limits (MAL) strategy to a risk analysis model, we investigated whether the Bio-Rad Mission:Control software program could guide our analytical risk assessment QC strategy. The objectives were to use the Bio-Rad program to analyze our current MAL strategy for its ability to detect SEc in selected analytes and to design a QC strategy that would reduce the number of QC events needed to detect a shift in assay performance.

Methods: Six high volume serum assays (Sodium, Potassium, Chloride, Bicarbonate, Creatinine and Hemoglobin A1c) were selected for analysis. Current QC data (running QC mean and SD) were loaded into the Mission:Control software. CLIA allowable total error limits and the repeat 1:2s QC rule were used to assess protocol performance. The expected number of QC events until detection (EQCE) of a critical systematic error condition for each assay were calculated by the Mission: Control program. Detection of SEc using the running QC mean and SD were compared to fixed QC mean and SD limits. Finally, new user defined fixed means and SDs were selected in order to improve SEc detection while minimizing false rejection.

Results: For all assays analyzed, assuming no bias in the measurement procedures, the least number of QC events needed to detect SEc were those QC protocols designed using the running QC mean and SD to define QC limits. For example, the sodium running mean(SD) values for QC levels 1 and 2 were 125mmol/L(0.847) and 152mmol/L(0.732). Using the running mean and SD in the QC rule, a SEc = +/-1.7 mmol/L was predicted to be detected in 1.3 QC events with a false rejection rate of 1%. Alternatively, if the measurement process is biased with correct values for QC levels 1 and 2 of 126 mmol/L and 153 mmol/L, then using QC rule fixed mean(SD) values of 126mmol/L(1.2) and 153mmol/L(1.2) is predicted to detect a SEc = 2.9 mmol/L in 4.2 QC events with a false rejection rate of 0.4%. Narrowing the fixed SDs to 1.1 mmol/L at 126mmol/L and 1.0 mmol/L at 153mmol/L reduced the expected number of QC events to 2.1 with a false rejection rate of 1.9%.

Conclusion: The Bio-Rad Mission: Control software program allowed us to assess the performance of our current QC strategy and to design new QC limits that are predicted to reduce the number of QC events needed to detect SEc. This strategy will also reduce the number of unreliable patient results that would be reported prior to detection of the SEc. Further implementation of a risked based QC strategy will optimize error detection without added expense of additional QC analysis.

B-376

Design of Autoverification Review Codes Using Historical Data

C. R. McCudden. *The Ottawa Hospital, Ottawa, ON, Canada*

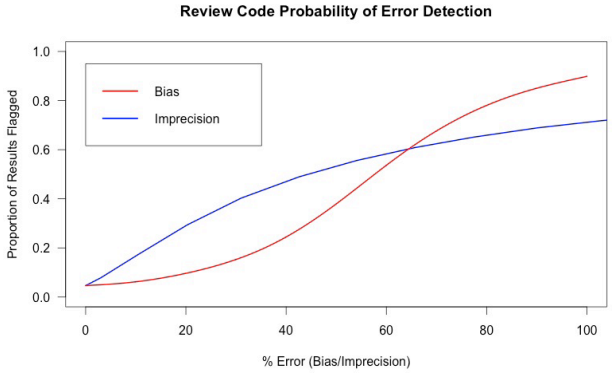
Background: Autoverification is used to automate the reporting of patient results, ensuring consistency and improving turnaround times. Autoverification is based on evaluating each result against pre-defined rules. These rules may include reference intervals, quality control results, analytical ranges, critical values, and delta checks.

Objective: The goal of this study was to establish a set of review limits for automated chemistry tests that don't have critical values or delta checks.

Methods: Two-years of laboratory results were extracted from the LIS database. Extracts included 286 unique reportable results from the Siemens Dimension Vista 1500; results included urine, serum, and plasma and calculated parameters, such as eGFR. Histograms were generated for each analyte and the 95% non-parametric confidence intervals were determined from the 2.5th and 97.5th percentiles. Visual inspection of the histograms and confidence intervals were combined to establish review codes where results outside of these historical limits would occur <5% of the time; for high volume tests, limits were widening to minimize the volume of false positives. Error simulation was used to model the efficacy of the review codes. Error was simulated by randomly introducing bias or imprecision (each separately). The modeled error was compared to the review code cutoffs to determine what proportion of results would be flagged.

Results: Review codes were established for 241 reportable tests. Error simulation models yielded error-dependent probability (see Figure showing error detection probability curves). The figure shows the % error introduced against the probability of error detection, where color denotes bias or imprecision. Real world implementation demonstrated a good trade-off between false-positive rate (y-intercept in figure) and error detection probability depending on the amount of bias or imprecision.

Conclusions: The review codes designed herein provide an additional quality tool for tests that do have critical values or delta checks. This approach is readily implemented in a typical autoverification workflow.



