SCIENTIFIC POSTER SESSION SCHEDULE

Posters of accepted abstracts were viewed in the Exhibit Hall of the Georgia World Congress Center on Tuesday, July 28 and Wednesday, July 29, 2015.

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 ..................... S21
Factors Affecting Test Results ........ A-127 – A-176 ..................... S39
Hematology/Coagulation .............. A-177 – A-204 ..................... S56
Immunology .................. A-205 – A-241 ..................... S66
Mass Spectrometry Applications .... A-242 – A-306 ..................... S78
Nutrition/Trace Metals/Vitamins ...... A-307 – A-331 ..................... S98
Cancer/Tumor Markers .............. A-342 – A-385 ................... S109

WEDNESDAY, JULY 29, POSTER SESSIONS

9:30am – 5:00pm

Cardiac Markers ...................... B-001 – B-040 .................... S121
Electrolytes/Blood Gas/Metabolites ... B-041 – B-057 .................... S133
Infectious Disease .................. B-058 – B-115 .................... S139
Lipids/Lipoproteins .................. B-117 – B-138 .................... S157
Management .................. B-139 – B-174 .................... S163
Molecular Pathology/Probes .......... B-175 – B-206 .................... S174
Pediatric/Fetal Clinical Chemistry ... B-208 – B-224 .................... S183
Point-of-Care Testing .............. B-225 – B-281 .................... S189
Proteins/Enzymes .................. B-283 – B-297 .................... S208
TDM/Toxicology/DAU .............. B-298 – B-344 .................... S213
Technology/Design Development .... B-345 – B-370 .................... S227
Automation/Computer Applications .. B-372 – B-390 .................... S234

Author Index ............................................................ S241
Keyword Index ........................................................... S251

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Tuesday, July 28, 9:30 am – 5:00 pm

Clinical Studies/Outcomes

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 ≥30 min, 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.


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 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...
Clinical Studies/Outcomes

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

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Clinical interpretation of laboratory test results is 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 in infants, children, and adolescents. The aim of this study was to establish reference values of serum ALP in infants, children, and adolescents up to 18 years using hospital data.

Nineteen thousand two hundred thirty-one test results belonging to infants, children and adolescents up to 18 years using hospital data. The data showed complex patterns in the concentrations of ALP during child growth and development, as well as sex differences.

A-004

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, kGH, prolactin, testosterone, DHEA (Beckman Coulter UniCel DxC 600 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 IGF, 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.

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**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 defined by a reduction in glomerular filtration rate (GFR) and increased urinary albumin excretion. GFR can be accessed or calculated by various methods. NGAL is a biomarker of acute kidney injury, but there is also evidence that its levels may reflect the acute 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 liability 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, Brazil, 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 (Biopoint Diagnostics) from -80°C preserved samples, until first-thawed. KDIGO G category was calculated according to creatinine and cystatin 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%) as 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), cystatin C (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 mg/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 mg/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 mg/mL, a AUC_ROC 0.829, 70.4% sensitivity and 79.7% specificity.

**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-010**

**Validation of anti-phospholipase A2 receptor antibody testing by ELISA and immunofluo escence**


**Background:** Recently, antibodies against phospholipase A2 receptor (PLA2R) in the urine of patients with biopsy-proven renal diagnoses have been identified. This antibody is a major target antigen among patients with idiopathic membranous nephropathy (IMN) and is absent in other forms of membranous nephropathy. As a result of the clinical potential of detecting anti-PLA2R antibodies, 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=21), IgA nephropathy (n=5), interstitial nephritis (n=9), minimal change disease (n=10), monoclonal protein-related diseases (n=13), vasculitis (n=20), other immune

**A-009**

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

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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 preeclampsia (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 preeclampsia, 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 preeclampsia 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 preeclampsia. 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.
complex mediated diseases (n=18) and other disorders (n=41). Among adult reference donors, IFA 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 IMA 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 IMA 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 101 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-1a, ICAM, L-selectin, E-selectin, TNFR1, TNFR1I, 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 principal 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 [logTNFR1, sTNFR2, MMP-9], 2 [sICAM-1, sL-selectin = L-selectin], 3 [log IL-6, log IL-8, log MCP-1], and 4 [MIP-1a, 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 χ² = 9.44, P<0.01: Log rank test χ² = 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-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 with 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 of a random and unselected set of negative bilirubin results from the same clinical populations. Reflex testing was conducted using one reagent lot of Icotest 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 Icotest 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 Icotest, 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 Icotest-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 UTIs

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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/jL. 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

L. C. Imoh1, O. O. Ogunkeye1, C. O. Isichei1, A. A. Gadzama2. ’Jos University Teaching Hospital, Jos, Nigeria, University of Abuja, Abuja, Nigeria

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 (GDMADA) and 21 (16.2%) of women had GDM by WHO criteria (GDMWHO). 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 GDMWHO women [OR=13.1, 95%CI(3.4-50.6)] compared to GDMADA 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 (p=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-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: 0.59-0.912) 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 ethnics 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 Tuja. 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% to 30.3%. 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 Tuja (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 Tuja 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 di fferent 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 determine prevalence and effect of food allergies for functional constipation in childhood.

**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 perennial disease (anil 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.

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**A-022**

**Diagnosis of Infection Utilizing Accellix CD64**

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Background:

Differentialing 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 cartridge 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 one-time use cartridge which includes onboard reagents and control material as well as an integrated flow cell. Infected (ICU) 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 (ICU-17, ICUc-13 and C-30). CD64 Index levels were higher (mean ± SEM) in ICU infection patients than 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.

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**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 lipopolysaccharide 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 ROC assay PATHFAST.

Results:

Mean presepsin concentrations of the patient group at presentation and of the control group were 1945 ng/L 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 1 st and the 4 th 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.

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**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, 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 nonelected 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 glycolytic 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 glycolytic 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-sorbant 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 (GTG) but not for groups 1 and 3 (GDM) respectively. 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 -1.6U/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 evaluating 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 aspirations (September 2014 to January 2015) collected from both adults and children tested simultaneously by both rapid Sofia Influenza A+B FIA immunofluoresce ence (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 Subtype H1. Only one out of 707 specimens was positive for Influenza B.

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 inflammatory 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, adiponecin, 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 reinfection, 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

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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, ethnic, 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 the 19,821 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-Mr (47.9%), followed by anti-E (17.8%), anti-Ec (6.7%), anti-P1 (6.1%), anti-Le-1 (5.5%), anti-M (4.9%), anti-Mr+E (2.5%), anti-Jk2 (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-Mr, anti-E, anti-P1 and anti-Jk2.

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 in 18 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 fifthday 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-029

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 (Elsys) >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, TNF-α), vasculopathy (VEGF), and fibrosis (endoglin, GDF-15, PDGFR and TGFβ) and cardiac biomarkers (NT-proBNP - Siemens Vista and hs-cTnT - Roche) by chemoluminiscence 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, anticientromere 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 relation was found between hs-cTnT, endoglin and TGFβ2 and Elsys. 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,
clinicalcytogeneticsstudyofdeletiononderivativeschrome9 inacml patientwithbcrlabr rearrangement

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Background: The BCR/ABL gene rearrangement is the causative factor in chronic myeloid leukemia (CML). In most cases, it is cytogenetically identified via translocation, common to 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 the Ph+ CML patients are thought to have prognostic value. This 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) and multiplex reverse transcriptase-polymerase chain reaction (MRT-PCR).

Methods: In September 2014, a 41-year-old patient with leucocytosis, severe anaemia, and thrombocytosis, was referred to our institution to perform a diagnostic evaluation. Cytogenetic analysis showed the presence of the translocation between chromosomes 9 and 22 as well as a possible deletion of the derivative chromosome 9, identified by the presence of the Philadelphia chromosome (Ph). A possible deletion on derivative 9 identified by conventional cytogenetic and confirmed by fluorescence in situ hybridization (FISH) and multiplex reverse transcriptase-polymerase chain reaction (MRT-PCR). The cytogenetic analysis showed the presence of the translocation between chromosomes 9 and 22 as well as a possible deletion of the derivative chromosome 9 identified by conventional cytogenetic and confirmed by fluorescence in situ hybridization (FISH). The multiplex reverse transcriptase-polymerase chain reaction (MRT-PCR) detected the presence of the BCR/ABL transcript.

Conclusion: This 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) and multiplex reverse transcriptase-polymerase chain reaction (MRT-PCR). The MRT-PCR detected the presence of the BCR/ABL transcript and a possible deletion on derivative 9 identified by conventional cytogenetic and confirmed by fluorescence in situ hybridization (FISH).

The Economic Burden of Confirmatory Testing for Cardiac Troponins and Human Chorionic Gonadotropins: 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. 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.

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.

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.

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 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.

| biomarker | in patients with or without MAEs and in survivors and non-survivors |
|-----------|--------------------------|-----------------------------|-----------------------------|-----------------------------|
| Medians (95% CI) | suPAR | Presepin | 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 | 11.08(2.17-7.7) | 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(1.3-13.3) | 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

A-033

The Economic Burden of Confirmatory Testing for Cardiac Troponins and Human Chorionic Gonadotropins: A Multi-Site Laboratory Database Analysis

A-034

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

Tuesday, July 28, 9:30 am – 5:00 pm

Clinical Studies/Outcomes

CLINICAL CHEMISTRY, Vol. 61, No. 10, Supplement, 2015 S11
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, Bintermicenx) 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.

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 tuberculosis pleural effusion.

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 a 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 AUROC 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.

Obesity and Oxidative Stress


Background:

Obesity is become one of the most common risk factor of metabolic syndrome. Obesity and its related 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.

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 polysomnography with apnea / hypopnea index - AHI). It was considered moderate apnea AHI > 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-
The Levels Of Vaspin And Chemerin Levels And Correlation Between Cardiologic Parameters In Patients With Psoriasis 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) concentration of soluble vaspin between psoriatic patients and the control group 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 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 significantly increased (0.33±0.13 cm vs. 0.25±0.12 cm; p=0.04) in patients with psoriasis compared with the controls.

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.

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 alteration, 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. The cytogenetic analysis demonstrated a 45,XX,rob(14;21)(q10q10) (fig.1) and 46,XX,+21,rob(21;21) (q10;q10) (fig.2). Discussion: Most de novo (14;21) trisomies originate in maternal or paternal meiosis. The maternal age is 29-22 years. In de novo (21;21) Down Syndrome the situation is different, in most cases the (21;21) is an isochromosome rather than the result of Robertsonian translocation caused by a fusion between 2 heterochromatidic chromosomes. About half were of paternal and half of maternal origin. In de novo (21;21) true Robertsonian trisomy 21 cases, the extra chromosome 21 was maternal. In this case the expected karyotype would be the presence of the translocated...
Double aneuploidy with a karyotype 48,XXY,+21

M. R. Cochak1, J. F. Abreu1, R. Kuhbauche2, G. Cury Neto1, M. P. Migliavacca2. 1DASA, Cascavel, Brazil, 2DASA, Barueri, Brazil

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.

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. This study demonstrates that the CLINITEK Novus analyzer provides strong result agreement allowing for consistent clinical decision making.

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.
Clinical Studies/Outcomes

High “Normal” Potassium Poses Mortality Risk for All Patients

A. B. SOLINGER, S. I. ROTHMAN, G. D. FINLAY. FAR INSTITUTE, SARASOTA, FL

Background: Recent studies show increased mortality in acute myocardial infarction (AMI) patients with serum potassium levels of 4.5-5.0 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 appears not to account for this increased mortality. Without prospective studies, our mortality risk for serum potassium levels above 4.5 mEq/L. All patients have an increased mortality risk above 4.5 mEq/L.

SARASOTA, FL

A. B. SOLINGER, S. I. ROTHMAN, G. D. FINLAY.

Monashed University, Cape Town, South Africa

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-045
Assessment of Hyperkalaemia in the Medical Emergency Unit

I. Y. Mohammade, R. Erasmus*, A. Zemlinin, 2Bayero University/ AKTH, Kano, Nigeria, 2Stellenbosch University, Department of Physiology, IZMIR, Turkey, 2SIF A UNIVERSITY Faculty of Medicine, Department of Physiology, IZMIR, Turkey, 2SIF A UNIVERSITY Faculty of Medicine, Department of Biochemistry, IZMIR, Turkey, 2SIF A UNIVERSITY Faculty of Medicine, Department of Biostatistics and Medical Informatic, IZMIR, Turkey

A. B. SOLINGER, S. I. ROTHMAN, G. D. FINLAY.

Tuesday, July 28, 9:30 am – 5:00 pm

Tuesday, July 28, 9:30 am – 5:00 pm

A-049
SEVERAL ANALYSIS IS USED TO IDENTIFY PATIENTS WITH PSORIATIC ARTHRITIS

T. ATABAY 1, S. KOBAK 1, M. AKYILDIZ 2, H. CENGIZ 2, H. MOLLAOGLU 1.

PSORIATIC ARTHRITIS

A-049

A. B. SOLINGER, S. I. ROTHMAN, G. D. FINLAY.

A-051

Performance evaluation of the cobas u 701 urine analyzer

D. D. Fuller, T. E. Davis, W. L., Veros, K. L. Newcomer, R. J. Buckner, R. J. Buckner, J. D. Talbott. Indiana University School of Medicine, Indianapolis, IN

Performance evaluation of the cobas u 701 urine analyzer

D. D. Fuller, T. E. Davis, W. L., Veros, K. L. Newcomer, R. J. Buckner, R. J. Buckner, J. D. Talbott. Indiana University School of Medicine, Indianapolis, IN

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 pathologic 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 used 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 ≥5%) within acceptable agreement rates. Recovery of defined concentration ranges was assessed for HAYA, 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 semi-quantitative and qualitative parameters confirmed reproducible recognition of negative and positive samples (distributed over 2 concentration ranges).

Results obtained for HAYA, 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 limits for a new urinalysis system. The agreement with visual microscopy without requiring reclassification provides improvement for routine workloads. 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-053
Severe acute pancreatitis and serum C-Reactive Protein
J. D. Santorotiorio, C. Cañavate-Solano, A. García-de la Torre, F. Arce-Matute, S. Pérez-Ramos. Puerto Real University Hospital, Cadiz, Spain

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 SA.

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 3.2 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.
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 ±3 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 blaKPC 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 blaKPC-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 blaKPC-2 gene expression by PCR. The genetic relationship among isolates was analyzed by PFGE using restriction enzyme XhoI.

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 blaKPC-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-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: 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.
Tuesday, July 28, 9:30 am – 5:00 pm

Clinical Studies/Outcomes

Serum MDA levels were measured by HPLC (Ultimate3000, ThermoDionex, USA) with a fluorescence detector. Within-run precision values were 1.8-5.5% and between-run precision values were 6.5-9% for 0.40-1.15 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 (DiaBeckmanCoulter, USA). Serum CRP levels were measured immunoturbidometrically (Integra400, Roche Diagnostics, 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 42.05 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 (<60 minutes and >90 minutes), 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 timeline 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-061

Analysis of allergens in patients with allergic skin 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 atop 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 not 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 (25.3%, 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 sLDL-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 sLDL-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 61 were 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/m2. 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 (sLDL-C) was quantified by modifie Tsutomu –Hirano method.
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 Log10 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 log10 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 APACHE II 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

APACHEII/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

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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-Cardiotid-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.

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

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Background:
The premature coronary heart disease (CHD) is strongly associated with familial 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 familial 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±0.10 mIU/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±6.3 mg/dL; p=0.001); Body Mass Index (kg/m²) (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±3.6 mg/dL; p=0.463); LDL-C (118.1±3.6 vs 111.4±3.4 mg/dL; p=0.184) and fasting blood glucose (95.5±2.4 vs 92.9±1.2 mg/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.

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 >3g/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.
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 hypothalamic-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 (AUCT3 = 0.664 ± 0.071 (SEM), p = 0.03; AUCT2 = 0.795 ± 0.088 (SEM), p = 0.01).

Conclusions: Hair cortisol successfully detected the expected increased 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.

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**Screen with Reflex to Bette 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 cost 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 patient. 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


**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 glycomic 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 extended two-compartment 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: d[T]/dt = -k1 * [T] (Eqn.1), where k1 is the AG ingestion rate (mg/day), and α = GFR (1-1/k1) / V, where GFR = glomerular filtration rate (mL/min), V is the plasma volume (nominally 3 L), and k1 is the fractional reabsorption in kidney of filtered AG (in normoglycemia, f = 0.9984). For steady-state (excretion rate = ingestion rate), k1 = GFR / (1-f) (Eqn.2). Normal distributions for k1 (4.62 ± 1.62 mg/day) and GFR (80-120 mL/min) are known. Assuming that model parameters other than α are unaffected by CT, then values for α 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 changes in [AG] upon initiation of CT (derived from Eqn.1): t1/2 = ln(0.5) / k1.

**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 = 0.988-0.995). Compared to normoglycemia (f = 0.9984), the decrease in f was ~5%. CT nonetheless decreases [AG] relative to the reference range ([AG] = 7.2-33.3 µg/mL). Decrease in [AG] after 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 glycomic control), the rapid effect on [AG] means that AG measurement could instead provide an early independent measure of effective CT.

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**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, involved in the menstrual cycle, pregnancy, and embryogenesis. It is generally used.

**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 calibrator was traceable to two different kinds of reference materials (ERMA-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).

Conclusion: As a result of evaluation with total 19 kinds of drugs and PRG derivatives, significant cross reactivity with almost all cross reagents were not observed.

**Conclusions:** 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.

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**Endocrinology/Hormones**

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**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)-A1, homocysteine, ferritin, and high sensitivity C-reactive protein (hsCRP). Apo-A1 levels were slightly higher in males, but remained relatively constant throughout lifetime, while vitamin B12 steadily decreased throughout childhood and then remained constant. ApoB, homocysteine, ferritin, 25-OH vitamin D, RAEC 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.
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=0.85±5.4, Spearman r=0.959 (n=87). For urine, Deming regression of the CLIA versus the RIA was y=0.60±113, Spearman r=0.952 (n=40). Using 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=1.00±0.015, r²=1.00; for urine was y=1.008±0.015, r²=1.00. 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 (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 (CV≤4.0%) and 4 hours in unpreserved urine (CV≤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=0.87±7.2, Spearman r=0.952 (n=14). Additionally, paired serum and heparinized plasma specimens taken during the same blood draw produced a correlation of y=1.00±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.

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.

Methods: 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 significantly different 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 17α-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 30 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 230.7 ng/dL at midnight to 566.4 ng/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)
laboratory turnaround time for CO₂ 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 CO₂ 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 CO₂ concentrations used as the gold standard for the diagnosis of DKA. CO₂ 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 (RENN-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 coefficient (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.
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 separation using 0.1% formic acid in water and acetonitrile as mobile phase. 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.

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 Validator, Deming Regression, 99% confidence interval to exclude outliers) are as follows: Y = 1.11 * X + 0.40, r=0.998, F=37.0, SE=14.03.

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.