B-377

Enzymatic HbA1c assay on ARCHITECT c8000: Effect of extended wait time prior to sampling

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Background: The enzymatic Hemoglobin A1c (HbA1c) assay on the ARCHITECT c8000 instrument is used to determine HbA1c levels. The results could be used not only for monitoring of patients with diabetes mellitus but also for diagnosis of diabetes mellitus. Therefore, precision and accuracy of results becomes extremely important. With this assay, HbA1c levels can be determined from whole blood samples without a manual pretreatment. Process efficiency can be increased by having this assay on the Automated Processing System (APS). The package insert for the assay instructs to mix all specimens thoroughly by low speed vortexing or by gently inverting 10 times prior to loading on the instrument. This raises the questions as to how long the mixed specimens can wait prior to sampling and if settling of blood cells due to extended wait time affect the HbA1c results obtained.**Methods:** To determine the effect of extended wait time before sampling on the HbA1c results obtained from the ARCHITECT c8000 instrument, several samples were mixed gently by inverting 10 times and loaded on to the APS. Each sample was tested immediately and then tested after various time points (every 30 minutes up to 5 hours) without further mixing. The results obtained at various time points were compared.**Results:** The results indicated that the HbA1c results were not affected by extended wait time prior to sampling (up to 5 hours after mixing) since the %CV between the results obtained at various time points was less than 1.5%. **Conclusion:** Process efficiency can be increased by having the HbA1c assay on the APS . The results indicate that there was no significant difference in the HbA1c result whether the samples were tested immediately after mixing or up to 5 hours later without further mixing.

Table 1: HbA1c results for 14 different samples tested at different time points after mixing.

Sample No.	0 hr	0.5 hr	1 hr	1.5 hr	2 hr	3 hr	4 hr	5 hr	%CV
Sample 1	5	4.9	4.9	4.9	4.9	5	5	5	1.3
Sample 2	4.5	4.6	4.7	4.6	4.6	4.7	4.7	4.6	1.45
Sample 3	5.2	5.2	5.2	5.3	5.3	5.3	5.3	5.3	0.89
Sample 4	5.2	5.2	5.3	5.3	5.3	5.3	5.3	5.3	0.77
Sample 5	6.5	6.5	6.5	6.5	6.5	6.6	6.5	6.5	0.62
Sample 6	5	5	5.1	5	5.1	5.1	5.1	5.1	0.92
Sample 7	9.8	10	10	10	10	10.1	10.1	10	0.83
Sample 8	5.6	5.6	5.6	5.6	5.6	5.7	5.7	5.7	0.92
Sample 9	6.4	6.4	6.5	6.5	6.5	6.5	6.5	6.5	0.62
Sample 10	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	0.00
Sample 11	4.9	4.9	4.9	4.9	4.9	4.9	4.9	5	0.82
Sample 12	5.5	5.5	5.6	5.6	5.6	5.6	5.6	5.7	0.96
Sample 13	7.9	7.8	7.8	7.9	7.9	7.9	7.9	7.9	0.51
Sample 14	6.9	6.9	7	7.1	7.1	7.1	7.1	7	1.16

B-378

New Solution to Patients Identification with Laboratory Information System

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Background:

Blood transfusion is a life-saving medical procedure and over 779,344 people have blood transfusions annually in Taiwan. However, it could lead to devastating consequences and fatality even if there is only one defect like miscollected samples (wrong-blood-in-tube, WBIT), mislabeled samples or misused patient samples in the laboratory. Given that laboratory errors accounted for approximately 30% of errors resulting in mistransfusion, this project applied an information system with barcode technology to avert the potential clerical errors in the transfusion laboratory.

Methods:

The study retrospectively reviewed the outcomes of laboratory information system introduced in 2011 and completed in 2014. The information system with barcode technology was designed to exactly match the facts from recipient to donor samples and verified reports of ABO typing, RhD typing, crossmatching and the antibody screening test. Once an error had been detected, a warning message was shown on the screen, the procedure was discontinued and then this record was kept. Additionally, a wireless information barcode system had been administered for inpatient identification and for checking the consistency of the wristband and the labeled tube.

Results:

A total of 24560 inpatients requiring blood transfusions were identified in a medical center. In 2010, the 14 mislabeled incidents of the18322 samples were found; however, in 2014, no mislabeled case was reported (N=19016). Besides, the records showed that the phlebotomists had good compliance with the use of the new system.

Conclusion:

The transfusion laboratory developed a zero-tolerance policy to identify patients and manage samples. The writer believed that a successful information system should be stable and user-friendly. After implementing the information system with barcode technology over a year period in the facility, the incidents of misidentified and miscolleced transfusion samples were totally eliminated.