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 is 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® c4000, Cobas® 6000 and Tosoh® G8 were 6.86 ± 2.16, 6.75 ± 2.16 and 9.69 ± 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® c4000 and 8 by Cobas® 6000), therefore were not included in the correlation & CE studies. Comparing Architect® c4000 to Tosoh® G8 rivaled r=0.9944, y=1.03 ± 0.7204 ± X at an TEa as low as 7% in the normal Hb samples and r=0.9710, y=1.19 ± 0.962 and CE at TEa as low as 13% in the abnormal Hb samples. Comparing Cobas® 6000 to Tosoh® G8 rivaled r=0.9932, y=0.86 ± 0.740 and CE at TEa as low as 9% in the normal Hb samples, and r=0.9716, y=0.88 ± 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.
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 2181 pg/mL. At 1000 pg/mL, the observed % cross reactivity for proinsulin and c-peptide was 0.08% and 0.14%, respectively. At 1000 pg/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

**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 D3 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 D3-d6. 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. 300 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 vortexcised for 10 seconds. 300 μL aliquot was injected onto an Agilent Poroshell 120 SB-C8 column where both 25-OH Vitamin D3 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 D3 and 389.3 → 211.2 for 25-OH Vitamin D3-d6. 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 D3 at all concentration tested. Accuracy ranged between 98% and 110%. Good linearity was also obtained within the concentration range of 1-25 pg/mL for free 25-OH Vitamin D3 (r ≥ 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 D3 concentration correlates excellently with the concentration of PTH and poorly with the total 25-OH Vitamin D3 concentration. A poor correlation was observed between total 25-OH Vitamin D3 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 D3 in human serum/plasma samples employing a high sensitivity tandem mass spectrometer. We can now evaluate the role of free 25-OH Vitamin D3 in patients with bone and/or a variety of malignant diseases.

**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: Hcrt1 (Orex A) and Hcrt2 (Orex B). Their neurons are located in the lateral hypothalamus, Hypocretin (Hcrt) or Orexin is a neuropeptide that binds to two major receptors: Hcrt1 (Orex A) and Hcrt2 (Orex B). Their neurons are located in the lateral hypothalamus, 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

**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 hypocalcemia 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 in 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 EP26-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.
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 Hct 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 = 24 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 Hct (1 and 2) by enzyme immunoassay method (EIA) using the reagent: Cusabio Human Orexin A and B (ELISA Kit Catalog Number CSB-E08595b).

Results:

Patients with active syphilis or treated showed significantly lower values Hct 1 = 63.5 ± 15.8 ng/mL vs 138.0 ± 46.4 ng/mL (p = 0.043) and Hct 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 had also significantly lower levels of Hct 1 (55.8 ± 24.3 ng/mL vs 106.6 ± 33.1 ng/mL, p = 0.006) and Hct 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 ± 6.7, 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 Hct 1 and the ES scale was r = 0.79, while for Hct 2 was r = 0.46.  te superior do formulário

Conclusion:

Our results demonstrated that Hct 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 Hct 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


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 D2 and 25(OH)vitamin D3 in both serum and plasma. Vitamin D Total LOCI reagents include a releasing reagent, biotinylated monoclonal antibody, and two synthetic head 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 D3 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 traceable to 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 = -0.200 ng/mL, r = 0.9172, and n = 112 over a concentration range of 7.8-71 ng/mL. Minimal cross-reactivity is observed with 125(OH)D2, vitamin D3, and D at 500 µg/mL, 3-epi-25(OH)D3 at 100 ng/mL, and vitamin D (ergocalciferol) and vitamin D (cholecalciferol) at 1000 ng/mL. This assay is equimolar for 25(OH)vitamin D2 and D3.

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

E. Romeu1, P. Melchionno1, N. Germain1, H. Fonteneau1, M. Navrati1. 1Beckman-Coulter, Marseille, France, 2Beckman-Coulter, Chaska, MN

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 line regression and Bland-Altman percentage bias. For all analysis, ID-GC/MS estradiol measurement was used as the reference. Limit of Quantitation (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 quantify 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.

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

S. Ray, P. Sibley, K. Wilson, E. Merebet. Siemens Healthcare Diagnostics, Tarrytown, NY

**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 2007/0632 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 dialyssis 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 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.

Analytical and Clinical Performance of IMMULITE 2000 TSI Assay*

D. Kiaei, N. Birmingham, S. Chapman-Montgomery, B. Zhang, S. Thompson, J. Lei. Siemens Healthcare Diagnostics, Tarrytown, NY

**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 TSI includes a determination of the autoimmune etiology of thyrotoxicosis, the specific, quantitative detection of TSI in serum and plasma. The clinical utility of TSI includes a determination of the autoimmune etiology of thyrotoxicosis, prediction of remission or relapse, confirmation of GD as Graves’ disease, and diagnosis and assessment of GD in patients with other thyroid disorders. The IMMULITE 2000 TSI assay was compared to the THYRETAIN TSI Reporter BioAssay using 244 serum samples from GD patients. The detection limits of the assay were determined in accordance with 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.

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 (EII) employing two biotinylated monoclonal antibodies (rabbit) that superseded EII assay employing a biotinylated polyclonal antibody (rabbit). Upper Reference Limits (URLs) proposed for EII in male adults and in females in follicular and luteal phases and menopause are very 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 EII 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. EII lower URL in ovulatory phase is consistent with the negative bias at high concentrations.
**A-092**

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

Q. A. Mojiminiyi, N. Abdella. *Faculty of Medicine, Kuwait University, Kuwait, Kuwait*

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 ($r = -0.07$), HOMA-IR ($r = 0.12$), triglycerides ($r = 0.22$), HDL-C ($r = 0.19$) 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, HOMA-IR, 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%, 43%, 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

H. Arakawa, S. Haraya, K. Karasawa, Y. Sano, H. Ohkuma. *Showa university, Tokyo, Japan; ‘Eiken Chemical Co. Ltd, Tochigi, Japan*

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 increasing 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 (ELIA), but the many reported methods lack sufficient sensitivity and specificity for oxytocin. We here report the production of a highly affinity and high specificity antibody towards oxytocin and its use in a highly sensitive ELIA using colorimetric and bioluminescence detection.

Methods: Antibody: Anti-oxytocin antiserum was obtained by immunizing rabbits with oxytocin-bovine thyroglobin 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 antisem 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

Y. L. Trull, J. A. Hashim. *University of Texas Southwestern Medical Center, Dallas, TX*

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 -20°C 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 3000g 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 prevalence of MetS from 1st to 4th quartile were 40%, 43%, 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: Macroprolactin was detected in 6% of samples from patients with elevated prolactin levels, as determined by laboratory-based immunoassay analyzer. 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 a 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**

A. Terra, K. Aoyagi, S. Kojima. FUJIREBIO INC., Tokyo, Japan

**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), hematocrit (52 g/dL), creatinine (1 g/dL), albumin (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**

A. Azizi, P. Sthaneshrar, S. Vethakkan. University of malaya, Kuala Lumpur, Malaysia

**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, specificit , 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, specificit , positive and negative predictive values were 88.2%, 100%, 90.2% 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%, PP 100% and NPV 86%.

**Conclusion:** Sensitivities, specificit es, 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**

**See Abstract A-097 on Page 38 at end of the Endocrinology section.**

**A-098**

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

B. S. Moura, L. G. S. Assunção, M. D. Farace, L. Moutinho. Lab Rede, Belo Horizonte, Minas Gerais, Brazil

**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.
Tuesday, July 28, 9:30 am – 5:00 pm

Imprecision studies were conducted using Liquichek® Immunoassay immunoassay.

Background: Clinical uses of human chorionic gonadotropin (hCG) include K. M. Ness, A. Algeciras-Schimnich. βhCG (5th IS) Immunoassay Evaluation of Analytical Performance of the Beckman Coulter Total and Lyphochek® Fertility quality control (QC) materials (Bio-Rad, Inc). Analyte interference in the total βhCG (3rd IS) assay. Method comparison studies with the antibodies interference was assessed by evaluating three samples with known containing elevated hCG concentrations. Susceptibility of the assay to heterophile interference was assessed by evaluating three samples with known containing elevated hCG concentrations. Limit of quantitation (LOQ) studies were conducted using a serum sample with low calibrators, different than what was used for calibration of the assay, as unknowns. Measurement range (AMR) studies were conducted by testing a lot of hCG 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 (r²=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=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 ≥20,000 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 follows: 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, reapline 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.

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**A-099**

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

K. M. Ness, A. Algeciras-Schimnich. Mayo Clinic, Rochester, MN

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 (r²=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=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 ≥20,000 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 follows: 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, reapline 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.

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**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

R. Fontes, O. P. Denardin, J. D. Souza, A. P. M. Almeida, A. C. B. Cerqueira, F. M. C. F. Silva, Y. Schrank, P. B. Araujo, D. M. Gomes, DAS4, Rio de Janeiro, Brazil

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-medications (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), amiodapine, 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 significantly higher in patients treated with more than one drug that could potentially increase its levels, like carbamazepine plus hydantoin or beta blockers plus one of this medications: 2.3 to 2.9 mU/L. TSH was lower in patients in use of amiodapine: 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 association increased TSH. Amlodipine in the diabetic group compared to a group of non-diabetic elderly evaluated under similar conditions. Some medications only in association 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 e fect of Amlodipine decreasing TSH levels.
Development of a New High Sensitivity Biochip Based Direct Immunoassay for the Measurement of Low Levels of Total Testosterone in Serum


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 (LC-MS) 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” isotest-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 assay 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 by Westgard). 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.

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

Y. Schrank, M. Freire, O. V. P. Denardin. DASA - Brazil, Rio de Janeiro, Brazil

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 chemiluminescence immunoassay 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. Overall we observed a tendency to higher GH peak levels and higher percentage of responsive tests according to IGF1-GH ST 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 IGF1 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.

Is free T3 useful to evaluate thyroid status?

S. Marui, A. L. N. Camilo, D. Waltrich, O. V. P. Denardin. DASA, Sao Paulo, Brazil

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 (57.5%) (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 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.

Conclusion: Comparison among total and free thyroid hormones using TSH to define thyroid status.
status. We conclude that FT3 and TT3 levels are less influenced by normal and borderline TSH ranges.

<table>
<thead>
<tr>
<th>Age (a)</th>
<th>Mean Age (±SD)</th>
<th>25(OH)D (±SD)</th>
<th>PTH (±SD)</th>
<th>25(OH)D status</th>
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</thead>
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<td></td>
<td>25(OH)D (±SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deficient</td>
<td>Insufficient</td>
<td>Adequate</td>
<td></td>
</tr>
<tr>
<td>12-18 (119)</td>
<td>15.5 ± 1.8</td>
<td>36.32 ± 20.88</td>
<td>30.37 ± 26.32</td>
<td>1.7%</td>
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<tr>
<td>19-30 (611)</td>
<td>25.75 ± 1.26</td>
<td>35.59 ± 25.23</td>
<td>34.27 ± 57.01</td>
<td>8.2%</td>
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<tr>
<td>31-60 (460a)</td>
<td>47.44 ± 8.63</td>
<td>34.52 ± 26.30</td>
<td>47.88 ± 90.84</td>
<td>7.6%</td>
</tr>
<tr>
<td>Total (5334)</td>
<td>44.24 ± 11.48</td>
<td>34.69 ± 26.07</td>
<td>45.93 ± 85.55</td>
<td>7.5%</td>
</tr>
</tbody>
</table>

A-108

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

P. B. Araujo, M. C. Freire, Y. Schrank, R. Fontes, D. M. Gomes. DASA, Rio de Janeiro, Brazil, Rio de Janeiro, Brazil

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 (<20.0 ng/mL), insufficiency (21.1-29.9 ng/mL), and adequate (>30.0 ng/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 relevance, since mean PTH level is lower among this age category. Therefore, a better definition on the optimal level of vitamin D is yet to be determined.
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 homeostasis?

S. Marui, F. Fukuoka, F. Prieto, D. C. C. Akiyama, O. V. P. Denardin. DASA, Sao Paulo, Brazil

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 25OHID (Chemiluminescence, Architect Abbott), intact PTH (Chemiluminescence, Immulite2000 Siemens), calcium and phosphorus (colorimetric CPC and phosphonomolydate/UV principle, Advia2400 Siemens, respectively) in the period from October till November 2014 (1,010 M: 373F) and grouped as young adults (16 to 35 years-old), adults (36 to 60 years) and old adults (>60 years). 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))


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 <0.01 µIU/mL, an LoD of ≥0.002 µIU/mL, and a 10% total assay CV LoQ concentration of <0.005 µIU/mL. Total assay precision 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 TSH (<0.005%), 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 hypothyroid patient samples down to 0.005 µIU/mL.

**A-115**

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

G. H. C. Souza, D. A. G. Zauli, E. Mateo, A. C. S. Ferreira. Hermes Pardini Institute, Vespasiano, Brazil

The Type I diabetes is characterized by insufficient production of insulin by the pancreas being called autoimmune destruction, affecting mostly 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 1 diabetes and can be tested previously to the disease establishment, during the period called prediabetes. This study aimed to validate threantibodies 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 - EUROIMMUNAG) 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 Cohen index was 0.333 (95% CI: 0.122 to 0.544). In both protocols, a proficient 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

E. P. Odum, O. Otokunefor. University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria

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 included in this study. Two hundred and seventy-eight pregnant women were included in this study. Mean (SD) TSH and FT4 were 1.0 (1.4) mIU/L and 12.0 (4.8) pmol/L respectively.

Results

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 included in this study. Two hundred and seventy-eight pregnant women were included in this study. Mean (SD) TSH and FT4 were 1.0 (1.4) mIU/L and 12.0 (4.8) pmol/L respectively.

The study was conducted in four health care facilities in Port Par...
Prevalence of subclinical hypothyroidism in adults without known thyroid disease: An epidemiological study in Chengdu, China

N. Xin, L. Guangquan, W. Haolan, L. Tongxing, H. Hengjian, W. Lanlan, L. Guixing, West China hospital, Sichuan University, Chengdu, China

Background:
Subclinical hypothyroidism (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>
<thead>
<tr>
<th>Age-strata (Years)</th>
<th>Male(%, n/N)</th>
<th>Female(%, n/N)</th>
<th>Total(%, n/N)</th>
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<td>18.0, 1455/8101</td>
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<td>≥80</td>
<td>26.1, 325/1245</td>
<td>33.8, 112/331</td>
<td>27.7, 437/1576</td>
</tr>
<tr>
<td>Total</td>
<td>11.8, 7850/66474</td>
<td>20.3, 9044/40410</td>
<td>15.3, 16894/10484</td>
</tr>
</tbody>
</table>

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 Capillarlys Flex Piercing® - capillary electrophoresis and Trinity Biotech-Premier HbR210® - boronate affinity HPLC) 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 (Capillaries Flex Piercing® values: 5.8 and 6.6%; Premier HB9210® values: 5.7 and 6.6%) and between-run (Capillaries 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 capillaries electrophoresis and boronate affinity HPLC, respectively. Comparison of both methods against Bio-Rad Variant Turbo II demonstrated significant correlation (Capillaries 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 NGSDF (http://www.ngsdf.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.
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 at 413 +/- 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: The Premier Hb9210 performs well with a short analysis time, so far no detected interferences and is very well suitable for routine analysis of HbA1c.

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.

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

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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 disease (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 multivesseles (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 mU/L; \(p<0.001\)). The comparison between single- and multivesseled groups showed higher TSH levels in PACD with multivesseled affected (TSH 2.3±1.5 versus 2.8±1.3 mU/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**


**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 functionality. Based on the index of Katz, which are classified in levels 1-6, the independent individual to perform all activities, and index 6 indicates dependenelle to perform 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 dependence to perform the six activities. TSH, FT4, TT3 and FT3 were measured by radioimmunossay. T3 was measured by radioimmunossay.

**Results:** There was a negative correlation of TSH with T3 (\(r = -0.1736, p = 0.0417\)), with TT4 (\(r = -0.2035, p = 0.0167\), and especially with T3 (\(r = -0.2331, p = 0.0059\)). 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 (\(r = -0.1143, p = 0.2066\), TT3 (\(r = -0.8712, p = 0.3301\)), 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.

**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 T4, T3, TSH, Insulin (ELISA), lipid profile, fasting blood sugar (FBS) (enzymatic) and apo B, apo A - I (Immuno-turbidimetric). 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/apoA1, 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 vs 28.73±5.12; p<0.0001). They showed gross dyslipidemia as compared to HC (HC vs Hypothyroid TC 175.49±16.25 vs 231.69±20.84 mg/dl; Triglyceride (TG) 126.49±23.1 vs 184.04±40.70 mg/dl; LDLc 110.41±17.9 vs 133.04±37.2 mg/dl; p<0.0001; HDLc 41.18±5.60 vs 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) as compared to group II (137.83±12.24 vs 126.69±9.39 mm Hg, p<0.0001) (92.09±8.06 vs 84.75±6.14 mm Hg, p<0.0001). FBS in group I was significantly higher than in group II (205.48±25.25 vs 92.58±1.68; p<0.0001), insulin resistance was observed in group I as compared to group II (31.37±1.30 vs 6.30±4.06, p<0.0001), T4, T3 significantly were reduced in group I than in group II (T 0.29±0.19 vs 0.61 ± 0.12; T4 2.47±1.07 vs 3.68±1.05 p=0.0001) (TSH significantly higher in group I than in group II (43.13±15.46 vs 25.65±24.8 p<0.0001). AI of group I was significantly higher than HC (0.64±0.66 vs 0.41±0.19, p<0.0001) and group II (0.64±0.66 vs 0.54±0.07, p=0.0001). The CVD risk ratios were significantly raised in group I than group II (apoB/apoA1 1.38±0.29 vs 1.23±0.36, p=0.02; TC/HDLc 5.82±1.18 vs 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), DM/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.
Factors Affecting Test Results

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 (r = 0.93 ± 0.05, R: 0.99)1) 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

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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%.

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


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

S40

filtrate testing on 5 samples with IP interference (average IP 4.08 mg/dL, 3.62-4.63)
Two-fold dilutions were not sufficient to abrogate the interference. Protein-free
The majority of samples (12/15) showed a positive interference for IP. The average
average MP concentration: 3.08 g/dL (1.84-8.66 g/dL; 9 IgG kappa, 2 IgG lambda,
multiple myeloma, 1 unknown; average total protein: 9.0 g/dL (7.2-14.7 g/dL); and
RESULTS: Patient characteristics were as follows: average age: 63 years (36-80, 9
phosphorus to determine the magnitude of the interference.
Ultrafiltration Devices, Millipore) were prepared and re-tested for inorganic
analyzed to determine presence or absence of interference. Where quantity allowed,
reaction curves were
results for P samples.
deviation of errors E(R) in predicted HCT, were numerically superior compared to
α(R)>>α(P); the overall ratio α(R)/α(P) was 1.51 ±0.15. For both P and R, α was highly
distributed: α(P) = 1.23±0.26 μmol/cm²; α(R) = 1.86±0.41 μmol/cm². Note that
combined
α(R)>>α(P); the overall ratio α(R)/α(P) was 1.51 ±0.15. For both P and R, α was highly
distributed: α(P) = 1.23±0.26 μmol/cm²; α(R) = 1.86±0.41 μmol/cm². Note that
differences in potassium per unit area between center circular punch
STUDY DESIGN: Remnant sera from 15 patients with serum protein electrophoresis
being submitted for complete blood count including HCT (Sysmex). FPB were formed by
treated as constant (0.314 cm²). R sample areas were taken as the difference between
total area and punch area. P and R samples were extracted in 490 μL 2.5 mM K-EDTA solution (+1 hour) with periodic vortexing. The resulting solution was measured for
K (Roche Cobas e501). After correction for extraction solution K, values of K/area
(α, μmol K/cm²), and correlations of α to HCT, were compared between results for
P and R samples.

A-130

Monoclonal Protein Interference with Chemistry Measurements
Performed on the Siemens Advia® 1800 Chemistry Analyzer
Beaumont Health System, Royal Oak, MI

BACKGROUND: Previous articles have reported monoclonal protein (MP)
interference with routine automated chemistry and immunoassay tests on a number of
assays affected included inorganic phosphorus, alkaline phosphatase, ALT, AST,
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®
filtration) were all found to be consistent with otherwise normal
Circumstances: Calculations described can be used to assess whether values for
Backround: Certain quantitative assays using filter paper bloodspots (FPB) are
adversely affected by hematocrit (HCT) as an unknown preanalytical variable. Capriau
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 cm²). R sample areas were taken as the difference between
total area and punch area. P and R samples were extracted in 490 μL 2.5 mM K-EDTA solution (+1 hour) with periodic vortexing. The resulting solution was measured for
K (Roche Cobas e501). After correction for extraction solution K, values of K/area
(α, μmol K/cm²), and correlations of α to HCT, were compared between results for
P and R samples.

RESULTS: A total of 43 patient samples were utilized. Bloodspots areas (A, cm²)
were normally distributed (A = 0.91±0.046 cm²) and were in aggregate negatively
correlated with HCT (A = -0.067; -0.00042 HCT(%) + 1.06 cm²; r = -0.97). 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/cm²; α(R) = 1.86±0.41 μmol/cm². Note that
(α) = 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/cm² (r² = 0.795); (α(R) = 0.052 HCT(%) + 0.010 (r² = 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) = 0.019 ±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-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

Background: Certain quantitative assays using filter paper bloodspots (FPB) are adversely affected by hematocrit (HCT) as an unknown preanalytical variable. Capriau et al. [Ann. Clin. Biochem. 2013;49:404] 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 cm²). R sample areas were taken as the difference between total area and punch area. P and R samples were extracted in 490 μL 2.5 mM K-EDTA solution (+1 hour) with periodic vortexing. The resulting solution was measured for K (Roche Cobas e501). After correction for extraction solution K, values of K/area (α, μmol K/cm²), and correlations of α to HCT, were compared between results for P and R samples.

RESULTS: A total of 43 patient samples were utilized. Bloodspots areas (A, cm²) were normally distributed (A = 0.91±0.046 cm²) and were in aggregate negatively correlated with HCT (A = -0.067; -0.00042 HCT(%) + 1.06 cm²; r = -0.97). 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/cm²; α(R) = 1.86±0.41 μmol/cm². 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/cm² (r² = 0.795); (α(R) = 0.052 HCT(%) + 0.010 (r² = 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) = 0.019 ±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.
Impact of Sample Handling on Intact Parathyroid Hormone (PTH) Concentrations in Specimens


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 4-5% 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.
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Blood Donation Temporarily Improves Glycated Hemoglobin (HbA1c) Status In Healthy Men

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Background: A significant change in glyemic 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 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.

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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 interpretations 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 up to 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-dectrions 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.
Factors Affecting Test Results

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 postanalytical factors 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 2000g). 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 index (II) of ammonia was 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 is 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.

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 their easy disposal and inexpensive nature. These products, however, are produced at varying levels of purity and then often sold as universally applicable to all sample preparation, with some being advertised as compatible with liquid-liquid extraction protocols using organic solvents. Differences in the manufacturing and/or cleaning process of each tube can lead to sample contamination from materials that leach from the tubes and jeopardize patient management through their impact on the identified 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) and VWR (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, acetophenone, methyl-t-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 μm) and gradient of Acetonitrile and 5 mM ammonium formate. Identification of analytes relied upon an Agilent 6460 LC/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% Dimethylpolysiloxane Capillary capillary column (0.25μm, 0.25 x 15000 mm) (Chrompack, Rhenen, The Netherlands, 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 1.06x or 100 times greater than blank solvents. Conversely, the 1.5 mL Rainin and Eppendorf tubestested for peak areas comparable to the blank controls; leaching was found not 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
Establishing Evidence-Based Thresholds for Pseudohyperkalemia Alerts


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, platelet counts above 500 x 10^9/L or WBC counts greater than 150 x 10^3/microL 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 spectra of hemoglobin and OHCob. The extent of OHCob interference varies between platforms and has not been assessed using the specific instrumentation/assays used in our laboratory.

Objective: 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, lactoglobulin, 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 (54g/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 affected 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.
Factors Affecting Test Results

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 -20 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 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 glomerular 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 3470 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 (93.4-94.8) when we compared MDRD to CKD-Epi. The mean eGFR of males was 83.4 (82.6-84.3) using MDRD compared to 93.2 (92.1-94.3) using CKD-Epi. The difference for females was less significant at 93.2 (91.9-94.2) for MDRD versus 95.0 (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 glomerular 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 for Malays improved from 82.2 to 88.0 using the CKD-Epi formula. The Malays also although an intra-ethnic coefficient for the Malay population may be indicated

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 was 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 SST aliquots 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.
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 failure 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 (65.1%) and viral cirrhosis (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 using 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 was 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 m², respectively, 113.5 mL/min/1.73 m². GFR < 90 mL/min/1.73 m² was obtained in 40 (63.5%) patients using the GFR_{Cys} formula and in 13 (20.6%) patients using GFR_{Cr} formula. In 7 (11.1%) patients using GFR_{Cr} formula. Significant differences (P > 0.05) were observed between Child-Pugh A and B stages (P = 0.007), while there was no difference between B and C stages (P > 0.05).

**Conclusion:** Cystatin C significantly higher in patients with cirrhosis than in the control group. More patients with decreased glomerular filtration rate (GFR) were identified based on CysCr GFR than on creatinine GFR. Serum cystatin C represent sensitive indicators of renal dysfunction in liver cirrhosis.

**A-152**

**Strong Negative Interference by Calcium Dobsesilate 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 and 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.

**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 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
Factors Affecting Test Results

Table 1. Recovery of chloride spiked on gauze.

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<th>Spiked Cl Conc. (mM)</th>
<th>Mean Yield without a Gauze Blank (mM)</th>
<th>Mean Yield with a Gauze Blank (mM)</th>
<th>Analytical Recovery without a Gauze Blank (%)</th>
<th>Analytical Recovery with a Gauze Blank (%)</th>
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</tbody>
</table>

Evaluation of Sweat Collection Material for Sweat Chloride Testing

C. A. Gallo, D. A. Payto, S. Wang. Cleveland Clinic, Cleveland, OH

**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. 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 0.7 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.
Factors Affecting Test Results

A-157

Protein Interference with Common Laboratory Tests

J. H. Contois1, J. P. Nagy2, R. Nguyen1,1 Sun Diagnostics, New Gloucester, ME; Health Diagnostics Laboratory, Richmond, VA

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 the following 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, LIP, PHOS, BUN, UA, Lp(a), sIDL-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), fructoseamine, 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.

Table 1. Difference in THC results.

<table>
<thead>
<tr>
<th>Samples Refrigerated in Plastic Tubes (mg/mL)</th>
<th>Samples Refrigerated in Plastic Containers with Metal Screw Caps (mg/mL)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1479</td>
<td>1207</td>
<td>-18</td>
</tr>
<tr>
<td>728</td>
<td>511</td>
<td>-30</td>
</tr>
<tr>
<td>312</td>
<td>151</td>
<td>-52</td>
</tr>
<tr>
<td>65</td>
<td>51</td>
<td>-22</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td>-50</td>
</tr>
</tbody>
</table>

A-160

Long-Term Stability of Novel Urinary Acute Kidney Injury Biomarkers

C. M. Adamo, A. A. Sethi, D. B. Schranz, C. King, A. Bergquist, T. Argonne, T. H. Carlson. Pacific Biomarkers, Seattle, A

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, 5, 15, 18 and 28 months. The between-run precision (BRP) of each biomarker assay was determined from QC's 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.

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Effects of Urine Specimen Containers with Metal Caps on Urine Test Results

C. A. Gallo, C. Heideloff, K. Pan, S. Wang. Cleveland Clinic, Cleveland, OH

Background: Specimen collection containers must be carefully evaluated to ensure that the analyses 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 aliquoted 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 expected 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

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.

<table>
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<tr>
<th>Samples Refrigerated in Plastic Tubes (mg/mL)</th>
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</tr>
</tbody>
</table>
Factors Affecting Test Results

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 10000 Daltons cut-off and results recorded post centrifugation. The next 6 samples were tested for plasma hemoglobin at baseline noting 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 index may be required. Result comments should include the information regarding icteric and lipemic interferences.

The effects of interfering substances on Free Plasma Hemoglobin values measured using Hemocue Plasma/Low Hemoglobin spectrophotometer

S. Eugene, T. Law, N. Heger, M. Kellogg. Boston Childrens Hospital, Boston, MA

Background:
Red blood cell hemolysis can be the result of a mechanical destruction with a venricular 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.

Results:
Table 1 shows the Sigma-metrics for a sample of the method groups that reported HbA1c results for the 3 samples from survey GH-2b. The metrics for sample GH-2b-4, 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 52.2 % 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.
Factors Affecting Test Results

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 immunooassay 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 c8000 and three ARCHITECT i2000sr instruments. Data presented here are from the following serum clinical chemistry analytes: ALT, AST, total bilirubin, chloride, total cholesterol, creatinine (pierce), glucose, potassium, total protein, sodium, triglycerides and urea; the following immunooassay 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 (pierce), glucose, potassium, sodium, total protein, sodium, triglycerides 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.

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 immunooassay 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 immunooassay analytes with no compromise to quality.

A-166  Evaluating the Effects of Preanalytical Factors on Common Biochemistry Parameters: Duration Before/After Centrifugation, Recentrifugation, and Storage Conditions

J. Litten1, J. Litten1, L. Nelson1, J. Johnson2, J. Shih2. 1Winchester Medical Center, Winchester, VA, 2Abbott Laboratories, Abbott Park, IL

Introduction: 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.

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.

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.

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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.

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.
Factors Affecting Test Results

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Stability of Extracted Samples for LC-MS/MS Analysis of Free Plasma Metanephrines

D. A. Payo1, J. Gabler2, S. Wang1, Cleveland Clinic, Cleveland, OH, 1Thermo Fisher Scientific, 2Palmetto Beach, FL

Background: Plasma free metanephrines is the recommended first line laboratory test 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 4, 24, and 48 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>
<thead>
<tr>
<th>Analyte</th>
<th>Test DB (%)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>6.54</td>
<td>2h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
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<tr>
<td>ALT</td>
<td>11.48</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
</tr>
<tr>
<td>GGT</td>
<td>11.06</td>
<td>6h</td>
<td>2h</td>
<td>6h</td>
<td>2h</td>
<td>6h</td>
<td>2h</td>
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<tr>
<td>Glucose</td>
<td>2.34</td>
<td>6h</td>
<td>6h</td>
<td>6h</td>
<td>6h</td>
<td>6h</td>
<td>6h</td>
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<tr>
<td>BUN</td>
<td>5.6</td>
<td>6h</td>
<td>6h</td>
<td>4h</td>
<td>4h</td>
<td>6h</td>
<td>4h</td>
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<tr>
<td>Creatinine</td>
<td>1.96</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
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<td>4h</td>
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<tr>
<td>T. prot.</td>
<td>1.36</td>
<td>2h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
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<tr>
<td>Albumin</td>
<td>1.43</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
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<tr>
<td>Na+</td>
<td>0.23</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
<td>4h</td>
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<tr>
<td>K+</td>
<td>1.81</td>
<td>4h</td>
<td>4h</td>
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<tr>
<td>Cl-</td>
<td>0.50</td>
<td>4h</td>
<td>4h</td>
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<td>4h</td>
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<tr>
<td>Ca2+</td>
<td>0.82</td>
<td>2h</td>
<td>4h</td>
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<td>4h</td>
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<td>4h</td>
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<tr>
<td>Mg2+</td>
<td>1.80</td>
<td>6h</td>
<td>6h</td>
<td>6h</td>
<td>6h</td>
<td>6h</td>
<td>6h</td>
</tr>
</tbody>
</table>

Table 1: Relative bias values for groups that exceeded desirable bias (DB)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Temperature</th>
<th>Timepoint (Hours)</th>
<th>n</th>
<th>Mean</th>
<th>%CV</th>
<th>%Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>4°C</td>
<td>2</td>
<td>3</td>
<td>310</td>
<td>3%</td>
<td>NA</td>
</tr>
<tr>
<td>Mid</td>
<td>4°C</td>
<td>2</td>
<td>3</td>
<td>318</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td>High</td>
<td>4°C</td>
<td>2</td>
<td>3</td>
<td>345</td>
<td>4%</td>
<td>8%</td>
</tr>
</tbody>
</table>

Table 1: Stability Data

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Improved testing options through evaluation of specimen temperature stabilities.

J. J. Rushton, A. Matheny, M. Rush, J. Parry, C. L. Wiley. Pathology Associates Medical Laboratories, Spokane, WA

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, Gluidin antibodies (IgG, IgA), Endomysial IgA , Tissue transglutaminase antibodies (IgG, 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

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Factors Affecting Test Results

Tuesday, July 28, 9:30 am – 5:00 pm

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Factors Affecting Test Results
Validation of lithium heparin tube for Cardiac markers in a Clinical Laboratory and its benefits

S. CARDOSO, K. K. C. SILVA, E. A. LIMA, A. C. B. ALMEIDA, J. D. CRUZ, M. AKYAMA, N. SILVA. DASA, BARUERI, Brazil

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 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 Elecsa Roche platform and fluorescence enzyme immunoassay (EL A), 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 1 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 36.6%. The troponin, T 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 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 decision time that has an interior surface similar to glass which do not contain surfactant carriers as evident by previous studies reporting significant differences in particular serum hormone concentrations collected in different types of BCTs. Surfaceactant(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 (T3), and total thyroxine (T4), 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, T3, and T4, were measured in random order on a Siemens Immulite™ 1000 analyzer and routine chemistry analytes were measured on a Siemens Dimension Rxl™ analyzer. The results of triplicate results for cortisol, T3, and T4 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.

Storage stability of selected nutritional biomarkers at -20°C for up to 12 months

H. Chen, M. R. Sternberg, R. L. Schleicher, C. M. Pfeiffer. CDC, Atlanta, GA

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 replications per condition. All replications 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

<table>
<thead>
<tr>
<th>Biomarkers (Serum)</th>
<th>Change, %</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin, ng/mL</td>
<td>1.22</td>
<td>0.0468</td>
</tr>
<tr>
<td>Transferrin, mg/L</td>
<td>-1.70</td>
<td>0.0233</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>-0.27</td>
<td>0.80</td>
</tr>
<tr>
<td>Water-soluble vitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate, umol/L</td>
<td>-11.7</td>
<td>0.0156</td>
</tr>
<tr>
<td>Vit B6 (PLP), umol/L</td>
<td>-33.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vit B12, pg/mL</td>
<td>1.97</td>
<td>0.09</td>
</tr>
<tr>
<td>Vit C, mg/dL</td>
<td>-49.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat-soluble vitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit A, ug/dL</td>
<td>-1.25</td>
<td>0.27</td>
</tr>
<tr>
<td>Vit E, ug/dL</td>
<td>-0.97</td>
<td>0.33</td>
</tr>
<tr>
<td>Vit D (25OHID), ng/mL</td>
<td>-0.75</td>
<td>0.65</td>
</tr>
</tbody>
</table>

The Performance of Chemically Modified Plastic Blood Collection Tubes To Achieve Interior Hydrophobic Surface on Clinical Chemistry Analytes

R. Bowen, S. Kim, R. Zare. Stanford University, Stanford, CA

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. Surfaceactant(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 (T3), and total thyroxine (T4), 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, T3, and T4, were measured in random order on a Siemens Immulite™ 1000 analyzer and routine chemistry analytes were measured on a Siemens Dimension Rxl™ analyzer. The results of triplicate results for cortisol, T3, and T4 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.
Factors Affecting Test Results

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-176

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.
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 (MRCP-Holland). The analysis was performed using the GeneMarker v2.6.2 software. In 11 cases, DNA was also evaluated by Comparative Genomic Hybridization analysis (a108K aCGH/SPNs; 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 complexities 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 called 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 assays. In conclusion, the detection of IKZF1 alterations 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 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 I on 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, hematologic 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 (PIGF) 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 PIGF 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/βthal 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 PIGF were measured in patients with HbS/βthal and controls using immunoenzymatic techniques.

Results: Levels of hs-CRP, vWF and PIGF were elevated in patients with HbS/βthal compared to controls (6.9±5.3 vs. 0.4±0.4mg/L, 70±178.3 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, PIGF and ADAMTS-13 (r>0.01). PIGF 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, PIGF, 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 PIGF suggesting a possible pathogenic role for iron load in SCD biology.
Preclinical validation of fluo escence 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 cells 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 i(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-hematologic patients with normal karyotypes who would be unlikely to harbor the AML1/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 (8q11) probes labeled respectively with red and green fluorophore. 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 test 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 3G1R (2,34%). The signal patterns and their cutoffs for blood samples were 1F1G1R (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.

High sensitivity Hepcidin-25 bioactive ELISA: a sensitive, fast and straightforward competitive Elisa for the quantification of Hepcidin-25 in human serum and plasma

M. Horkert 1, B. Uelker 1, T. Dudek 1, C. E. Geacintov 2. DRG Instruments GmbH, Marburg, Germany. 2. DRG International Inc., Springfield, NJ

Hepcidin is an iron homoostasis 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 state 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 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/dL), bilirubin (up to 0.5 mg/dL) and triglycerides (up to 7.5 mg/dL). 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 straightforward procedure with ready-to-use reagents, no shaking, high sensitivity, and a short total assay time of 1.5 hours.

Lipid effect on antithrombotic and prothrombotic activities of thrombomodulin (TM)

V. Grudzys, X. Sun. Cleveland State University, Cleveland, OH

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 (TAFIa) 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 TAFIa 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 TAFIa were generated by incubation with liposomal TM and thrombin in otherwise identical conditions. APC and TAFIa levels were measured by hydrolysis of spectrozyme PCAs (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 (PtdEtn) liposomes (153 ± 5%) in comparison to free TM (69 ± 8%), where TM in phosphatidylcholine (PtdCho) was set as a reference (100%). Incorporation of TM into 5% phosphatidylerserine (PtdSer) liposomes showed a decrease in APC amount (82 ± 18%). Further decrease in APC was observed in 10% PtdEtn and PtdSer liposomes (70 ± 21 % and 86 ± 26%, respectively). Competitive studies using co-incubation with TAFI showed significant differences of APC in PtdCho-TM liposomes (58 ± 14%) and in 10% PtdSer as well as 10% PtdEtn liposomes (24 ± 21 % and 34 ± 31 %, respectively). On the other hand, increase in TAFIa was observed in 5% PtdEtn-TM (207 ± 16%) and 5% PtdSer-TM (234 ± 21%) liposomes compared to PtdCho-TM (100 %). Reduction of TAFIa generation in competitive conditions was observed (from 207 ± 16% to 83 ± 30 %) in 5% PtdEtn liposomes.

Hematology/Coagulation

Tuesday, July 28, 9:30 am – 5:00 pm

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Conclusion: We determined that 5% PtSer and 5% PtEtn liposomes showed highest increases in TAFlr 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 antiagulant and anti-inflammatory potential of the endothelium.