B-379

Enhancing Quality Control in Clinical and Point of Care Settings

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OBJECTIVE

Published documents by the Clinical and Laboratory Standard Institute including EP18 and EP23 and by the International Organization for Standardization have provided guidance for managing quality in clinical devices. Suggested methodologies rely on understanding sources of error and designing integrated QC methods for rapid error detection and correction. One approach for designing an integrated QC method is to identify error patterns and devise targeted corrective actions. Timely error detection is necessary and can only be achieved through continuous monitoring of the measurement system. Methods for gathering and identifying error patterns can be accomplished through detailed examination of past end-user data. Such end-user data analysis can be achieved reliably only if the measurement system is closed. The measurement system should also be capable of collecting extensive information for proper identification of error patterns.

METHODOLOGY

GEM Premier 3000 blood analyzer (Instrumentation Laboratory, Bedford, MA, USA) has a single cartridge (GEM PAK) that includes all the analytical components needed for testing and, therefore, fits the criteria for a closed analytical system. The analyzer is designed to collect a large amount of data during its operation. Years of investigating cartridge data from healthcare facilities has provided an extensive library of identifiable patterns associated with specific error modes. The methodology has allowed the development of Intelligent Quality Management (iQM), replacing the use of traditional external quality controls. Collected data from several GEM PAKs at a clinical site were used for analyzing error detection capability of internal versus external controls. The iQM corrective action report was used to identify any analytical issue and corrective action performed by the analyzer.

RESULTS

More than 1000 samples including 300 QC's were evaluated among four GEM PAKs. Sigma values as total allowable error divided by standard deviation were used to assess error detection capability of the internal and external QC methodologies. Sigma values greater than four indicated a high probability of detecting abnormal analytical change. There were only three cases for internal controls with sigma less than 4. In contrast, there were eight cases for external controls with sigma less than 4. Furthermore, high frequency of internal control checks in iQM allowed faster error detection time even for parameters having less than 4 sigmas. As a result, iQM was capable of detecting several transient failures including four micro-clots while no failure was detected with external QC in any of the PAKs. All clot-related errors were detected within 1-2 minutes after sample introduction and followed by immediate user-notification and automatic start of corrective action.

CONCLUSION

Data analysis of the GEM PAKs in clinical use demonstrated the effectiveness of iQM in timely detection of errors that would otherwise go undetected with traditional external QC method. Errors were detected immediately after the sample that caused them. Real time and continuous monitoring of iQM allowed for immediate and automatic error detection and corrective action, further enhancing the analyzer's quality assurance at the clinical or point-of-care settings.

B-380

Performance Evaluation of Automated Clinical Chemistry Analyzer for Indocyanine Green (ICG) R15 Test

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Background: Indocyanine green retention rate at 15 minutes (ICG R15) test has been used to predict the residual liver function before hepatic resection or the prognosis of patients with liver cirrhosis. Conventional ICG R15 test is inefficient and inconvenient because it requires spectrophotometer manually and four samples in a patient. Here, this study aimed to establish the automation of ICG R15 test using automated clinical chemistry analyzer and evaluate the calculation of ICG R15 with small number of samples.

Methods: Performance of AU5832 analyzer (Beckman Coulter, Brea, CA, USA) for measuring ICG concentration was evaluated in accordance with the guidelines of the Clinical Laboratory Standards Institute (CLSI). Precision, linearity and carry-over were tested using standard materials prepared with five different concentrations. Plasma samples obtained from the patients were used for the comparison between manual and automation method. A total of 54 patients were requested for ICG R15 test at two university hospitals from July to October in 2014. We measured concentrations of ICG at the wavelength of both 800nm and 805nm by spectrophotometry, and at 800nm by AU5832. R15 of 54 patients analyzed by the two methods was compared using the Bland-Altman method and paired t-test. We evaluated the calculation of ICG R15 with three samples, except for the one of samples obtained at the time before injection, or 5, 10 and 15 minutes after injection of ICG to the patient, compared to that with 4 samples. The agreement of each method was assessed according to the three categories of R15 (Category A, R15 < 15%; Category B, 15% ≤ R15 <30%; and Category C, R15 ≥ 30%, respectively).

Results: The automated ICG test by AU5832 established in this study showed the proper performances according to CLSI. All coefficients of variation of ICG concentration showed values below 3% with linearity in the 0.1~1.0 mg/dL range

($r^2 \geq 0.999$). The test value of percentage sample carry-over was less than 1%. The significant correlation was found between the ICG concentration by automated method (800nm) and manual method (805nm) ($r^2 = 0.954$). Although the difference of ICG R15 results between the two methods was within 95% confidence interval, R15 was adjusted by the regression equation for being slightly lower by automated than manual method. R15 with 3 samples (0, 5 and 15 minutes) showed the best correlation with conventional R15 with 4 samples ($r^2 = 0.997$). Compared to manual method, R15 of AU5832 showed the excellent agreement with 4 samples (kappa value 0.891) and also with 3 samples (kappa value 0.820).