The Activity of Recombinant Endothelial Nitric Oxide Synthase Oxygenase Domain on Human Apo A1 Derived Discoidal Lipid Particles

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Background: Cardiovascular diseases (CVDs) are the number one 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 A1). 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 phosphatidylethanolamine/detergent micelles and apo A1 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. The specific 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 H2O2 by eNOSoxy and eNOSoxy-OH in the form of nitrite through a standard calibration curve (R2=0.9979). The activity of eNOSoxy/nanodisc or free eNOSoxy is calculated in nmol NO/min/nmol bound nanodisc were assayed in 96-well microplates. Samples were run in triplicate.

Results: The specific activity of eNOSoxy/nanodisc or free eNOSoxy is calculated in nmol NO/min/nmol bound nanodisc were assayed in 96-well microplates. Samples were run in triplicate.

Conclusion: The activity of eNOSoxy was determined using Tris-buffer at 0.5 ml/min. eNOSoxy concentration in nanodisc was verified by applying to a Superdex 200 10/30 gel filtration column on ÄKTA FPLC system. The specific 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 H2O2 by eNOSoxy and eNOSoxy-OH in the form of nitrite through a standard calibration curve (R2=0.9979). The activity of eNOSoxy/nanodisc or free eNOSoxy is calculated in nmol NO/min/nmol bound nanodisc were assayed in 96-well microplates. Samples were run in triplicate.

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Validation of fluo escence 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

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Molecular characterization and genotyping of alpha and beta thalassemias among anemic patients

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Purpose: To study the prevalence of thalassemia with or without sickle-cell disease and its clinical picture in a defined population. Methods: The study included blood samples of 3540 patients from January 2011 to December 2014, from the general population and some patients were from the special clinical units. The data was collected and the patients were classified into four groups: a) thalassemia; b) thalassemia with sickle-cell disease; c) sickle-cell disease with thalassemia; d) nor of both thalassemia and sickle-cell disease. Conclusion: 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.

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 4% 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 gene 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 1-5 (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 fluo escence in situ hybridization assay for detection of the t(11;14) (CCND1/IGH) translocation in a clinical laboratory
index and poor quality of metaphases, making it difficult to chromosome 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) we 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%), 1F2G2R (3.3%), 1F1R (1.4%), 2G1R (3.7%), 1G2R (4.4%), 1G1R (2.3%), 2G3R (1.4%), 3G2R (2.3%), 4G3R (2.3%) and 4G4R (2.3%). The signal patterns and its cutoffs for blood samples are 1F1G1R (6.2%), 2G1R (3.1%), 1G2R (3.1%), 1G1R (2.3%), 3G2R (1.4%) and 2F1 (1.4%). 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.

### A-185

**Performance of the Alpha-1-Antitrypsin assay for use on the Binding Site Optilite® turbidimetric analyser**

K. MacDonald, J. R. Kerr, D. J. Matters, S. Harding. The Binding Site Group Ltd, Birmingham, United Kingdom

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 cirirosis 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 124 samples; 102 from disease state patients and 22 from healthy blood donors (total range 0.40-4.73g/L). This demonstrated poor agreement with existing assays. We conclude that the A1AT assay for the Binding Site Optilite analyser is reliable, accurate and precise and shows good agreement with existing assays.

### A-186

**Performance of a Haptoglobin assay for use on the Binding Site Optilite® turbidimetric analyser**

E. Hardy, J. Kerr, A. Alvi, D. J. Matters, S. Harding. The Binding Site Group Ltd, Birmingham, United Kingdom

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 analyser. Precision was determined with a CLSI protocol using 148 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 - 0.00 (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 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 <6.3% 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.

### A-187

**Performance of serum IgA Kappa and IgA Lambda assays for use on the Binding Site Optilite® protein analyser**

L. D. Southan, A. Brown, M. D. Coley, A. Kaur, P. J. Showell, S. J. Harding. The Binding Site Group Ltd, Birmingham, United Kingdom

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 IgAκ and IgAλ and lambda and give a more accurate representation of tumour production. Furthermore, calculation of the IgAκ/IgAλ 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 IgAx and IgAλ assays (Hevylyte®, The Binding Site) for use on the Binding Site’s Optilite® analyser. The assays have measuring ranges of 0.18-11.20g/L for IgAx (reference interval 0.60-12.40g/L) and 0.155-10.40g/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 (IgAx) 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 <1.5% was seen with haemoglobin and bilirubin, for intralipid and triglycerides the mean bias -0.8% (minimum -2.5%, maximun 1.5%).

### Table 1: Precision of IgD assay

<table>
<thead>
<tr>
<th>Analyte concentration (g/L)</th>
<th>25.27</th>
<th>110.20</th>
<th>165.27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total precision (%CV)</td>
<td>4.0%</td>
<td>3.2%</td>
<td>3.2%</td>
</tr>
<tr>
<td>Between day precision (%CV)</td>
<td>2.7%</td>
<td>2.1%</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

**A-189**

### Multivariable Graphic and Numerical Statistical Techniques for Comparing the Performance of Two Hematology Instruments: A Practical Example with Sysmex EX-5000® and Sysmex XT-4000®

V. M. Genta1, W. Tang1, J. White1, D. Washburn2, 1Sentara Virginia Beach General Hospital, Virginia Beach, VA, 2Sentara Healthcare, Norfolk, VA

**Background**: Sysmex EX-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 simultaneously compared. We employed multivariable statistical models and their graphic representations to assess simultaneously the performance of the two instruments operating in the two modes. Methods: The precision was evaluated by assaying quality control material (E-CHERE® (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® (Version 16, 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 8.5%) and no clinically significant bias for reticulocytes (mean 0.01, minimum -0.5, maximum 0.4). Since the EX-5000 was considered our reference instrument the results were extrapolated to both instruments. After recalibration, comparison with patient specimens showed no clinically significant differences for XT-4000; RBC mean bias 1.5% (minimum 0%, maximum 3%); HCT mean bias -0.8% (minimum -2.5%, maximum 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 laboratory to perform numerical and graphical data analysis simultaneously on several instruments and parameters.
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 Labor.

Methods: Presently, 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 reaction positive for Myeloperoxidase Deficiency” because they presented 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 ADVIAHematology 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 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 6.9% (0.02g/L), 1.0% (0.030g/L), 4.0% (0.056g/L), 1.7% (0.289g/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=0.992x+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 underdetected analyte. 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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The frequency 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 identified 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 homozgyous 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 homozgyous states, and its association in the same individual was evaluated.

Results: The prevalence of the mutation, searched in 1973 samples was 5.06% (n = 100) in heterozygous and 0.05% (n = 1) in homozgyous state. The mutation of the prothrombin gene (1092 samples) was heterozygous in 4.39% (n = 48), homozgyous 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).
Tuesday, July 28, 9:30 am – 5:00 pm

Hematology/Coagulation performance.

Conclusions: from ~2900Pa to ~300Pa (4.5% CV), equivalent to a 90% reduction in clot stiffness. Increasing the amount of abciximab in the sample progressively reduced clot stiffness (2% CV), while average clot stiffness with thromboplastin was 2944 Pa (4.75% CV). Average clot stiffness was 690 Pa with thromboplastin with 3.5 and 9.4% CVs respectively. Average clot stiffness was 2,090 Pa with kaolin or whole blood (n = 11 for kaolin, n = 12 for thromboplastin) from activation with a novel blood-derived control sample (n = 21 for kaolin, n = 28 for thromboplastin). QuantraDx reproducibility was assessed with kaolin and thromboplastin (n = 21 for kaolin, n = 28 for thromboplastin) and whole blood (n = 11 for kaolin, n = 12 for thromboplastin) from

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-cardiac average clotting time for kaolin activation was 3 minutes (2% CV), while average clot stiffness with thromboplastin was 2944 Pa (4.73% CV). Increasing the amount of abiciximab 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.

Heparin Interference in Anti Xa Assays for Rivaroxaban and Apixaban

K. Finnegan1, G. A. Cutsforth1, 2Stony Brook University, Stony Brook, NY, 2Diagnostica 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 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.

DxH PROService Solutions: A Case Study - Proactive Monitoring of System Data

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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 improper 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. PROService 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 PROService servers. The PROService 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 PROService 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 PROService 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 PROService 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.
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. 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 R²=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.

Development of Hemoglobinopathies

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 trains 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. From 148,317 individual hemoglobin HPLC results, 21,451 showed abnormalities of hemoglobin, affecting its functionality, that occurs when there are changes in genes encoding trains 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. We calculated the prevalence and diversity of hemoglobinopathies in Brazilian population, and its distribution among country regions.

Results: 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 postoperative patients, 2,753 (1.86%) in South and 1,126 (0.76%) in North of Brazil.

Application of Procalcitonin and neutrophil VCS parameters in the diagnosis of bacterial infection in non-small cell lung cancer postoperative patients

Background: We explored the predictive value of procalcitonin and VCS parameters of neutrophil in infected non-small cell lung cancer patients after operation.

Methods: To analyze treatment of 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 di ferences (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.

Variant hemoglobin prevalence in Brazil and its regions: An update from a large laboratory database.

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 trains 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. From 148,317 individual hemoglobin HPLC results, 21,451 showed hemoglobinopathies, and were classified by hemoglobin found and country region.
Evaluation of Serum IgM Hevylite® assays; IgM Kappa and IgM Lambda for use on the Binding Site Optilite® analyser

J. R. Kerr, S. K. Ahalirwal, 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 (IgMk) and IgM Lambda (IgMλ) may give a more accurate representation of tumor production. Moreover, calculation of the IgMk/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 IgMk 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 ≤5% CV for IgMk and ≤5.0% CV for IgMλ at all levels tested. Within run precision was ≤1.7% CV for IgMk and ≤3.5% 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 IgMk and 0.16-6.98g/L for IgMλ. This validated measuring ranges of 0.2-5.0g/L for IgMk and 0.18-4.50g/L for IgMλ. At 1/10 with sensitivity of 0.020g/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/L sodium and lambda gave y=1.01x - 0.00. We conclude that the Optilite IgM Hevylite assays are reliable, accurate & precise method for quantifying IgM Kappa & IgM Lambda and show good agreement with existing turbidimetric assays.

Conclusion: Decreased levels of intracellular adenosine may provide clues regarding the mechanisms of aging and age-related diseases. Further research is needed to elucidate the role of adenosine in maintaining cellular health in aging tissues.

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

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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 participants aged 3 to 79 and obtained fresh whole blood specimens. Sixteen hematology markers (including calculated parameters) were measured using the Beckman Coulter DxH1200 or ImmX 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 CLSI 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 on-line coagulation testing and jeopardize patient safety

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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 posture 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 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 posture during blood collection by venipuncture. The second and third tubes were drawn after the patient was sitting in a comfortable sitting position, and the third after 20 min standing in an 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 < 0.05.

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.
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.
Application of Assay Appropriate Statistical Analysis Dramatically Improves Turn Around Time

L. J. Overson, 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 StatLitia (Brendan Scientific). The MFI for each bead is compared to the MFI of the standards via a SPL 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 parallel calculations. An alternate to StatLitia was identified at http://www.ist.nist.gov/div898/handbook; this approach relates the weight as a function of the variability of the response at each standard (W=1/StDev). 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 StatLitia 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 StatLitia 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.

Quantitation of Eculizumab in serum by microLC-ESI-Q-TOF MS


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 measurement 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 20ml 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 at 55°C. A 10µL 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 portion of the mAb could be used to quantify eculizumab in patient serum using microLC-ESI-Q-TOF mass spectrometry.
CEREBROSPINAL FLUID FREE KAPPA CHAINS AS A POSSIBLE BIOMARKER TO ASSAY RISK CONVERSION TO MULTIPLE SCLEROSIS

P. Menendez Valladares, M. I. Garcia Sanchez, J. L. Garcia De Veas Silva, C. Bermudo Guturte, G. Izquierdo Ayuso. Virgen Macarena University Hospital, Seville, Spain

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 which 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 Assay. The predictive value of free kappa chains was calculated using the Barkhof-Tintore criteria, MRI and oligoclonal bands (OCGB) criteria. Statistical analysis was performed using the SPSS®20. p<0.05 was considered significant.

Results:
Using a cut-off value of 0.38mg/L determined from the mean CSF-FKC levels of 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 the table 1.

Conclusion:
In our cohort, high CSF-FKC values above 0.38mg/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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1: patients with CSF-FKC &gt; 0.38 mg/L</th>
<th>Group 2: patients with CSF-FKC &lt; 0.38 mg/L</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>45</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Gender (female %)</td>
<td>37 (82.2)</td>
<td>23 (71.9)</td>
<td></td>
</tr>
<tr>
<td>Age [mean (CI95%)]</td>
<td>34.6 (31.8-37.3)</td>
<td>36.2 (31.9-40.6)</td>
<td></td>
</tr>
<tr>
<td>Oligoclonal IgG bands in CSF (+/-)</td>
<td>45/0</td>
<td>3/29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IgG index (&gt;0.5:&lt;0.5)</td>
<td>45/0</td>
<td>17/15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>≥3 Barkhof-Tintore criteria (%)</td>
<td>29 (44)</td>
<td>7 (10.6)</td>
<td>0.0040</td>
</tr>
<tr>
<td>Fullfilled MRI and OCGB criteria (yes/no)</td>
<td>29/14</td>
<td>3/20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Conversion to MS (%)</td>
<td>51.5</td>
<td>4.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
**Immunology**

A-212

The incidence/prevalence of autoimmune diseases is increasing worldwide

A. Lerner, P. Jeremias, T. Matthias. Aesku.Kipp Institute, Wendelsheim, Germany

Background: 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 geopiodemiological 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, IIDD and IBID, with 17.4, 12.2, 3.7, 2.6 and 2.4%, respectively. Geopiodemiologically, the following countries had high to low %/year AD frequencies 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 (IIDD) and gastrointestinal (CD, IBID) 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.

A-214

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 to preserve sample integrity, enabling shipping from remote locations to a central laboratory for analysis.

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).

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A-215

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

M. Q. Ansari, M. K. Gupta. Cleveland Clinic, Cleveland, OH

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 SPE and IFE. Free light chains were measured using immunobass on Beckman Image platform. In both cases serum light chains and ratios were normal and the urine free light chains 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 (IgM) with normal albumin, calcium, CBC. He now has a normal SPEP and normal serum FLC [K 12.1 and L 11.9 mg/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 chemio and radiation. He has anaemia 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


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-, 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- cohort it was 2.0±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 provided 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+ 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 intrathetically.

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 37°C. The presence of cryoglobulins in vivo are associated with visual disturbances, headache, vertigo, loss of consciousness and stroke. These 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 4°C and 37°C for up to 72 hours. Cryoglobulins, if present, will precipitate at 4°C while the aliquot at 37°C 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 4°C but remain clear at 37°C 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 any 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 500 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 redissolved 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.
Tuesday, July 28, 9:30 am – 5:00 pm

Immunology

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SLE-Key™ 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 autoimmune diseases, but currently there is no reliable serologic diagnosis. Each autoimmune body 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™ 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 range 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-Key™ 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™ chip.

Results: The SLE-Key™ 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-Key™ classified 50-80% of these samples as healthy control depending on classification method chosen.

Conclusion: The SLE-Key™ multiplex test can be used to assist physicians in ruling out serologically a diagnosis of SLE with a sensitivity of >90%. The SLE-Key™ test can take years of monitoring.

A-220

Comparison of NK Vue, quantitative IFNγ ELISA, with flowcytometric NK activity tests

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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 51Cr 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°C CO2 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 flowcytometer, 83 participants were enrolled with informed consent to study. For flowcytometer, PBMC separated from the matched heparinized blood were incubated with K562 cells for 6 hours and stained with CD45-FITC, CD3-ECD, CD16/C56-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 flowcytometer.

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 (R² = 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 correlate with cytoplasmic IFNγ by flowcytometer.

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 compatible than traditional NK cell activity measurement, could be a feasible method to estimate and monitor the NK cell function in clinical setting.

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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
Circulating BCMA Binding to Its Ligand BAFF Prevents Normal Antibody Production in Multiple Myeloma Patients


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, IgG 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 IgG-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 IgG (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 IgG 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 = 0.0026) 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.

Analytical Evaluation of The Helena SPIFE 3000 Electrophoresis/ Immunofixation System


Background: Protein Gel electrophoresis (PEG) 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 gels for 17-gamma systems routine clinical use, the Sebia Hydrasys (Norchess, GA) and Helena SPIFE (Beaumont, TX). The Helena SPIFE 3000 system has the ability to run more PEG (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 PEG/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 lambda bands. 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.

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 F2 α (2,3-BPG) and leukotriene E4 (LTE4). Patients often present after taking over-the-counter antihistamines 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 > 200 ng/mL, LTE4 > 104 pg/mg creatinine, NMH > 200 ng/mg creatinine, and 2,3-BPG > 313 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 sensitivity and specificity for SM diagnosis was determined using cutoffs previously established (tryptase >200 ng/mL, LTE4 >104 pg/mg creatinine, NMH >200 ng/mg creatinine, and 2,3-BPG >313 pg/mg creatinine).

Application of Bioenergetic health index (BHI) in Healthy Individuals and Chronic Kidney Disease


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 × ATP-Linked Respiration)/(Proton Leak × 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
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 IgG-IgG (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.
Tuesday, July 28, 9:30 am – 5:00 pm

Immunology

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: Aeskulip A® Giladin (AGA), Aeskulip A® Tg (TG: RUO), Aeskulip A® DGP (DGP) and Aeskulip A® Tg New Generation (Neo-epitope Tg complexes to gladin=Tg-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 Tg neo IgA (r2=0.986, p<0.0025) and Tg neo/DGP IgG (r2=0.989, p<0.0001, respectively) stood out, significantly, as the best indicators of the intestinal damage in CD.

Conclusions: It is suggested that Tg neo IgA/IgG antibodies should be preferably used to reflect intestinal damage during screening, diagnosing and monitoring compliance in childhood CD.

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-γ (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 IL-18 secretion was elicited by TB antigen in TB infected patients. Quantification of IL-18 in addition to IFN-γ is not useful in improving Quantiferon test interpretation.

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,
The presence of this antibody allows us to diagnose the patient’s symptoms as LE.

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 Hydrosys 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 with 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.

A-236

A rare case of paraneoplastic limbic encephalitis associated with squamous cell lung carcinoma

J. L. García de Veas Silva, T. De Haro Muñoz, L. Jaimez Gamiz, J. V. García Lario, V. Fernandez Varela. Complejo Hospitalario Universitario de Granada, Granada, Spain

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 °C). 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: 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.

Hematology study
CBC without significant alterations.

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.

Microbiological analysis (Study of meningitis and viral encephalitis)
PCR negative for Herpes simplex, Varicella-zoster, Toscana virus, Enterovirus.

Autoimmune study
Antinuclear antibodies (ANA), DNA and ENAs screening: negatives
Onconeural 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 allows 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 paraneoplastic limbic encephalitis with anti-amphiphysin antibodies positives associated to squamous cell lung carcinoma.

Conclusion: The presence of anti-amphiphysin antibodies allows us to diagnose a squamous 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.

The Grace Bio-Labs ArrayCAM Protein Microarray System surpasses the diagnostic throughput and accuracy shortfalls of ELISA and Multiplex Bead Assays


Background: Simultaneous multiplexed profiling of protein markers will become a 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 consistence.

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 Bio-Labs Microarray Assay System components begin with antigens arrayed onto ONCYTE high porous nitrocellulose slides producing multiple co-localized assay reaction sites within each well. Sample analyze 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 image and automated software, which quickly 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, SMRP, SSA60 kDa, SSA52 kDa, SSB, SCL-70, J01, 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, ImmunooConcepts). 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 singleplex 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.