Conclusions: Beckman Coulter AU5832 for the determination of ICG concentration and R15 test performed well and showed good correlations with conventional spectrophotometry. Thus, ICG R15 test using AU5832, even with three samples, may be comparable to conventional method in the clinical use.

B-381

Overall Equipment Effectiveness (OEE) as a Tool to Improve Productivity in a Clinical Laboratory (Dasa, Brazil)

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Background:

Improving productivity, with focuses on the efficiency of the production, should be fostered in clinical laboratories. By doing so the provision of higher amount of goods and services to different health stakeholders, such as (i)patients, (ii)physicians and (iii)health insurance companies, can be translated into higher profits. Currently, productivity is considered a competitive advantage to clinical laboratories. One methodology to measure productivity is the OEE (Overall Equipment Effectiveness) metric which states the effectiveness of manufacturing process, using three different components: (i)availability (down time loss, which includes any events that stop planned production), (ii)performance (speed loss, which includes any factors that cause the process to operate at less than the maximum possible speed) and (iii)quality (in terms of unnecessary tests performed). It is unlikely that any manufacturing process can run at 100% OEE, therefore the vast majority of manufacturers benchmark their industry to set a challenging target of 85% OEE.

Objective:

This study aims (i)to demonstrate the improvement of productivity through the implementation of OEE, (ii)to understand the most common causes of efficiency loss in clinical laboratories ("Six Big Losses": stops, setup and adjustment, idle time and short stops, reduced speed, quality errors and reaching and startup errors) and (iii)to provide management tools to control and eliminate losses.

Methods:

The largest DASA Central Laboratory that is located in Rio de Janeiro (60 Million tests annually) was chosen for the implementation of the OEE analysis, focusing on the fully automated serum working area, comprised of clinical chemistry, immunology and hormones. The OEE index was calculated using the formula: (Availability)*(Perf ormance)*(Quality). Responsibilities were assigned to the production team, suppliers and support areas in order to (i)measure the three components of the aforementioned formula and (ii)to define the goals for these components and therefore of the OEE approach as a whole. These metrics composed the OEE dashboard that was analyzed on a weekly basis by the team and action plans were developed whenever the goals were not achieved. On a monthly basis, strategic meetings were held with the executive board of the company in order (i)to guarantee that the action plans were fully implemented, (ii)to supervise deviations and (iii)to brainstorm regarding new possibilities of how to improve productivity in that setting.

Results:

After two months of implementation, the OEE percentage of the serum working area increased by 11%, representing 23% improvement in productivity. The most significant increase was in "Availability". One of the main focus of the project was to improve the main losses, such as breakdowns, setup and adjustments, reducing the downtime of equipment. The OEE of immunology increased by 31%, clinical chemistry by 20% and hormones by 13%. These results allowed the enhancement of the production capacity without adding new assets to the production site.

Conclusion:

The results have shown that productivity increased thanks to OEE implementation. The method helped in terms of enhancing production capacity and therefore avoided wrong investment decisions, ensuring that the processes were adequate. In essence, OEE worked as the missing link between capex implementation and production processes.

B-382

Implementation of equipment management and its impact on a Clinical Laboratory production

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Background: Recent technological development on clinical laboratory tests led to higher production effectiveness and security. Meanwhile, a great number of equipment must be managed regarding to preventive and corrective maintenance. Thus, an effective system to manage equipment, technical resources, services, and cycle of life as far as aiding in viability studies for new technologies and risk management is necessary.

Methods: Case-study applied to a clinical laboratory in Brasilia, Brazil. To further manage the technological park and in order to improve productivity a sector of clinical engineering was created. Aiming an secure and sustainable process we elaborated a procedure and a software for equipment management was afforded (Engeman 7.6, Engecompany, Itaúna, Brazil). All analytical equipment were catalogued as well as its maintenance and critical information. The new software permitted to manage calibration, preventive maintenance and to monitor corrective maintenances. Information about documentation and the working team qualification was also recorded in the database. A training schedule for all professionals involved with equipment handling and maintenance was established.

Results: After the implementation of the new procedures the analysis of performance-related indicators such as: mean time between fails (MTBF), service-level agreement (SLA), preventive maintenance plan compliance, number of calibrations per test per equipment, effective availability of each equipment during working hours, could be promptly accessed from the database. Following these indicators led to better equipment management, reduction of unexpected failures and higher agility on problem-solving. Productive efficiency raised from 75% in 2013 (before implementation) to 98% in 2014.