**A-238**

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?

M. F. Mantovani¹, P. S. Osório², L. G. S. Carvalho², C. F. A. Pereira¹, ¹Faculdade Assis Gurgacz, Cascavel, Brazil, ²Universidade Estadual do Oeste do Paraná - UNIOESTE, Cascavel, Brazil, ³Diagnósticos da América - DASA, São Paulo, Brazil.

**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 screening 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 (Molecular Dynamics 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 determine 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.

<table>
<thead>
<tr>
<th>HTLV Blot</th>
<th>Neg</th>
<th>Pos</th>
<th>INC</th>
<th>POS</th>
</tr>
</thead>
<tbody>
<tr>
<td>rHTLV I/II CMIA</td>
<td>0,0 - 1,0</td>
<td>16</td>
<td>741</td>
<td></td>
</tr>
<tr>
<td>1,0 - 5,0</td>
<td>0</td>
<td>13</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>5,0 - 10,0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10,0 - 20,0</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>20,0 - 50,0</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>50,0 - 100,0</td>
<td>80</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&gt; 100,0</td>
<td>163</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

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.

**A-239**

Reduction of Immunoglobulin Carry-over on an Automated Tecxan Pipetting Platform

L. J. Ouverson, M. R. Snyder. Mayo Clinic, Rochester, MN

**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 Tecxan 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-IgG 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. A recent publication demonstrated that 0.17M NaOCl washes could eliminate carry-over of IgG molecules on the Tecxan 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 wash 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 0.83% to 6.0% which equates to about 600 fewer repeats per year. **Conclusion:** Carry-over can be a source of laboratory error 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

S. Thouless, J. Overton, P. Kenny, S. Harding. The Binding Site Group Ltd, Birmingham, United Kingdom

**Background:** The 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

<table>
<thead>
<tr>
<th>Analyte</th>
<th>QC</th>
<th>Mean</th>
<th>CV (%)</th>
<th>Sample range</th>
<th>Range of r*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freelite Kappa</td>
<td>High</td>
<td>26.418 mg/L</td>
<td>4.0</td>
<td>0.991 – 1189 mg/L</td>
<td>0.767 – 0.995</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>14.924 mg/L</td>
<td>5.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freelite Lambda</td>
<td>High</td>
<td>61.934 mg/L</td>
<td>7.0</td>
<td>1.37 – 14.248 mg/L</td>
<td>0.761 – 0.983</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>28.132 mg/L</td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG1</td>
<td>High</td>
<td>7122.160 g/L</td>
<td>4.3</td>
<td>1628 – 5392.6 g/L</td>
<td>0.925 – 0.965</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>2938.048 g/L</td>
<td>6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG2</td>
<td>High</td>
<td>4169.356 mg/L</td>
<td>3.2</td>
<td>1093.39 – 4480.43 mg/L</td>
<td>0.983 – 0.997</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>2394.085 mg/L</td>
<td>3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG3</td>
<td>Elevated</td>
<td>1642.858 mg/L</td>
<td>5.1</td>
<td>225.97 – 1145.28 mg/L</td>
<td>0.926 – 0.978</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>701.955 mg/L</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>350.000 mg/L</td>
<td>6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG4</td>
<td>Elevated</td>
<td>1868.307 mg/L</td>
<td>3.5</td>
<td>92.71 – 2527.53 mg/L</td>
<td>0.999 – 1.000</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>435.890 mg/L</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>237.559 mg/L</td>
<td>3.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A Range of r is calculated from 6 combinations of platform comparisons by Pearson Product-moment correlation coefficient*

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**A-241**

### Total Vs Antigen Specific Pneumococcal Antibody Response: A Comparison of Two Different Assay Types

M. M. Reynolds¹, D. L. Murray¹, M. A. V. Willrich¹, A. Parker², B. K. DuChateau², M. R. Snyder¹. ¹Mayo Clinic, Rochester, MN, ²The 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 Luminex™-based LDT (Mayo Clinic) which quantifies individual responses to 23 common Pneumococcal antigens.

**Results:** There was moderate agreement between the summed 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

W. D. Curtin¹, B. Holmquist¹, R. P. Grant¹, D. W. Chandler¹, ¹Endocrine Sciences, Laboratory Corporation of America® Holdings, Calabasas, CA, ²Laboratory Corporation of America® Holdings, Center for Esoteric Testing, Burlington, NC

Background:
Congenital Adrenal Hyperplasia (CAH) is most often caused by mutation of the 21-hydroxylase gene (CYP21), which results in underproduction 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 deplet ed serum (Golden West Biologica ls). Sample preparation consisted of isolate dilution using a cocktail of three heavy isotope internal standards followed by Liquid-Liquid Extraction. A Fluosphere® 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 5,000 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.
Tandem mass spectrometry-based molecular networking to detect drugs of abuse and analogues

J. Y. Yang1, D. A. Herold2, R. L. Fitzgerald1. 1UCSD, La Jolla, CA, 2UCSD/ 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 compared pairwise and cosine similarity scores were assigned per pair.
Tuesday, July 28, 9:30 am – 5:00 pm

Mass Spectrometry Applications

**A-252**

*Pre-column double derivatization method to quantify >35 clinically relevant amino acids by HPLC-TripleTOF™ 5600*


**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 isoas 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 2 mM 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 carbosyl - and amino + groups except phosphoethanolamine and taurine. The isotopic compounds were well separated. The method of 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.

**A-253**

*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 SerLab (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® 1-Class system, samples were injected onto either a 2.1 x 50mm Waters ACQUITY UPLC BEH Phenyl column (Method 1) or a 2.1 x 100mm Waters CORTECS UPLC C18 µm 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 36-720 pg/mL. Method 2 improved chromatographic resolution of aldosterone from interfering steroids in comparison to Method 1, which included baseline resolution of the 18-hydroxy cortisol/cortisone 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 Metabolism (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-Exactive 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-Exatives alternated between MS scans from 70-1000 m/z and MS² 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 R². 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 clinical 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 analytic. 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-HSA) 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-eHSA internal standard at approximately 22.0 µg/mL 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 1298 Series LC system) in the positive ion mode under multiple reaction monitoring (MRM) MS/MS conditions.

Results: The multiplexed urine albumin assay displayed good linearity over a concentration range of 5 to 300 µg/mL with 2 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.
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 986c, 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.

Development And Validation Of A High Sensitivity MicroLC-MS/MS Undervatization Method For Estradiol In Human Serum


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 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 multianaltes, 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-butanol-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 multianaltes 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 multianaltes 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 precision 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.

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 ESI+ mode, six 10-sec scans were summed from 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*, αα vs α, ββ vs β, βγ vs β*, p where γ = glycated). For samples with low HbA1c, boronate affinity concentration of glycated fraction was performed 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.

Vedolizumab Quantitation in Serum using SRM And microLC-ESI-Q-TOF Mass Spectrometry


Background: Vedolizumab is a humanized IgG 1κ therapeutic monoclonal antibody (mAb) that inhibits T-cell migration to the gut mucosa by selectively targeting α4β7 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 spectrometry (MS) methods to quantitate vedolizumab using: (1) tryptic peptides and intact light chain MS, but more importantly, showed more accurate 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.

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, R²>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 (s=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 (R²=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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**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 efficient to separate and measure 25OHD2, 25OHD3, as well as 3-epi 25OHD3, was different, which may cause variation between methods.

Methods: Our objective was to evaluate the effects of 25OHD2 and 3-epi 25OHD3, on Roche, Siemens, Abbott, IDS and DiaSorin immunoassays 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 25OHD2, 111 serum samples contained both 25OHD2 and 25OHD3, 32 serum samples contained 25OHD3, and 3-epi 25OHD3, and analyzed with these methods.

Results: In the results with no 25OHD3, or 3-epi 25OHD3, 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 bias less than 2.5 ng/mL while Abbott and IDS showed positive bias less than 2.0 ng/mL. 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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**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 Zer sample, the 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 ~ 18000 seconds) for a final value in ng/L. Angiotensin I (Proteochrom) 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 R² > 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: 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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**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 lack specificity 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. A 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.
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, glipizide, glipizide, and gliquidone. **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 Aglient HPLC with an ABSciex 3200 LC-MS/MS in positive ESI mode. Compounds were identified by a combination of retention time, two MRMs 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). **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.

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 (hHAS) 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 calibration concentrations was described by the Deming equation $y = 1.048x + 6.14$ with a correlation coefficient $r = 0.9995$. 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 $r = 0.9995$. **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.

A Simple and Sensitive Method for Quantification of 5HIA 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 diagnosis 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 of 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 40μg/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 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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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

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 ESU/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 ng/mL 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.
Mass Spectrometry Applications

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 Sciex 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 11 min 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 our measurements were below 31 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.

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 Cushings 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 Cushings 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.

METHODHOUDS: A novel method (LC-MS/MS) collected using a Salivette device (Sarstedt, Newton, NC) was mixed with internal standard (50 µL; d5-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 35°C. 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 d5-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.
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 (r2 > 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.

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Method development for the determination of 17α-hydroxyprogesterone in human serum by stable isotope dilution liquid chromatography tandem mass spectrometry

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Background: Hormones are an important class of clinical diagnostic markers. Accurate measurement of one or more 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-d4 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: weight ratio. After equilibration for 1 hour, 5 mL of CH3Cl2 was added to sample. Sample was shaken for 5 min and centrifuged at 3,000 rpm for 5 min. CH3Cl2 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°C 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 CH3Cl2, 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-38 min (30% A)-43 min (30% A). 17OHP and 17OHP-d4 were monitored at mass transfer m/z 331/337.3 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.
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, alkalized to convert specific metabolites to stable forms, and acidified and filtered prior to analysis. A UHPLC system with a solid core C18 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 ≥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.

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 263>121, cortisol d4 m/z 267>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.

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 ABBisec 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. 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 methanol 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 = 0.999. 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 = 36).

Conclusion: This model successfully combines batch and “random-access” type methods on a single multiplexing LC-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 APCl 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 Hydroxydeoxycholic acid (HDCA), 93.4-105% for Taurochenodeoxycholic acid (TCDA), 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% -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 quantitation of bile acids with an 0.05 µM limit of quantitation. Absolute quantification provides accurate results.

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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 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 Hydroxydeoxycholic acid (HDCA), 93.4-105% for Taurochenodeoxycholic acid (TCDA), 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% -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 acids 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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Mass Spectrometry Applications

Tuesday, July 28, 9:30 am – 5:00 pm

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 (2.6μm, 3.0 x 50mm) at a flow rate of 500μL/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 50μL of the final sample, derived from an original sample volume of 400μL 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 50μL 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.

A-286

Development of a serum based exosomal biomarker signature to distinguish indolent and aggressive prostate cancer


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.
Tuesday, July 28, 9:30 am – 5:00 pm

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 the over-the-counter and prescription medications such as Vick’s inhaler and Desoxyn may yield positive methamphetamine results. Chiral separation of D and L stereoisomers can help determine if the source was putatively illicit or illicit.

Cycloedextrins 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 Nor-(2,4-Dinitro-5-fluorophenyl) L-alaninamide (Marley’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™ 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 °C. Samples were reconstituted in a solution of sodium bicarbonate and the Marley’s reagent (Sigma-Aldrich) was added. The derivatization reaction was performed at 56 °C 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 µm 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 MRMs 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 Marley’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 control levels 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.

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, Hydroxylorazepam, Hydroxytriazolam, Oxazepam, Oxazepam) were included in this method. Urine sample (50 µL) was mixed with an enzyme solution (50 µL, 10,000 U/ml H. rugosus β-gluconidase 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 BZD. It was validated to test urine samples for pain management service.

Recent Postsenopausal Women Have Elevated Urinary 8-isoprostaglandin 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.

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. 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).

CONCLUSION More research is needed to determine the role of oxidative stress in the pathogenesis of diseases that are prevalent in postmenopausal women.
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 containing 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 7.6% 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 amines and has applied for clinical analysis

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 the micro-vial and evaporated to dryness under nitrogen. The dried extract was reconstituted in a 30:70 water:methanol solution and injected on to the 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 the 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 of 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.

The Analysis of Vitamin D and Metabolites in Plasma by LC-MS/MS

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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 do-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 (5mL) 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 ≤50%. 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.

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 Seisman 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 immunoassays. Methods: We verified performance claims of the Waters MassTrak assay and performed a patient concentration 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 MassTrak assay at three different concentrations over the measured analytical range were (mean ± CV): 0.6 ng/mL ± 20%, 16.0 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 MassTrak 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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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.

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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 (Asomycin (5 ng/mL), Cyclosporin A-d4 (250 ng/mL), Ramycin-d3 (5 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-buty 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 LDT 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.

**Reference Intervals for Midnight and Morning Cortisol in Saliva Measured by an LC-MS/MS Method**

D. R. Bunch, L. Kennedy, S. Wang. Cleveland Clinic, Cleveland, OH

**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 6:00AM and midnight samples were collected between 11PM and midnight. The Salivettes were centrifuged and the samples were frozen at -70°C 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.

**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 to its 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 and raspberry. 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 Gass) 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 limit of quantification (LOQ), linearity, precision, accuracy and comparability with Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Instrumental linearity was verified in the range of 0.01 - 100.0 µg/L for Chromium, 0.1-100.0 µg/L for Manganese and 0.5-100.0 µg/L for Nickel. The medium range of recovery over an interval of 0.5-100.0µg/L for the Chromium was 99.9-106.4%, for Manganese it was 95.7-102.3% and for Nickel it 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 congenital 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. Congenital 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 of 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 radioimmunoassays, 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, with 17-hydroxyprogesterone (331.0 → 97.1/109.1 [H-85, Ce(nalf, Round Rock, Texas, USA]) and 17-hydroxyprogesterone-d8 (339.2 → 100.0/112.1 [H-96, 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 250 000 ng/L. Across this linear range all calibration curves displayed excellent linearity of R2 > 0.999 and satisfied accuracy and precision requirements for ±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 100-250 000 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-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 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 ±1.1ppm, 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 100 µg/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 x103), 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 (PrEP).

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 was 1 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-[13C]-[15N2 (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 3.20, 0.400, and 0.0600 ng/ml hair (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
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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 Urin

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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$_4$ (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.
Development of a Reference Material for the Determination of Vitamin B₁₂ 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 B₁₂, which is involved in DNA synthesis and red blood cell formation. Vitamin B₁₂ deficiency can lead to anemia and permanent nerve damage. Vitamin B₁₂ 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 B₁₂ based on radioassay measurements at the Centers for Disease Control and Prevention (CDC), no material specifically designed for vitamin B₁₂ 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 B₁₂ value assignment. NIST, in collaboration with the National Institutes of Health-Office of Dietary Supplements (NIH-OIDS), has developed a new material to fill this void.

Methods: Candidate SRM 3951 Vitamin B₁₂ in Human Serum was designed to have three concentration levels of vitamin B₁₂: 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 B₁₂, 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 B₁₂ 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 isotopic-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 B₁₂. The recent availability of ¹³C-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 B₁₂ 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 B₁₂, 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 B₁₂ measurements are accurate and that patients with deficiencies are treated appropriately.

The role of oxidized metabolites of linoleic acid in alcohol-mediated hepatic inflammation and injury in a mouse model of alcoholic liver disease

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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 polysaturated 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-α and 13-αHODEs). 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 knockout (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 deletion 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 deletion 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 Ca²⁺. 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/njury, further supporting an important role for dietary lipids in ALD.

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 urban 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°1’ N, longitude 39°58’1 E), representing one of the holy places highly crowded in Haj 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 16.1/m/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/mL 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/m³ respectively in summer months. While in autumn, the mean concentrations were 0.06, 0.001, 0.002 and 0.01 μg/m³, respectively. The EC8s for Asafrat area, were found to be (1.08x10^4, 7.21x10^4, 4.10x10^4, 6.6x10^6 and 2.4x10^6) 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-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 D3 (D3) 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 D3 (D3) in the second PC, and D2 and the Centaur results are negatively correlated in the third PC. 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).

**Conclusions:**
The data shows a negative bias of the Centaur compared to the LC-MS/MS. The PCA results show a high influence of 25(OH)vitamin D2 (D2) in the second PC, and a negative bias of the Centaur compared to 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**

<table>
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<th>Data Sets</th>
<th>Corr. Coef.</th>
<th>Deming Slope</th>
<th>Deming Intercept</th>
<th>Average Bias</th>
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**A-312**

**The first decade of a global external quality assurance program for serum-based nutritional biomarkers**

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**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% 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 was as follows: 18 retinol, 5 25-hydroxyvitamin D3, 7 C-reactive protein, 9 ferritin, 6 soluble transferrin receptor, 7 vitamin B12, and 7 folate.

**Conclusions:** The number of labs benefiting 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**

X. Fu1, Y. Xu2, T. Vu2, P. Pattengale1, M. O’Gorman1. 1Children’s Hospital Los Angeles, University of Southern California, Los Angeles, CA, 2Children’s Hospital Los Angeles, Los Angeles, CA

**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 (Methanol: 2-propanol, 9:1 v:v) was added and vortexed for 15 seconds, 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.

**Results:** 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 nm. 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 95% 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 µg/mL, 0.28 µg/mL and 0.7 µg/mL 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 µg/mL. For γ-tocopherol, 17y 0.33-4.19 µg/mL.

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 associated with high urinary excretion of mercury in healthy individuals.

R. Hasanat1, W. Tamimi2, Z. Shaikor1.

King Khalid University Hospital, Riyadh, Saudi Arabia, 1King Abdulaziz Medical City, Riyadh, Saudi Arabia

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 simultaneous 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 93/246 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 meal per week (median 1.92 µg/g creatinine; IQR: 2.97). Similarly individuals (174/246) residing in urban areas had higher urinary 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=0.0006). 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 methanol 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 pentfluorobenzyl 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 a-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 ± 4.3] 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 ± 1) 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 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.

Clinical utility of serum folate measurement in tertiary care patients

G. Singh1, V. Singh2, H. Hamdan3. 1Georgia Regents University, Augusta, GA; 2 Moffit Cancer Center, Tampa, FL; 3 Univ of Missouri-Kansas City, Truman Medical Center, Kansas City, MO

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 <3.0ng/mL. Clinical parameters in 128 patients with serum folate levels <3.0ng/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 ≥13.0ng/mL.

**A-318**

<table>
<thead>
<tr>
<th>Serum folate</th>
<th>Georgia</th>
<th>Patients</th>
<th>Samples</th>
<th>Missouri</th>
<th>Patients</th>
<th>Samples</th>
<th>Total</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3.0ng/mL</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>&lt;3.4ng/mL</td>
<td>21</td>
<td>18</td>
<td>18</td>
<td>&lt;5.5ng/mL</td>
</tr>
<tr>
<td>&lt;5.5ng/mL</td>
<td>153</td>
<td>100</td>
<td>90</td>
<td>&lt;25.7ng/mL</td>
<td>304</td>
<td>202</td>
<td>184</td>
<td>&lt;7.0ng/mL</td>
</tr>
</tbody>
</table>

**A-319**

Antioxidant Status of Subjects with Metabolic Syndrome in Port Harcourt, Nigeria

E. P. Odum, C. G. Orluwene, A. A. Ejilemele, V. C. Wakwe. *University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria*

**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 ± 0.28µmol/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 ± 0.28µmol/L, 44.3 ± 7.3µmol/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**

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Subjects(N=100) Mean ± SD</th>
<th>Controls(N=100) Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (µmol/L)</td>
<td>27.5 ± 7.36</td>
<td>44.3 ± 7.30</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Vitamin E (µmol/L)</td>
<td>16.9 ± 4.86</td>
<td>30.8 ± 6.12</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Total Antioxidant Status (µmol/L)</td>
<td>1.2 ± 0.28</td>
<td>1.6 ± 0.28</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

* 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

A. Lee, Y. Kim, S. Joo, K. Lee. *Seoul Medical Science Institute, Seoul, Korea, Republic of*

**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 immunoassays (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 and 20.0-29.9 ng/mL respectively.
Tuesday, July 28, 9:30 am – 5:00 pm

Nutrition/Trace Metals/Vitamins

Fat percentage which did not significantly change in any of the studied groups manifested by lowering the levels of both fetuin A and Hs-CRP, irrespective to body in any group. Conclusion: Ramadan fasting has a potential anti-inflammatory effect.

Ramadan fasting, there was a significant decrease in the body weight of males > 35

Technique) and High sensitive C reactive protein by (ELISA technique) Results: After for determination of; serum Fetuin A by (ELISA technique), serum TNF-α by (ELISA technique) and Hs-CRP by (ELISA technique). 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 before Ramadan and on the 28th day of Ramadan fasting. The following parameters were measured: a) body composition was determined by bioelectrical impedance using InBody-220; the fat mass, skeletal muscle mass, total body weight 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:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Ramadan</th>
<th>After Ramadan</th>
<th>Mean difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetuin A</td>
<td>7.187 ± 0.081</td>
<td>5.355 ± 0.081</td>
<td>-1.832 ± 0.081</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.395 ± 0.012</td>
<td>0.648 ± 0.778</td>
<td>-0.253 ± 0.778</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.196 ± 0.225</td>
<td>0.080 ± 0.625</td>
<td>-0.116 ± 0.625</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>-0.043 ± 0.793</td>
<td>-0.119 ± 0.466</td>
<td>-0.076 ± 0.466</td>
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</tr>
</tbody>
</table>

**A-321**

Ramadan Fasting Reduces Serum Fetuin A And High Sensitive C Reactive Protein Levels

H. M. Demerdash1, N. M. EL-Azhary2, D. M. Samy3, 1Pharos University, Alexandria, Egypt, Alexandria, Egypt, 2Faculty of Medicine-Alexandria University, Egypt, Alexandria, Egypt, 3Faculty of Medicine - Alexandria University, Egypt, Alexandria, Egypt

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 bioelectrical impedance using InBody-220; the fat mass, skeletal muscle mass, total body weight 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.

**A-322**

Simultaneous Determination of Retinol and α-Tocopherol in Serum by Ultra Performance Liquid Chromatography

R. A. Munaier, V. T. Jeronimo, B. S. Dauanny, A. C. M. Vidal, E. Mateo, A. C. S. Ferreira. Hermes Pardini Institute, Vespasiano, Brazil

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.

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°C. 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 Assy Company.

Results: There was a significant difference between the TOS, TAS and NO levels of the groups of lactation days (p=0.480, 0.290, 0.426 respectively), without considering the gestational age. According the post hoc 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

**A-323**

Redox Modulatory Factors In Human Breast Milk

A. TAS1, F. YESILDAL1, T. OZGURTAS1, E. KOC2, H. OZTURK1
1Gulhane Military Medical Academy Department of Medical Biochemistry, Ankara, Turkey, 2Gazi University Faculty of Medicine Department of Pediatrics, Ankara, Turkey

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).

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Nutrition/Trace Metals/Vitamins

Tuesday, July 28, 9:30 am – 5:00 pm

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Performance and Certification of the ADVIA Centaur Vitamin D Total Assay

R. Spears, N. Parker, J. Freeman, K. Wilson, P. Sibley, Siemens Healthcare Dx, Tarrytown, NY

Background: Vitamin D helps regulate calcium in the development and maintenance of healthy bones. The National Institutes of Health of 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 ion Program (VDSC) 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 (VDSC), in which a set of 10 samples was evaluated each quarter against the CDC and University of Ghent Vitamin D3 and D2 Reference Measurement Procedure (RMP). Samples were tested blindly in replicates of four over 2 days, first 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 VDSP 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%. A linear regression of the blinded samples demonstrated a slope of 1.01 and an intercept of -1.89 nmol/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 VDSP 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.

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 AAAnalyst 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-100 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 0.097 / 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:
Altering the metals analyzed, Al is what attracts more attention, by the contamination of sample facility. Factor noticed in a lesser extend 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-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

A-325

The impact plastic tube EDTA K2 metal free in the analysis of trace metals

A. Pulchinelli, D. Duarte Pereira, G. Vicente, V. Reis. Fleury, Sao Paulo, Brazil

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 due to biosecurity issues its use was discontinued.

Table 1: TAS, TOS and NO levels in human breast milk samples on 3rd, 7th and 28th days of lactation.

<table>
<thead>
<tr>
<th></th>
<th>3rd day (Mean ± SD)</th>
<th>7th day (Mean ± SD)</th>
<th>28th day (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (μmol Trolox Eqv/L)</td>
<td>0.944 ± 0.254</td>
<td>0.928 ± 0.272</td>
<td>0.810 ± 0.221</td>
</tr>
<tr>
<td>TOS (μmol H2O2 Eqv/L)</td>
<td>0.673 ± 0.588</td>
<td>1.196 ± 1.121</td>
<td>1.096 ± 0.744</td>
</tr>
<tr>
<td>NO (μM)</td>
<td>0.382 ± 0.098</td>
<td>0.322 ± 0.092</td>
<td>0.282 ± 0.089</td>
</tr>
</tbody>
</table>

Discussion and Conclusion:
Among the metals analyzed, Al is what attracts more attention, by the contamination of sample facility. Factor noticed in a lesser extend 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.
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 whole folate concentration is achieved in
women of reproductive age for prevention of neural tube defects.

Associations between Vitamin D Status and Serum Cardiovascular Risk Markers in an Adult Brazilian Population.

M. F. M. C. Pinheiro, O. V. P. Denardin, M. C. Freire, T. S. P. Souza, M. L. L. Moreira, S. V. L. Argolo. DASA, Rio de Janeiro, Brazil

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-I 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 lipid profiles values (p<0.01) as well as for apolipoprotein A-I levels (p<0.01). Results were not able to show correlation with apolipoprotein B and homocysteine. Conclusions: Our data support the literature wherein 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 lipid profile and ApoA-1, markers that contribute to cardiovascular diseases, suggesting that Vitamin D may be important in maintaining cardiovascular health.

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 the possible 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 Acuity 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 Acuity UPLC BEH C18 1.7 μm 2.1x50 mm column kept under thermostatisation at 30 °C. The eluate absorbance was monitored at 453 nm to quantify β-carotene. The chromographic 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 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.

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 and 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.
The Inhibitory Effect of DENND1B Overexpression on the Release of Cytokine IL-4 in a Murine Ovalbumin-induced Asthma Model


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 C57BL/6 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma IgE (μg/mL)</th>
<th>BALF Leucocyte</th>
<th>IL-4 (pg/mL)</th>
<th>IL-5 (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
<th>TNF-α (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>12.4±0.3</td>
<td>133±7.5</td>
<td>184±5.4</td>
<td>215±7.2</td>
<td>170±5.2</td>
<td>186±5.7</td>
</tr>
<tr>
<td>OVA</td>
<td>14.5±0.6</td>
<td>148±7.4</td>
<td>204±6.7</td>
<td>235±8.9</td>
<td>190±6.8</td>
<td>205±7.3</td>
</tr>
<tr>
<td>OVA + Empty vector</td>
<td>16.8±0.7</td>
<td>165±8.2</td>
<td>221±8.5</td>
<td>255±9.2</td>
<td>205±7.3</td>
<td>225±8.5</td>
</tr>
<tr>
<td>OVA + DENND1B vector</td>
<td>10.5±0.3</td>
<td>115±6.7</td>
<td>150±5.2</td>
<td>175±6.8</td>
<td>165±6.7</td>
<td>185±7.3</td>
</tr>
</tbody>
</table>

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

J. Kirpich, W. Feng, C. McClain. University of Louisville, Louisville, KY

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 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 ilealine 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 EIOH 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 EIOH-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 EIOH) 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 EIOH feeding significant liver injury and steatosis were observed in USF+EIOH compared to pair-fed group. These effects of EIOH were blunted by SF diet containing MCT. Serum ALT levels, as a marker of liver injury, was significantly higher (p<0.05) in USF+EIOH group (44.9±1.2 to 84.2±1.8 IU/L) compared to SF+EIOH fed animals (27.2±1.9 to 92.1±2.4 IU/L). Hepatic triglyceride content was also higher in USF+EIOH compared to SF+EIOH fed animals (100.2±8.1 vs 68.7±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 inflammatory 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

1Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey, 2Turgut Ozal University, Medical Faculty, Ankara, Turkey.

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 Trichrome), there were seen periglomerular fibrosis, segmental sclerosis and thin interstitial fibrosis in the 15 min IR group. Also, when compared to 15 min IR group; a marked fibrosis in glomerules periglomerular fibrosis, segmental sclerosis and thin interstitial fibrosis in the 45 min IR group. Also, when compared to 15 min IR group; a marked fibrosis in glomerules periglomerular fibrosis, segmental sclerosis and thin interstitial fibrosis in the 45 min IR group. Additionally, connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), α-SMA, and HIF-1α tissue expressions were examined through IHC method. Histological staining revealed that there were remarkably a higher number of vacuoles in the kidney of RNaseL deficient mice than in wild type mice although the biological significance of the observation is largely unknown. Using Western blot 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 regulates the shedding of EGF precursor through inhibiting some specific proteases responsible for the event.

Conclusion: 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.

A-338

A comparison of the Jaffe creatinine and Enzymatic creatinine in pre-clinical species


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 pre-clinical species.

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) / EGRF 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 microbial infection and cell proliferation, mediated EGF/EGRF 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 Western blot 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 regulates the shedding of EGF precursor through inhibiting some specific proteases responsible for the event.

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A-337

Role of RNase L in Kidney

N. Alghamdi, Q. Zheng, D. Liu, A. Zhou. cleveland state university, Cleveland, OH.

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Conclusions: 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.
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**

F. Armutcu¹, M. Namuslu¹, U. Yıldırım², M. Yağmurca³, K. Demircan².

1Galatasaray University, Cerrahpasa Medical Faculty, Istanbul, Turkey,
2Turgut Ozal University, Medical Faculty, Ankara, Turkey

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 visualized in the renal tissue glomerular and tubulointerstitial areas of rats with ADR-induced nephropathy. 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**


University of Texas Medical Branch - Galveston, Galveston, TX

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 cardiac 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 **objectives** 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 myocardiits 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.
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) 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 significant.

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.
Possible target genes and pathways that CTCF regulated. Results: CTCF expression in MCIF-10A was the highest, and decreased gradually in MCIF7, 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-346
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 sensitivity 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 compare we mutant cross-reactivity to the Qiagen Therascreen assay.

Material and Methods: PASS primers consist of target specific regions separated by a universal 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.

A-347
Prognostic biomarker of the combination of TROY and LGR5 in patients with colorectal cancer

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Background: Wnt signalling 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 (TROY), which is expressed in the majority of advanced colorectal tumors. TROY LGR5 marks stem cells in multiple adult tissues and cancers including CRC, and TROY is produced in fast-cycling 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.

A-348
Determination of Reference Intervals for Catecholamine and Metanephrine Excretion Using Archived Patient Data

K. Doyle1, E. L. Frank1. 1University of Utah, Salt Lake City, UT, 2ARUP Laboratories, University of Utah, Salt Lake City, UT

Objective: Fractionated urinary metanephrines and catecholamines are measured for diagnosing and monitoring treatment of pheochromocytoma tumors and used infrequently to investigate dysautonomia. 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.

<table>
<thead>
<tr>
<th>Catecholamines</th>
<th>Age</th>
<th>RI</th>
<th>Metanephrines</th>
<th>Age</th>
<th>Gender</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(y)</td>
<td>(ug/d)</td>
<td></td>
<td>(y)</td>
<td>(ug/d)</td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>4-6</td>
<td>95 - 221</td>
<td>Metanephrine</td>
<td>1-7</td>
<td>F</td>
<td>42 - 135</td>
</tr>
<tr>
<td>7-12</td>
<td>76 - 371</td>
<td>≥18</td>
<td>39 - 143</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13-17</td>
<td>137 - 393</td>
<td>7-12</td>
<td>M</td>
<td>45 - 179</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-69</td>
<td>57 - 324</td>
<td>13-17</td>
<td>81 - 202</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥70</td>
<td>56 - 272</td>
<td>≥70</td>
<td>62 - 207</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td>4-7</td>
<td>1 - 9</td>
<td>Normetanephrine</td>
<td>7-12</td>
<td>F</td>
<td>32 - 247</td>
</tr>
<tr>
<td>16-69</td>
<td>4-7</td>
<td>1 - 7</td>
<td>13-17</td>
<td>73 - 266</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥70</td>
<td>1 - 5</td>
<td>≥70</td>
<td>109 - 393</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>4-12</td>
<td>6 - 45</td>
<td>7-12</td>
<td>M</td>
<td>70 - 273</td>
<td></td>
</tr>
<tr>
<td>13-17</td>
<td>15 - 57</td>
<td>13-17</td>
<td>92 - 312</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-69</td>
<td>16 - 71</td>
<td>18-29</td>
<td>95 - 379</td>
<td></td>
<td></td>
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</tr>
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<td>≥70</td>
<td>11 - 60</td>
<td>≥70</td>
<td>125 - 510</td>
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</tr>
</tbody>
</table>

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.
Cancer/Tumor Markers

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-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 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: 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: 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 + 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.

Tuesday, July 28, 9:30 am – 5:00 pm


**Tuesday, July 28, 9:30 am – 5:00 pm**

**Cancer/Tumor Markers**

**A-353**

**Value of dFLC data in monitoring response to therapy in a patient with Primary Amyloidosis**

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**Background:** Recent evidence has 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=6235 ng/L, Bence Jones Proteinuria (BJP) positive for free kappa (16.8 mg/L), 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 dFLC 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/L. 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/L. 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 / 3.71) 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.

**A-354**

**Verification and Validation of LUMIPULSE G CA125II Assay under Development**


**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'-spiroadamanate)-4-methoxy-4-(3"-phosphoryloxyphe)nyl-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 non-spiked samples. No high dosehook 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.

**A-358**

**Validation of Fluorescence In Situ Hybridization assay for detection of TP53 gene deletion**


**Background:** Fluorescence in Situ Hybridization (FISH) provides an important adjunct to conventional cytogentic 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 TP53 probe deletion 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 specificity and sensitivity. 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 TP53 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 we identified six atypical signal patterns: 2G1R, 1G1R, 3G2R, 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, 1G1R, 3G2R, 3G3R, 4G4R. 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, 1G1R, 3G2R, 3G3R, 4G4R. The clinical validity of FISH should showed presence of the TP53 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.
A familial adenomatous polyposis case with a novel (c.4091delG; p.Ser1364Metfs*51) mutation

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1Hermes Pardini Institute, Vespasiano, Brazil, 2Hermes Pardini Institute (Progenética), Vespasiano, Brazil, 3Hermes Pardini Institute (Progenética), Vespasiano, Brazil

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited colon cancer syndrome caused by mutations in the adenomatous polyposis coli (APC) gene (OMIM#175170). 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 than 1315 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 aunt 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 intrinsic 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_000083.5). 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://doros.charité.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.

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.

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 also the 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 genes 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 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 mutations were detected in four patients and 8 patients presented variants of unknown clinical significance (VUS) in one or more genes. We considered as VUS undescribed 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 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 mutation was detected in four patients and 8 patients presented variants of unknown clinical significance (VUS) in one or more genes.

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.

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 cytoplogic 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 parapneumonic, 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/ were MPE.

Conclusions: HCYS levels in pleural fluid showed high diagnostic efficacy to predict whether a pleural effusion is benign or malignant.

Measurement of Human Epididymis Protein 4 (HE4) may be Closely Associated with Breast Cancer Progression

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Background: Human epidymis 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 using EIA (Enzyme Linked Immunosorbent Assay). Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC) using the software MEDCALC®.

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 15.6, 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.
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**

J. L. García de Veas Silva, T. De Haro Muñoz, L. Jaimez Gamiz, J. V. García Lario, V. Fernandez Varela. Complejo Hospitalario Universitario de Granada, Granada, Spain

**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 in 1%-5% 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 defines 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.

<table>
<thead>
<tr>
<th>Paraneoplastic neurological syndromes, onconeural antibodies and tumors associated</th>
<th>Onconeural antibodies</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Paraneoplastic neurological syndromes</th>
<th>Tumor associated</th>
<th>Survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-Hu</td>
<td>Male</td>
<td>67</td>
<td>Paraneoplastic encephalitis</td>
<td>Lung adenocarcinoma</td>
<td>19 months (still alive)</td>
<td></td>
</tr>
<tr>
<td>anti-Hu</td>
<td>Female</td>
<td>50</td>
<td>Paraneoplastic encephalitis</td>
<td>Small cell lung cancer</td>
<td>7 months (exitus)</td>
<td></td>
</tr>
<tr>
<td>anti-Ma-2</td>
<td>Female</td>
<td>65</td>
<td>Acute cognitive impairment</td>
<td>Breast cancer</td>
<td>2 months (exitus)</td>
<td></td>
</tr>
<tr>
<td>anti-amphiphysin</td>
<td>Male</td>
<td>79</td>
<td>Limbic encephalitis</td>
<td>Squamous cell lung carcinoma</td>
<td>2 months (exitus)</td>
<td></td>
</tr>
</tbody>
</table>

**A-365**

**A quick protocol for the identification of Multiple Myeloma in patients attending Emergency Services with severe bone pain**

J. L. García de Veas Silva, T. De Haro Muñoz, R. Rios Tamayo, J. V. García Lario, P. Navarro Álvarez. Complejo Hospitalario Universitario de Granada, Granada, Spain

**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, vertebral 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 I (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%, PP of 94% and NPV 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.
Utilization of specific intact human choric 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 (HCGC) 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 iHCG >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 samples analyzed for iHCG during the time period. Of them, 32 had iHCG >14 mIU/mL, and 25 between 5-14 mIU/mL. Of the 32 with >14 mIU/mL, iHCG, 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 iHCG (iHCG of 0.7 and <0.5, and iHCG 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 bHCG 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 at 14 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.

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

D. C. C. Lin1, D. Colantonio2. 1University of Toronto, Toronto, ON, Canada, 2Hospital for Sick Children, Toronto, ON, Canada

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 µg/L for AFP, below conventionally used diagnostic cut-off values for CNS germ cell tumors of 5 IU/L and 10 µg/L. Linearity was confirmed from 2.8 to 27.8 IU/L for beta-hCG and 2.4 to 45.2 µg/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 0.05 µg/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.