Conclusion: Implementing an electronic management process to the equipment led to higher confidence to end-user and also to patients, since a predictable platform permit lower delays and trustful results.

B-383

Development of an Automated Solid Phase Extraction of Procainamide in Serum Samples

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Background: In determining patient compliance and assessing dosage of antiarrhythmic drugs like procainamide ($C_{13}H_{21}N_3O$), quantification in the patient serum or plasma samples is essential. As the demand on sample processing and analysis increases, laboratory automation solutions become necessary for a testing facility. Automated solutions not only facilitate greater sample processing throughput, but these solutions also minimize the variation and error between samples. However, robotic systems can be subject to positional or spatial biases - intra-performance differences between positions on the deck of the system. Here we investigated potential positional biases of solid phase extraction (SPE) column positions on the VERSA 10 SPE deck.

Methods: A fully automated assay on VERSA 10 SPE Workstation was used for water and serum samples spiked with procainamide using 3mL columns (C8 / SCX). A set of 12 columns were simultaneously processed for activations, wash and elution steps of the protocol. The samples and reagents were mediated through the columns by an automated 12 channel positive pressure module. The eluted samples were subjected to drying with an automated nitrogen dryer in combination with automated heating and shaking. The dried pellet was reconstituted in 1mL of mobile phase and transferred to HPLC vials for high performance liquid chromatographic (HPLC) analysis. The reconstituted procainamide HCl samples (10µL) were analyzed on a Plastisil ODS column.

Results: Positional biases were investigated on the VERSA 10 SPE by examining the recovery of 20µg of procainamide HCl serum samples after SPE on all column positions. Four batches of twelve samples were tested (11 procainamide samples and 1 negative control). The average procainamide HCl recovery per batch (n=11) shows a recovery range between 87.86% - 98.35%. Analysis of Variance (ANOVA) and Tukey-Kramer HSD analysis on post SPE procainamide HCl recovery indicated no statistically differences between column positions on the VERSA 10 SPE deck. Furthermore, the average coefficient of variance (CV%) across column positions was 4.05% (±0.859%).

Conclusion: The presented data and analysis show that there are no positional biases in column positions across the deck of the VERSA 10 SPE automation system. The overall coefficient of variance within each batch was low indicating high precision across each column position that suggests a well-suited solution for increasing throughput and reproducibility while minimizing hands-on sample preparation time.

B-384

Comparison of Two Automated Microscopy Image-Based Urine Sediment Analyzers

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Background:

Urinalysis is one of the most commonly requested and performed tests in clinical laboratories. The microscopic analysis of urine gives valuable information about many clinical conditions encompassing kidney and urinary tract diseases. Neverthless, traditional microscopic examination is time consuming, not suitable to analyze large number of urine samples, and poorly standardized. Therefore, automated urinalyzers have been widely used instruments especially in high-throughput laboratories in recent years. The objective of the present study was to compare the performances of two automated urine sediment analyzers.

Methods:

A total of 151 fresh urine samples sent for urinalysis were assessed using IQ200 (Iris Diagnostics, USA) and FUS200 (Dirui Industry, China) analyzers. Precision, linearity, and carry-over studies were carried out for IQ200 and FUS200. Also, we evaluated the performance of FUS200 in accordance with IQ200 for detection of red blood cells (RBC) and white blood cells (WBC) in high power field (HPF). Detected urine particles were classified by both two automated systems and images edited by same laboratory technician.

Results:

The cell count results were compared by Passing-Bablok regression analysis and Bland-Altman plots. Comparative analysis with these automatic systems, IQ200 and FUS200, revealed concentration-dependent differences for the counted cells. Bland-Altman plots showed slightly higher results by IQ200 for both RBC and WBC counts. Passing-Bablok regression parameters for RBC and WBC are showed in Table 1.

Conclusion:

Both of the urine analyzers showed similar performance and clinically insignificant variability of RBC and WBC counts. Standardization of urine analyzers and evaluation of particle images are needed to improve and ultimately the quality of patient care.

Passing-Bablok regression						
	n	Intercept	95% CI	Slope	95% CI	Significant deviation from linearity
FUS200-IQ200 (RBC)	151	0,14	0,00-1,00	1,28	1,00-1,63	p<0.01
FUS200-IQ200 (WBC)	151	1,00	0,00-1,00	1,00	1,00-1,30	p<0.01

B-385

On-line flagging monitoring - A new quality management tool for the analytical phase

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Background: Traditionally, it is difficult to demonstrate the influence of analytical quality on daily medical decision making. This is partly due to the fact that analytical quality specifications should be related to the highest hierarchical model, which is their effect on clinical decisions. Strictly, this would require complex and expensive outcome studies which, however, still are lacking in the field of laboratory medicine. We, therefore, looked for other tools that could translate analytical quality, in particular, assay stability problems into their influence on daily medical practice.