Cancer/Tumor Markers

Tuesday, July 28, 9:30 am – 5:00 pm

S115
Cancer/Tumor Markers

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 K2EDTA or Streck Cell-Free DNA blood collection tube that contained blood that was then 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 ifCs and a ratio of ifCs between the 100 and 200 groups 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 -0.045, -0.0129, -0.084 and -0.0154. 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 -0.323 and -0.51. 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 quality with reported QC ratios of -0.023 and -0.051. Of the 4 cells determined to have poor quality DNA, only 2 (33%) were found to be of high quality with QC ratios of -0.045, -0.0129, -0.084 and -0.0154. Of the 4 cells determined to have low quality scores, the QC ratios were reported as 0.056 or indeterminate.

Use of Prostate-Specific Antigen Test in National Samples of Privately-Insured, Medicare and Medicaid Patients, 2007-2012
S. Shahangian. CDC, Atlanta, GA

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 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 preserving patient timeline 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 rates 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 insured (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
Background: To evaluate Chromogramin A assay on the BRAHMS KRYPTOR COMPACT PLUS analyzer and compare with the current manual ELISA assay.

Methods: We evaluated the Chromogramin A assay based on its performance in the precision, 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: On the 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 was 10.7µg/L. Comparison between the two methods showed 91.5 % concordance for test results.

Conclusion: The performance of the automated Chromogramin 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.

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)

J. H. Rim¹, J. Yoo¹. 'Yonsei University College of Medicine, Severance Hospital, Seoul, Korea, Republic of. 'National Health Insurance Service Ilsan Hospital, Goyang, Korea, Republic of

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 obliged 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 result of FOBT ranged from 0.0 % 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 63.3 %, 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.
Tuesday, July 28, 9:30 am – 5:00 pm

**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-lactate 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% (ranged 5.8-9.3%) at concentrations of 29.71, 60.85 and 119.4 mg/dL. The imprecision for HP also produced CV of <10% (range 5.7-8.0%) at concentrations of 78.6, 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 (r²=0.978) (SAA) and y = 1.013x-3.205 (r²=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 coefficient 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 were analyzed. The FREND-LC Fluorescence Immunoassay for Quantitation of SAA and Haptoglobin in Lung Cancer performances of the FREND™-LC assay.

**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 MIKE/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.**

U. Ray1, S. Bank2, P. Jana3, P. Jana2, S. Bhattacharyya1, A. Sinha1, Y. Byron1, P. Roberts-Thomson1, P. Roberts-Thomson1, 1Royal Hobart Hospital, Tasmania, Hobart, Australia, 2Sinha Institute of Medical Sciences & Technology, Calcutta, India

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)]. Triglycerid, 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)throughout 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. Triglycerid, 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 Triglycerid, 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**

Q. Zheng, N. Alighamdi, D. Liu, A. Zhou. Cleveland State University, Cleveland, OH

**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**

N. ILHAN, H. GUNGOR, N. ILHAN, F. H. GUL. Firat University Medical Faculty, Elazig, Turkey

**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
**A-383**

**Comparison of K2EDTA Tubes and a Specialized Tube to Stabilize RNA for Rare Single Cell Gene Expression Analysis**

L. Strotman1, L. Milliner1, K. Goudy2, R. Valdes3, M. Linder1, 1University of Louisville, PGXL Technologies, Louisville, KY, 2PGXL Technologies, Louisville, KY

**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 GAPDH 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 ±2SD of the mean Ct value for xenorRNA expression (28.5±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**

S. Vardanyan1, E. Sanchez2, M. Li1, C. Wang1, A. Gillespie1, M. Spitzer1, A. Shvartsur1, N. M. Harutyunyan1, G. Garzo1, G. Tang1, J. Said1, H. Chen1, J. R. Berneson1, 1Institute for Myeloma & Bone Cancer Research, West Hollywood, CA, 2Geffen School of Medicine at the University of California at Los Angeles, Los Angeles, CA

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 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=0.003, respectively). Untreated WM patients (n=9) also had significantly higher BCMA levels than healthy individuals (n=14; p=0.001). 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.
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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**

R. Bray, P. Collinson, P. Lim, A. Prasad, S. Firoozi, A. Ntalianis. St. George’s Hospital, LONDON, United Kingdom

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 or sex, 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**

A. H. Wu1, J. Estis2, P. Helestine3, K. Bui4, J. Todd5, P. Kavask6. 1University of California, San Francisco, San Francisco, CA, 2Singulex, Inc., Alameda, CA, 3McMaster University, Hamilton, ON, 4University of Eastern Finland, Kuopio, Finland, 5Helsinki University Central Hospital, Helsinki, Finland, 6University of Zagreb, Zagreb, Croatia, 7Regionale Kliniken Holding RKH GmbH, Ludwigshurg, Germany, 8University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom, 9Haukeland University Hospital, Bergen, Norway, 10AZ St-Jan Hospital Bruges, Bruges, Belgium, 11Clinical Center of Serbia, Belgrade, Serbia

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 studying 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.

**B-003**

**Changes in the utilization of evidence-based recommendations for the biochemical diagnosis of acute myocardial infarction.**

P.O. Collinson1, A. Hammerer-Lercher2, M. van Dieijen-Visser3, K. Pulkki4, J. Suvisaari5, A. Staviljen-Rukavina6, H. Baum7, C. Duf8, A. Aakre9, M. Langlois10, S. Stankovic11, P. Laitinen12. St George’s Hospital, London, United Kingdom, 2University Hospital Innsbruck, Innsbruck, Austria, 3Maastricht University Medical Center, Maastricht, Netherlands, 4University of Eastern Finland, Kuopio, Finland, 5Helsinki University Central Hospital, Helsinki, Finland, 6University of Zagreb, Zagreb, Croatia, 7Regional Kliniken Holding RKH GmbH, Ludwigshurg, Germany, 8University Hospital of North Staffordshire, Stoke-on-Trent, United Kingdom, 9Haukeland University Hospital, Bergen, Norway, 10AZ St-Jan Hospital Bruges, Bruges, Belgium, 11Clinical Center of Serbia, Belgrade, Serbia

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 (Chi2 2.83 ns).

An increase in use of the 99th percentile from (35.4% 2006, 37.9% in 2010, 52.1% in 2013) Chi2 89.4, p<0.0001. In acute units in 2013, there was a significant 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) Chi2 89.4, p<0.0001.
Cardiac Markers

B-005

Association of Blood Homocysteine levels with Subclinical Coronary Atherosclerosis in Asymptomatic subjects


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, 1,594 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 CAC scores 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) 3.900 (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

A. H. Wu1, J. Estis2, P. Heselton2, T. Dang2, J. Todd2, P. Kavkač2. 1University of California, San Francisco, San Francisco, CA, 2Singulex, Inc., Alameda, CA, 3McMaster University, Hamilton, ON, Canada.

Background: Endothelin (ET-1, (a1a-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.
Cardiac Markers

<table>
<thead>
<tr>
<th>Endothelin results</th>
<th>Median, pg/mL</th>
<th>95% CI, pg/mL</th>
<th>99th percentile, pg/mL</th>
<th>%&lt;95th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire cohort</td>
<td>2.4</td>
<td>3.9</td>
<td>5.5</td>
<td>5.9</td>
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<tr>
<td>Females</td>
<td>2.4</td>
<td>3.8</td>
<td>5.4</td>
<td>5.4</td>
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<tr>
<td>Males</td>
<td>2.5</td>
<td>4.0</td>
<td>5.9</td>
<td>6.6</td>
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<td>&lt;=50 y</td>
<td>2.2</td>
<td>3.4</td>
<td>4.7</td>
<td>2.1</td>
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<tr>
<td>HbA1c &lt;5.9%</td>
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<td>3.8</td>
<td>5.4</td>
<td>5.2</td>
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<td>HbA1c &gt;=5.9%</td>
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<td>4.4</td>
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<tr>
<td>LDL &lt;= 129 mg/dL</td>
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<td>4.0</td>
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<tr>
<td>LDL &gt; 129 mg/dL</td>
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<td>3.7</td>
<td>5.4</td>
<td>4.9</td>
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<tr>
<td>HDL at target†</td>
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<td>3.8</td>
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<td>&lt;=45 mg/dL</td>
<td>&gt;=35 mg/dL</td>
<td>p&lt;0.001</td>
<td>&gt;P=0.05</td>
</tr>
</tbody>
</table>

**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.


**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), returns were obtained from 72% (E) and 39.5% (NA). Of those markers no longer recommended, in Europe 91 responses (63.7% Central or University hospitals, 19.8% community hospitals) and in North America 177 responses (65.2% community hospitals, 34.8% from local universities). From Europe 17% (E) and 46.8% (NA) were from academic departments. In Europe 72% (E) and 55.5% (NA) were from academic institutions.

There were significant differences in the choice of decision limits and their derivations. Decision limits (E vs NA) were: 80% 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, adoption 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


**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 subjected to magnetic separation and the assay is based on the addition of the mixture 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. A 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 ≥ 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 gamma globulin, 5 mg/dL creatinine, 5 mg/L whole blood lystate, 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-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 hypotension. Changes in gene expression of cardiovascular pathways by micro RNAs (miRNA) have been identified as potential biomarkers of CVD. miRNAs 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-012
Retrospective analysis of 160,000 BNPs shows that initially low NT-pro BNP is highly predictive of negative NT-proBNP
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Background:
Low BNP or 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 NT-proBNP.

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 €.

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 analysis 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 rapid concentration decreases (t1/2 < 5 hours; %Δ[cTnI] ≤ -20%), 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 rapid concentration decreases (t1/2 < 5 hours; %Δ[cTnI] ≤ -20%), we sought to quantify the frequency of false-positive cTnI results and develop strategies to prevent them.

Conclusions: Retroactive 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.
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, HFrEF, nor HFpEF; nor were there significant differences between controls, HFrEF, and HFpEF. 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 HFrEF, 6 HFpEF), 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.

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 and hs-cTnT assays. Results: Unadjusted hazard ratios by quintiles for both hs-cTnI and hs-cTnT by vintage were significantly more predictive of death compared to hs-cTnI in vintage <1 year (p <0.0001) and trending at p=0.07 at ≥1 y. 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.

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 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 measurement 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, homoglobin 1 mg/mL demonstrated no interference. Similarly, analysis of drugs at high/low concentrations in mmol/mL, including acetaminophen 33/1324, aspirin 720/3600, atorvastatin 2/20, Diphenhydramine 2/20, fenofibrate 42/125, lisonopril 0.25/0.74, niacin 480/4800, tolnbutamide 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 0.999-1.0, 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.
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 99.9th percentile was used in 198 (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. The decision limit used varied from 2 ng/L to 700 ng/L with peaks of utilisation at 14 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 at 30 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.
Cardiac Markers

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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 almost completely 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 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.
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- and 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 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 (3.9-9.2). 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 inter-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 variability 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, LDL, ApoA1, ApoB, and Lp(a), 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.

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 tool for early detection and monitoring of cardiovascular disease. With growing

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 &8496]]-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.

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.

Development of an Enhanced Chemiluminescent High Sensitivity Troponin I assay* on VITROS® 5600 Integrated and VITROS® 3600 and EC/ECIQ Immunodiagnostic Systems


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 LoD, 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 cTnI, the 99th percentile was 23 pg/mL. Correlations of the VITROS® High Sensitivity Troponin I assay and both a commercially available high sensitivity assay and the VITROS® Troponin I assay were obtained using 111 patient samples from a variety of clinical categories. The comparison of VITROS® Troponin I assay and both a commercially available high sensitivity assay and the VITROS® Troponin I assay were obtained using 111 patient samples from a variety of clinical categories. The comparison of VITROS® Troponin I and VITROS® High Sensitivity Troponin I resulted in a slope of 1.00 pg/mL, an intercept of -20 pg/mL and a R of 0.99.

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.
Relative contribution of high sensitivity cardiac Troponins I and T in cardiovascular risk stratification in patients with OS 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/m² 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 AccuTnDx (Access/Beckman Counter) with detection limit 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 of ferences.

**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 no 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 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.

**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 70 subjects (males and females) in the age group of 30-70 years 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 (m²). Two ml of fasting sample collected in plain vacutainers and serum was separated and stored at -70ºC 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 the hip ratio of 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-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 MÉRIEUX, 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 pairs proportions of BNP and Gal-3. The tests were 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.
Characteristics of the New Beckman Coulter High-Sensitive Cardiac Troponin I Assay (hfTnI)


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 tnl 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/dl albumin, 1000 mg/dl fibrinogen, 28.8 U/m 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.

Increased Incidence of Cardiac Troponin I Abnormalities in Women Utilizing a High Sensitivity Assay


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® or 2000®). 99th percentiles were as follows: contemporary cTnI 30 ng/L (0.030 μg/L); hs-cTnI 16 ng/L for males and 236 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 than 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.

Validation of lithium heparin tube for Cardiac markers in a Clinical Laboratory and its benefit

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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
Cardiac Markers

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 (EL A), 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 uptake 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 sexes with premature coronary disease in period between 2010-2015. We measured high-sensitivity C-Reactive Protein, homocysteine and lipoprotein (a). Framingham 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) μg/mL 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.
Tuesday, July 28, 9:30 am – 5:00 pm

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 ERENNIA® 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, which is supported by 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. The assay was linear throughout the reportable range, which extends to 5000 pg/mL, and there is no high dose hook effect up to 100,000 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).

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-039

Validation and Correlation study of the Values for the Beckman cTnI and TnI+3 Assay on the DxI 800 and Access-2 Analyzers.


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 Immununoassay analyzer in October 2010, Beckman Coulter (BC) recently reintroduced 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 cTn 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). Linearity scores were performed using BC calibrators.

Results:

Table. 1

<table>
<thead>
<tr>
<th>Analyzers</th>
<th>No.</th>
<th>Mean x/y</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access-2 ED/ML</td>
<td>109</td>
<td>0.117/0.108</td>
<td>0.981</td>
<td>-0.008</td>
<td>0.9939</td>
</tr>
<tr>
<td>Access-2 ED/DxI800</td>
<td>115</td>
<td>0.120/0.117</td>
<td>0.791</td>
<td>0.015</td>
<td>0.9776</td>
</tr>
<tr>
<td>Access-2ML/DxI800 ML</td>
<td>115</td>
<td>0.131/0.111</td>
<td>0.735</td>
<td>0.069</td>
<td>0.9589</td>
</tr>
</tbody>
</table>

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.
Evaluating the accuracy of total hemoglobin (tHb) measurements is important for patient care management. 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. Different 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.

Background: The 2014 Ebola epidemic, the largest in history, revealed the need of inter-instrument harmonization of tHb results. tHb methods with calibration traceable to the CNmetHb reference procedure support interval of ± 0.59 g/dL. 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 residual whole blood from heparinized whole blood samples (n=110) sent to the lab for routine tHb testing.

Methods: The evaluation study included precision, interference, linearity, and correlation with a commercially available Cyanmethemoglobin Reference Procedure and a Hospital Lab Reference Method. The study included precision, interference, linearity, and correlation with a commercially available Cyanmethemoglobin Reference Procedure and a Hospital Lab Reference Method.

Results: Linear regression analyses of tHb results yielded the following equations: [GEM Premier 4000] = 1.001[CNmetHb] + 0.0497, (r² = 0.9993, Range 3-21 g/dL); [GEM Premier 4000] = 0.9608[ABL 800] + 0.4885, (r² = 0.9688, 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: 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 advantages of having a small bench-top footprint, requiring a small sample volume in a self-contained reagent disc. The complete test menu comprises of 31 different tests divided among 16 different panels. Methods: 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 MeLac 12 ALB (ABL, BU, Ca, Cl−, CRE, GLU, K+, LAC, Mg, Na+, Phos, iCO2) 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 interval-precision accuracy for all assays varied from <0.01-16.4% CV for the results and high QC material. The correlation 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; iCO2: 11-39 mmol/L; ALP: 20-1758 U/L; ALT: 17-140 U/L; AST: 16-1558 U/L; DBIL: 0.5-15.4 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), iCO2 (y = 1.20x - 2.12), ALP (y = 0.85x + 0.16), ALT (y = 0.89x + 5.33), AST (y = 0.93x - 8.80), and DBIL (y = 0.46x + 0.16). The calculated among gap using the Piccolo Xpress values was significantly different from the laboratory mainly due the positive biases in Ca²⁺ and iCO2. The reference ranges for ALB, Cl−, iCO2, 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 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.
Tuesday, July 28, 9:30 am – 5:00 pm

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 ≤ 9.9% (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 fructoseamine 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 Fructoseamine 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® CI- Slide Assay∗

S. J. Danielson, T. Grupp, J. Ramerman. Ortho Clinical Diagnostics, Rochester, NY

Background: * The use of the VITROS CI- Slide with urine specimens is in development

VITROS Chemistry Products CI 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 CI 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

VITROS 5,1 FS System = 1.001* ADVIA 1800 + 1.66; (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 two commercial methods: titration using a Corning 9265 Chloridometer and indirect potentiometry with the Chloride assay on the Siemens’ ADVIA 1800 Chemistry System.

Results: The VITROS CI Slides assay showed excellent correlation with both methods. VITROS 5,1 FS System = 0.999* Corning 9265 + 3.08; (r) = 0.999 and VITROS 5,1 FS System = 1.001* ADVIA 1800 + 1.66; (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 Siemens’ 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 dilute 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 CI 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

S. Bae, D. Ko, E. Cho, T. Jeong, W. Lee, S. Chun, W. Min. Asan Medical Center, Seoul, Korea, Republic of

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 0.790, 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 0.985 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 and precision. 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 (σb) 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: γb,analytic = σb2 + σanalytic2

Using an equivalent equation for the Radiometer and simple algebra, the imprecision of the two GEM’s can be calculated from: σGEM = σb2 + σanalytic2

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 points grouped into 2 hr intervals. The Table compares the directly measured saGEM, the calculated sGEM and corresponding sigmas, calculated from (biologic variation)3 (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.
High Throughput Immunoassay for Kidney Function Biomarker
Symmetric Dimethylarginine (SDMA)


Symmetric dimethylarginine (SDMA) is a dimethylated derivative of arginine that results from intranuclear 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 351 canine 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:

<table>
<thead>
<tr>
<th>Test</th>
<th>SDMA assayed</th>
<th>SDMA GEM</th>
<th>Sigma ABL</th>
<th>Sigma GEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride, mmol/L</td>
<td>0.44</td>
<td>0.52</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>0.11</td>
<td>0.41</td>
<td>4.7</td>
<td>1.3</td>
</tr>
<tr>
<td>HCO₃, mmol/L</td>
<td>0.22</td>
<td>0.60</td>
<td>3.6</td>
<td>1.3</td>
</tr>
<tr>
<td>ionized Ca, mmol/L</td>
<td>0.0042</td>
<td>0.033</td>
<td>3.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>0.0244</td>
<td>0.044</td>
<td>7.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>0.38</td>
<td>0.77</td>
<td>2.7</td>
<td>1.7</td>
</tr>
<tr>
<td>pCO₂, mmHg</td>
<td>0.34</td>
<td>0.40</td>
<td>6.6</td>
<td>5.6</td>
</tr>
<tr>
<td>pH</td>
<td>0.00148</td>
<td>0.029</td>
<td>15.0</td>
<td>0.8</td>
</tr>
<tr>
<td>pO₂, mmHg</td>
<td>1.36</td>
<td>5.34</td>
<td>9.9</td>
<td>2.5</td>
</tr>
</tbody>
</table>

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.

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 (AIC_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.