Methods: We investigated the effect of analytical shifts on so-called "surrogate" medical decisions, such as flagging of laboratory results using local cut-offs. We developed an on-line tool for monitoring of daily flagging rates, which we called "The Flagger" (www.theflagger.be). The time course of the data is followed by variable moving medians (n = 5, 8, 16). Instabilities are mainly assessed from limits based on biological variation. State-of-the-art limits are used for analytes with low biological variation.

Results: We report our first experiences about the value of flagging monitoring with the "surrogate" medical decision "hypercalcemia". For example, an analytical shift of ~0.06 mmol/L (~2.5%) is "translated" by the Flagger application into a 3-fold increase of the flagging rate (from ~3% to ~9%). Clinical chemists indeed considered this increase in flagging rate important. Currently, this tool is programmed by local IT-departments, however, laboratory information system providers are interested to develop generally applicable solutions. Moreover, with our Flagger platform, we are able to perform peer-group monitoring of flagging rates opening all the benefits of peer group comparisons.

Conclusion: We consider on-line flagging monitoring in the individual laboratory and external by peer group monitoring an interesting quality management tool for the analytical phase. It is particularly useful because it directly translates analytical quality into quality of medical decision making using locally important cut-offs.

B-386

Efficiency of Automated Online CAP Proficiency Testing Submission in a Multisite Laboratory System.

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Background: Proficiency testing (PT) from the College of American Pathologists (CAP) requires that sample challenges for all tests reported by a CLIA moderately complex laboratory be received three times per year and handled in a manner identical to patient testing. The PT process within the laboratory is often a laborious manual process with transcription steps subject with error that may result in PT failure. **Methods:** Multiple Roche instruments supported on a wide area network by virtual server-based Data Innovations (DI) middleware were integrated into a CAP driver application, e-LAB Solutions Connect, via online database connectivity (ODBC). A single core chemistry lab was utilized as a pilot site for the entire Geisinger lab enterprise consisting of 6 hospital labs and 6 rapid response outpatient labs. Instrument operators were blinded as to which specimens were CAP PT samples; hence, a much closer emulation of patient testing was achieved. Workload mapping was performed and side by side comparisons of manual versus automated CAP PT processes compared for time efficiency. Cost/benefit analysis for rolling this system out to our multisite system was performed factoring in costs for software installation as well as estimated time savings. Troubleshooting/remediation costs were also estimated for CAP PT failures due to manual clerical errors.

Results: Installation of this middleware-mediated CAP connectivity was straightforward with the exception of integrating DI driver software on PC workstations with the Windows 7 Enterprise 64-bit/citrix operating system. Once a patch was installed the system has worked flawlessly for 10+ months. Current CAP method codes and other demographic information were automatically uploaded by e-LAB Solutions Connect and a CAP PT sample identification and labeling system established in the laboratory information system (LIS). 30 CAP PT challenges performed on Roche instruments since April of 2014 have been submitted by this automated connectivity as survey samples were accessioned into the LIS and interspersed with tested patient specimens by supervisory personnel. An average time savings of 45 minutes was estimated for each PT survey challenge. The number of clerical errors was reduced from 3 to zero for comparable PT surveys over a comparable time period. The time savings for each PT error investigation/remediation was estimated at 115 minutes.

Conclusion: CAP PT testing with automated results submission was found to be more efficient and less error prone than the previous manual method. Time savings justify installation cost of the automated system for DI connected Roche instruments in typical hospital labs. Installation of the automated system in rapid response labs performing routine chemistry were also judged as justified from a quality and regulatory compliance standpoint with an extended payback period. The CAP e-LAB Solutions Connect system will be rolled out in the future to all Roche instrumented labs for chemistry/immunochemistry testing and to other lab areas as vendor middleware automated solutions are developed. The aggregated enterprise time savings is expected to grow as a total of 363 PT testing events/year impact the Geisinger Lab system. This stable DI-ODBC extraction capability offers tremendous opportunities for expanding similar real time data management functions in the future.

B-387

Desire to Stem Lab Test Overuse Leads to Software Development

K. Russell, D. Maennle, Z. Uddin. *St. John Macomb-Oakland Hospital, Warren, Warren, MI*

The spiraling cost of laboratory tests (currently estimated at $60-70 billion/year) has been a major concern to healthcare administrators. A significant increase in testing costs is also anticipated as a consequence of the Affordable Care Act. Several cost-containment plans were initiated during the last decades but with limited success. The objective of our study is to develop two solutions for the cost-containment of laboratory tests. They include: a) building of an informatics structure to support utilization management; b) organization of the physician education program starting from the residency level.

A computer database was created to house > 4400 tests. The test names, test code, cost and respective turn-around time (TAT) are listed based on various reference laboratories in the area. The test is orderable by the physician if the cost is ≤ "Gatekeeper" value, e.g. $200.00. If the cost of the test is >$200.00, then the physician must contact the medical director or pathologist of the hospital laboratory for his or her approval before ordering the test. This "Gatekeeper" value is determined by the Medical Executive Committee of the hospital and may vary from one hospital to another.

We have developed two independent software applications to support utilization management of laboratory tests. They include native code computer programming and web site based application software.

A retrospective data analysis for a period of six months for laboratory send-out tests by a 410 bed tertiary care hospital with an active residency program provided the following information: a) Total costs of all the send-out tests = $671,054.90 b) Cost of the send-out tests > $200 i.e. the "gatekeeper value" = $271,003.54 c) Cost of tests which were reported after the discharge of the patient = $137,918.13.

All hospitals strive to minimize the length of stay of the patient in order to reduce the cost of doing business. However, there is no mechanism to control the ordering of send-out laboratory tests with long turnaround times. This means that the result of the send-out test has no relevance in the immediate treatment rendered by the physician. We recommend that regardless of the cost of the send-out test, the physician should consider the turnaround time of the test result before ordering. We have no way to know the role of the "gatekeeper value of > $200" in our retrospective data analysis. This question can only be answered after the institution of the application software in the laboratory information system (LIS), as this involves consultation between the physicians and pathologist.

The second step in the cost-containment process is physician education. This will contain a detailed synopsis of the software including: an explanation (Google search), clinical significance, turnaround time, and cost of all tests and lab support for non-orderable tests.

We believe that this two-pronged effort, an informatics structure and the physician education program, shall contribute significantly towards controlling the sky-rocketing cost of laboratory tests.

B-388

A novel PPT courseware tool for teaching clinical laboratory specialty English

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Background: Currently, PowerPoint(PPT) is the most frequently used tools for generation and presentation of the courseware for teaching clinical laboratory specialty English in China due to its diverse functions and convenience in operation. Unfortunately, it is not possible to select English word, or sentence on a presenting PowerPoint slide show, and give the standard pronunciation without using supper-link technique. So, almost every English teacher whose mother language is not English have experienced straits in such situation-they have forgotten linking the English word or sentence to an audio file, and unfortunately they don't know how to read it when the slide is being presented in the class room. Thus it is important to develop a tool to resolve the difficulties. In this study, we design and implement a PPT courseware tool based on text to speech (TTS) engine, which is able to automatically read the selected English word or sentence on a being-played slide in real time mode, without using the traditional supper-linking technique.

Methodology: The tool consists of three components: a macro, a buffer, and an attendant program. The macro was built with visual basic application editor embedded in Microsoft PowerPoint, the role of the macro is to collect the selected English word or sentence in the being-presented slide, and send it to the buffer.. The buffer is a memory map file used to be a cache to preserve the texts from the macro. While the attendant program is a executable file which was compiled with Microsoft Visual C++ development kit. Because the text-to-speech library was included into the program, it is able to convert the texts in the buffer to speech.

Usage: In edit stage, create a new PPT document, add a button in the master slide, and then link the button to the macro. In presenting stage, when need help, select English word or sentence , click the button on the slide, the attendant program will give the standard pronunciation of the selected English word or sentence

Conclusion: We have developed a PowerPoint courseware tool which is driven by text to speech engine, and able to read the random selected English word or sentence on a presenting PowerPoint slide show without using the traditional supper-linking technique. The key components of the tool consists of a VBA macro, a buffer and an attendant program. The role of the macro is to collect the selected English word or sentence in the being-presented slide, and then send them into the buffer, while the function of the attendant program is receiving the texts in the buffer, and convert it to speech. This novel tool is mainly used in English teaching in non-English speaking countries, it can not only help clinical laboratory specialty English teaching, but also help English teaching at all levels of schools.

B-389

Quality specification calculation: Total Error and imprecision in Cerebrospinal fluid (CSF) Analysis in a reference laboratory in Brazil

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Background: Since the end of the 19th century, Cerebrospinal Fluid (CSF) analysis has been used as an aid in diagnostic and monitoring of Neurological diseases and, through the years, its ordering to clinical laboratory is increasing, so that it is very important to advance in the laboratorial quality control for this biological matrix, assuring continuous quality control improvement. Total Error and imprecision are different for each laboratory test, and establishes test performance so that it fits the purpose of use. The Analytical Total Error can be calculated by different approaches; the most common form is the sum of Random Error and Systematic one. Total Error Limits defines how much results can vary and/or approach targets values aimed at clinically acceptable performance for these laboratory tests.

Objective: To propose a quality specification value for total error and imprecision for 10 different analyses in cerebrospinal fluid.

Methods: Total Error was calculated by the sum of Random and Systematic Errors of 10 different analyses in cerebrospinal fluid that were previously analyzed with Advia 2400 - Siemens® from January to December 2014. For the Random Error we used the coefficient of variation (CV) of each test multiplied to 1,65 for a desired confidence level of 90%. For Systematic Error calculation, we used results from two Proficiency Test providers: Control Lab® and PNCQ® - National Program of Quality Control.

Results: The medium CV of the period for each CSF analyte obtained was 2,77% for lactate, 2,48% for albumin, 1,25% for chloride, 1,42% for glucose, 4,99% for IgG, 6,22% for IgM, 1,32% for lactate dehydrogenase, 1,19% for potassium, 1,89% for protein total and 0,88% for sodium. Total Error obtained was 10,64% for lactate, 6,90% for albumin, 3,63% for chloride, 4,99% for glucose, 12,31% for IgG, 22,18% for IgM, 4,68% for lactate dehydrogenase, 3,73% for potassium, 6,30% for protein total and 2,28% for sodium.

Conclusion: Taking into account the medium CV of the period for each analyte, when compared to the CV reported by Advia 2400 Siemens® manufacturer package insert, we realized that all our obtained CVs were smaller than the informed ones. As our Proficiency Testing performance were all inside acceptable limits, we can consider that the quality specification values of total error and imprecision are adequate for use as an aid in quality continuous improvement in monitoring of laboratorial analytic quality control.

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Assessment of utility of daily patient results averages as adjunct quality control in a weekday-only satellite chemistry laboratory

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Background: Our department operates a weekday-only (8AM-5PM) satellite laboratory in an infusion center with a menu of 18 chemistry tests on a Roche c501

analyzer. We examined whether daily patient results averages (PRA) in this setting might be useful as a patient-based quality control (PBQC) adjunct to standard daily liquid quality control (LQC) measurements. First, we evaluated the reproducibility (coefficient of variation, CV) of daily PRAs for each analyte, and compared these to CVs of LQC. Second, for select analytes found to have relatively low PRA CVs, we evaluated the extent to which use of daily PRA measurements could improve detection of analytical errors when combined with LQC.

Methods: Patient results data for approximately one month (21 weekdays) were obtained from the Sunquest laboratory information system. For calculation of patient results averages (PRA), qualifying results were restricted to those within the reference range for each analyte. PRA and standard deviation (S) of PRA across 21 days was calculated for each analyte. Coefficients of variation for PRA (CV-PRA) were compared to those observed for standard liquid quality control (LQC) measurements (CV-LQC). For those analytes for which CV-PRA was less than CV-LQC, we evaluated the potential advantage of addition of PRA to daily LQC. For each analyte, a presumed PRA shift was determined such that probability of detection (P) was 0.5 when using LQC alone (viz., using high LQC and low LQC measurements), according to criterion that at least one 1-2S deviation from mean was obtained. For this same PRA shift, P = 0.5 for LQC alone was compared to P obtained for LQC + PRA (viz., using high LQC, low LQC, and PRA measurements), according to the same criterion.

Results: Across 21 days, the number of results per day per assay ranged from 23 ±4 (uric acid) to 75 ±21 (electrolytes). Qualifying results (results within the reference range) ranged from 70 ± 6 % (LDH) to 99 ± 1 % (Cl). Seven analytes had CV-PRA < CV-LQC (analyte, CV%): albumin, 1.25%; Ca, 0.67%; Cl, 0.62%; CO2, 1.13%; creatinine, 3.44%; K, 1.14%; Na, 0.65%. The remainder did not meet this criterion: ALP, 3.7%; ALT, 5.2%; AST, 5.1%; BUN, 4.6%; glucose, 1.4%; LDH, 2.0%; Mg, 1.4%; P, 2.5%; protein, 0.9%; TBIL, 6.1%; uric acid, 4.3%. Among the seven analytes for which CV-PRA < CV-LQC, probability (P) of shift detection by LQC for circumstances as described in Methods (LQC P = 0.5) was increased substantially by inclusion of PRA (analyte, shift in analyte concentration, P): CO2, ±1.07 mmol/L, 0.97; creatinine, ±0.099 mg/dL, 0.93; albumin, ±0.126 g/dL, 0.85; Ca, ±0.14 mg/dL, 0.80; K, ±0.097 mmol/L, 0.76; Cl, ±1.24 mmol/L, 0.74; Na, ±1.48 mmol/L, 0.68.

Conclusions: For 7 analytes, daily PRA demonstrated CVs less than those for LQC. For these analytes, calculations demonstrated that daily PRA can increase probability of detection of small results shifts when used as an adjunct to LQC. Daily PRA is a simple and essentially cost-free form of PBQC that may be useful for certain analytes in part-time laboratory settings.

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