Validation of a Novel High Throughput Immunoassay for the Quantitation of Symmetric Dimethylarginine (SDMA)


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.
The method comparison table below shows Weighted Deming fit against the ADVIA 2400 Clinical Chemistry System.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Matrix</th>
<th>n</th>
<th>Slope</th>
<th>Intercept</th>
<th>r</th>
<th>Sy.x</th>
<th>Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIC_3</td>
<td>Whole Blood</td>
<td>75</td>
<td>1.00</td>
<td>0.13</td>
<td>0.990</td>
<td>0.030</td>
<td>4.50-12.80</td>
<td>%</td>
</tr>
<tr>
<td>CA_2</td>
<td>Serum</td>
<td>130</td>
<td>0.99</td>
<td>-0.19</td>
<td>0.999</td>
<td>0.021</td>
<td>1.49-15.22</td>
<td>mg/dL</td>
</tr>
<tr>
<td>CA_2</td>
<td>Urine</td>
<td>102</td>
<td>0.98</td>
<td>-0.17</td>
<td>0.999</td>
<td>0.031</td>
<td>2.16-29.08</td>
<td>mg/dL</td>
</tr>
<tr>
<td>CHOL_2</td>
<td>Serum</td>
<td>99</td>
<td>0.98</td>
<td>0.61</td>
<td>0.997</td>
<td>0.033</td>
<td>28-549</td>
<td>mg/dL</td>
</tr>
<tr>
<td>CREA_2</td>
<td>Urine</td>
<td>142</td>
<td>1.05</td>
<td>-0.05</td>
<td>0.998</td>
<td>0.040</td>
<td>3.47-290.92</td>
<td>mg/dL</td>
</tr>
<tr>
<td>GLUH_3</td>
<td>Serum</td>
<td>97</td>
<td>0.99</td>
<td>-0.55</td>
<td>0.999</td>
<td>0.032</td>
<td>43-659</td>
<td>mg/dL</td>
</tr>
<tr>
<td>GLUH_3</td>
<td>CSF</td>
<td>104</td>
<td>0.98</td>
<td>-0.55</td>
<td>1.000</td>
<td>0.019</td>
<td>16-620</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>

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-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 certified value for reference materials.

Objective:
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 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 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.
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 r² > 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.

B-053
Performance of the NephroCheck® for VITROS® Test** on the VITROS® 3600 Immunodiagnostic System


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 mg/mL for IGFBP-7 yielding an AKIRISK™ Score range of 0.0235 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 0.23 ng/mL for TIMP-2 and 1.994 ng/mL for IGFBP-7 resulting in LoD and LoQ for the AKIRISK™ Score of 2.8x10⁻⁸ 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 20.0 ng/mL resulted in within-laboratory %CV of 5.8%, 6.6%, 7.5%, and 8.0% respectively.

B-054
Asymptomatic severe hypophosphataemia in acute T-cell lymphoblastic leukaemia

N. Zakaria, P. Sihaneshwar, H. Shanmugam. University of Malaya, Kuala Lumpur, Malaysia

Introduction:
Hypophosphatemia is a metabolic disorder that is commonly encountered in critically ill patients. Hypophosphatemia is defined as plasma phosphate level below 0.80 mmol per litre (mmol/L), and can be further divided into subgroups of 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 than 0.32 mmol/L). Phosphate has many roles in physiological functions, thus the depletion of serum phosphate could lead to impairment in multiple organ systems, which include respiratory system, cardiovascular system, 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 hypophosphataemia in a child with acute 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 anaemia and increased total white cell count of 183 x 10⁶/L. His peripheral blood picture showed numerous blast cells. Bone marrow examination and immunophenotyping confirmed 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 regimen 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-CL/MS

H. Nah, J. Won, S. Lee, J. Kim. Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, Republic of

Background:
Serum creatinine is measured in order to estimate glomerular filtration rate. For the measurement of creatinine, alkaline pircate 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 from the problem of bilirubin interference, reagent based on the enzymatic result can be alternative assay. The aim of this study is to compare enzymatic and kinetic Jaffe methods with isotope dilution-liquid chromatography mass spectrometry (ID-CL/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 DxC800i (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 kinetic 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.

Assessment of serum indices implementation on ADVIA Chemistry 2400 System

L. K. Tirado, C. F. A. Pereira, N. F. da Cruz. DASA, Bauru, Brazil

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 interferences in a short time and with a high quality score.

Electrolytes/Blood Gas/Metabolites

Evaluation of Electrolyte Performance on the Siemens ADVIA Chemistry XPT System
H. Mindicino1, D. Kaminski2, C. Conarpe1, D. Desai1, M. Healy1. ‘Siemens Healthcare Diagnostics, Tarrytown, NY, ’Siemens Healthcare Diagnostics, Glasgow, DE

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.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Matrix</th>
<th>n</th>
<th>Slope</th>
<th>Y-intercept</th>
<th>r</th>
<th>Syx</th>
<th>Range (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>Serum</td>
<td>117</td>
<td>1.01</td>
<td>0.7</td>
<td>0.998</td>
<td>0.015</td>
<td>50-191</td>
</tr>
<tr>
<td>Cl</td>
<td>Urine</td>
<td>137</td>
<td>0.99</td>
<td>2.8</td>
<td>0.999</td>
<td>0.035</td>
<td>15-375</td>
</tr>
<tr>
<td>Na</td>
<td>Serum</td>
<td>102</td>
<td>0.98</td>
<td>2.4</td>
<td>0.996</td>
<td>0.012</td>
<td>101-196</td>
</tr>
<tr>
<td>Na</td>
<td>Urine</td>
<td>139</td>
<td>0.96</td>
<td>4.13</td>
<td>0.999</td>
<td>0.036</td>
<td>10-377</td>
</tr>
<tr>
<td>K</td>
<td>Serum</td>
<td>122</td>
<td>0.97</td>
<td>0.11</td>
<td>0.998</td>
<td>0.021</td>
<td>1.0-9.7</td>
</tr>
<tr>
<td>K</td>
<td>Urine</td>
<td>142</td>
<td>1.00</td>
<td>0.07</td>
<td>1.000</td>
<td>0.013</td>
<td>2.4-272.3</td>
</tr>
</tbody>
</table>

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 0.5% 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.
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 often 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 efficiency 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 (InflA & 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.

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.
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), Trechomonas 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(pPCR) was ≥94%. Analytical 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 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.

**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, any run time can be further shortened as it is readily translatable to hospital-deployed automated immunosassay 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.

Validation and Correlation of VIDAS® IgGII and IgMII Lyme Antibodies vs VIDAS® Lyme IgG/IgM in the Laboratory Evaluation of Lyme Disease: Clinical Implications.

U. L. PRISCO, K. J. PRISCO. FINEYARD MEDICAL CARE, VINEYARD HAVEN, MA

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 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 immunosassays (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 LYT 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.

CLINICAL CHEMISTRY, Vol. 61. No. 10, Supplement, 2015 S141
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 methods for 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, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma urealyticum, 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. gonorrhoea, C. trachomatis, U. urealyticum; MI: M. hominis, M. genitalium, U. urealyticum. The primers and probes selected in this study were specific for conserved regions of the genes encoding glycerolaldehyde 3 phosphate dehydrogenase (GAP/GAPDH) (M. hominis), MgPsa 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 (pD1) (N. gonorrhoeae). The selected primers were considered adequate 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 TagMan 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-068

MULTI-ARRAY Assay to Discriminate Recent from Long-Standing HIV Infection


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 sroconversion 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® QuickFLEX SQ 120, and all MESO SECTOR® Imagers.

### B-071

**Rapid Detection of Microbial Resistance to Lactam Antibiotics by LC-MS/MS**

M. J. Y. Jarvis1, A. Romaschin2, L. Matukas2, Y. Chen3, N. Crawley3.

1SCIEX, Concord, ON, Canada, 2St Michael’s Hospital, Toronto, ON, Canada, 3University of Toronto, Toronto, ON, Canada

**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 β-lactamase 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 OImmuno 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, interferring substances, cross-reactant reactivity, and prozone effect. Donor saliva (human) was collected using the SuperSal™ Universal Saliva Collection Device (OImmuno 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. Specificty was 100% (20/20; CI 93.6 - 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 e forts.

### 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**

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**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 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 validated 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 0.0075 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 p4 controls assayed in replicates of three at two separate times per day for five days using a single calibration curve. ANOVA gave CVs <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.

**Results:** The LOD of the RDT is 3.00E+04 PFU/test for EBOV Zaire virus and 1.88E+04 PFU/test for recombinant VP40 antigen when spiked into whole blood. Specificity is 97.5% (39/40; CI 88.6 - 99.9%) for fingerprint (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**

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**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'-AGCTTGCAACTTTGTTAA-3' (M1) and 5'-TCCGACTTATACCGGCAGTC-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 NH4OH 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 of *B. burgdorferi* sensu lato isolates, 15 had a 100% ID match with a 16S rDNA signature sequence of *B. burgdorferi* sensu lato, 3 had a single nucleotide mismatch against any closest species of the *B. burgdorferi* sensu lato group. 16S rDNA (Figure 1). **Conclusion** Direct DNA sequencing of the PCR amplicon of a highly conserved segment with hypervariable regions of the *B. burgdorferi* sensu lato 16S rDNA may reveal greater strain diversity in the brorreliae 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 10 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 ECOI 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 mic organisms 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 LRTIs in our region. This knowledge can contribute to 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% toaztreomycin; 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 aureus/methicillin-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 60% 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 microbista 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-079

Improved Detection of Histoplasma capsulatum Antigen in Urine Specimens Utilizing Ultrafiltration and Connoctially 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-Mylogics, Inc (Immuno®, 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-Mylogics assay demonstrated high specificity, but low sensitivity when compared to Mira Vista Diagnostic’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 next with reagents from Immn to establish a baseline on a Dynex DXS 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 then compared to Mira Vista’s results.

**Results:** Correlation between Mira Vista’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% near 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


**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 40% 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 ± 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.
Infectious Disease

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 hemolysis 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 terms 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, 0.25, and 0.05 ppm respectively for K98, and 123%, 97%, and 101% for 10, 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 was 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.
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® MALTD-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/mm³.

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.

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 as syphilis and HIV, with an increase of cases of co-infection between HIV and syphilis among young patients at risk of contracting HIV, and in turn, HIV can change Syphilis natural course.

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 immunosassay - ECLIA (HIV Combi PT COBAS®, Roche) and chemiluminescent microparticle immunosassay - 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 immunosassay - 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.

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 specificit, 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 interfering 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...
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 assay 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%.

Results:

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B-091

Analyzing Amino Acids Profile in Influenz 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 relatively 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) prolonging 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.

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 naïve 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 mouse 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.
Evaluation of maternal and congenital syphilis in six maternity hospitals in São Paulo, Brazil.


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 laboratory serology of women during the first and third trimester of pregnancy, and of newborns at birth and at 2–3 months of age. Syphilis infection in women during pregnancy is associated with a 10% increased risk of preterm delivery, with complications in up to 20% of live births, and an increased risk of congenital heart defects. CS is a preventable condition and 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 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 F. A-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%.

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, p70 S6K, and 4EBP1 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.

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/mL, 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 specifict , simple to use and user friendly. We believe that this kit will be a useful tool for a rapid diagnostics of acute pharyngitis.

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 horse-radish 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 carbosylated fluorescent microspheres using EDC - Sulfosuccinimidyl 6- (biotinamido) hexanoate (BSH) chemistry. 0.25” DBS punches were extracted into 1ml of PBS, 0.5% tween 20, 50% 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 R² 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 94.7, 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.

Disclaimer: The Multiplier Flex and M2 MMRVT assay is For Research Use Only. Not for use in diagnostic procedures M². Multiplier-FLEX are registered trademarks of Dynex Technologies Inc.

Comparison of DiaSorin HBeAg and anti-HBe ELISA Methods: Generation of Combinalbe 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 B Antigen (HBeAg) and Antibody to Hepatitis B 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 GenS 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 analyses, 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 GenS 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, genotype 1a) 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.
Infectious Disease

D. A. G. Zauli1, C. L. P. Menezes2, C. L. Oliveira1, E. Mateo1, A. C. S. Ferreira1, 1Hermes Pardini Institute, Vespasiano, Brazil, 2Linhagen Produtos em Biotecnologia Ltda, Belo Horizonte, Brazil

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 are infected with HCV worldwide. According to the World Health Organization, it is estimated that 30% of infected patients develop liver cirrhosis, and/or hepatocellular carcinoma (HCC). Diagnosis of hepatitis B and C infection is usually 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 detection of nucleic acid-based markers related to hepatitis-causing viruses. The aim of the study was to develop an in-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 serum using QIAamp Virus Spin MiniElute Kit and Reverse transcription qPCR viral RNA was performed with high Capacity cDNA Reverse Transcription Kit. The amplification reactions were performed 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 0.99 and efficiencies of 90–100% for both HBV and HCV. In 116 clinical samples with a range of viral loads, the detection limit was 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.

Wednesday, July 29, 9:30 am – 5:00 pm

CLINICAL CHEMISTRY, Vol. 61, No. 10, Supplement, 2015

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Prevalence and antimicrobial susceptibility profile of mic organisms 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 examining these infections. Methods: This study was carried out on samples of blood from 1,394 patients 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 baumanii (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 aureus aureus/methicillin-resistant (MSSA). 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 to the 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 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.

Development and Evaluation of a Serological Chikungunya Antibody Detection Assay

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Background: Chikungunya (also named breakdown fever) is a highly emerging disease in many tropical settings with great socioeconomic impact. Casuative 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 develop. Both take advantage of native antigens produced with a proprietary technique which was exclusively developed for this serology and led to a sensitive and specific test 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 areas.

To further improve Chikungunya diagnostic a Lineblot is currently under development over the world. It will assist diagnosis of travel returners with unknown fever 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.
Multiplex sensitive type-specific detection of 14 high-risk HP 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 quantify 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.

Blood culture candida isolates in tertiary public hospitals in southeast brazilian region

C. Q. P. Oliveira, F. C. S. Roseiro, A. A. Silva, O. V. P. Denardin, DASA, Barueri, Brazil

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 study was to evaluate the distribution of Candida species of candidemia from eleven tertiary public hospitals in Brazil.

Methods: Data were collected from October 2014 till January 2015 from blood specimens 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 (chromID 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.

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
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 outpatient 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 years-old (4.5), < 1 year old (1.5) and over 60 years-old patients (2.0). Gram-negative bacteria represented 85% of total 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. mirabilis 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 than 1 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.

Epidemiology of Respiratory Virus among Patients Attending Private Hospitals in São Paulo, Brazil.


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, Syncyrial Respiratory Virus (SRV) A and B, Rhinoviruses (HRVs), Adenoviruses (Ad), Bocavirus (HBVo), Metapneumovirus (HMPV), Coronavirus (CoV), and Enterovirus (Echovirus, Coxsackievirus 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 viruses were detected were SRV (A and B, 27%), HRVs (24%), HBVo (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 were SRV (A and B, 27%), HRVs (24%), HBVo (12%) and HMPV (9%). 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.

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 aspergillosis 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
Infectious Disease

Wednesday, July 29, 9:30 am – 5:00 pm

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.

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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


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 Long-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 death has been reported.

### CLINICAL CHEMISTRY

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<th>November</th>
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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.

S156
B-117

Quantitative Characterization of Protein Content from HDL and LDL Size Fractions

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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 to HDL and LDL cholesterol (HDL-C and LDL-C). The higher relative CVD risk associated with LDL-P and HDL-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-clases 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 a 2% 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 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 size profile overlap in multiple serum samples with wide range of cholesterol and triglyceride levels.

Conclusions: We demonstrate an advanced lipoprotein particle sizing and sub-class particle number analysis approach which has the specific 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 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 0.908 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 efficac . Furthermore, the approach can be used in Life Science Research for biomarker discovery, validation and development of new diagnostic tests.

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/m². 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 sibings 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.

B-123
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-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, 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 of 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, and the obesity treated.

**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/S HDL-C ratio 13.

**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/HDL3-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/HDL3-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, homogeneous enzymatic methods have been proposed as an alternative.

**Methodology:** LDL-C measurements for PUC, a direct homogeneous 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 |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Category (mg/dL) | N | Mean | Mean | % Diff,a | Mean | Mean | % Diff,b | Mean | Mean | % Diff,c |
| ≤25 | 322 | 18.1 | 18.9 | 8.8 | 12.3 | -32.9 | 14.6 | -19.7 |
| 26-50 | 538 | 16.0 | 14.3 | -4.3 | 28.5 | -20.9 | 32.8 | -6.3 |
| 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 | 129.2 | -7.3 | 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 | 31.2 | 31.1 | 0.1 | 25.1 | -24.4 | 28.9 | -12.2 |
| >100 | 1023 | 36.1 | 35.0 | 0.6 | 29.2 | -23.1 | 33.0 | -11.4 |

*Percent difference = 100*(Calculated LDL-C – BQ LDL-C)/BQ LDL-C, % Diff,a = 100*(Calculation LDL-C by Friedewald – PUC)/PUC, % Diff,b = 100*(Calculation LDL-C by Hopkins – BQ LDL-C)/BQ LDL-C, % Diff,c = 100*(Calculation LDL-C by Hopkins – BQ LDL-C)/BQ LDL-C. a, Percent difference = 100*(Direct LDL-C – 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 Hopkins – BQ LDL-C)/BQ LDL-C. P-values are from a one sample t-test performed on percent difference.
**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-metalic 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 laboratory 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 present in 7% and 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 descendiant 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.

**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 characterization. **Results:** 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-metalic outline and incidence of cardiovascular risk factors in patients (age below 45 years) with AMI diagnosis. **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 present in 7% and 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 descendiant 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.

**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 dimer 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 (57AS and 88BS) 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, apolC1, apolC3, apoE and other plasma parameters.

**Results:** Two LPL-ELISA systems were established. One assay used two different monoclonal antibodies against LPL (57AS and 88BS) 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 (88BS) 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 post-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 significant high LPL activity. Significant amount of apolC1 and apolC3 were detected in RLP suggesting the inhibition of LPL activity.

**Conclusion:** Sensitivity and specificity of 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. ApolC1 or C3 in RLP may inhibit the LPL activity in spite of dimmer structure.
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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 µg/ml) in the studied population, two groups were established (n=60 each): High ADP levels (ADP-HL) ≥18.0 µg/ml (range: 18.0 to 33.2) and low ADP levels (ADP-LL) <18.0 µg/ml. Both groups did not differ in age (61±14 years-old vs 62±19; p=0.99), gender (F/M: 32/28 vs 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 1.0 ± 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 (4.3-24.6) pg/mL). 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.

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=12). 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.
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 kri ngle 4 type 2 repeats by linear regression analysis. Results: The LOB and LOD were 1 and 13 mmol/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 mmol/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 kri ngle 4 type 2 repeats. Conclusion: The Sun Ds 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: 2.8% (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.

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### Table 1. Average Method Bias and peer group CV values

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Ideal Bias (%)</th>
<th>Ideal CV (%)</th>
<th>Actual Bias (%a)</th>
<th>Actual CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOL</td>
<td>0</td>
<td>10</td>
<td>-0.1 (58)</td>
<td>2.3 (58)</td>
</tr>
<tr>
<td>TRIG</td>
<td>0</td>
<td>10</td>
<td>-0.7 (46)</td>
<td>1.7 (46)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0</td>
<td>10</td>
<td>-0.6 (36)</td>
<td>1.9 (36)</td>
</tr>
</tbody>
</table>

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**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 biases 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 HDL 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 CCPD-II, and UV-A4020 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-rich 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 inhibited HTGL activity, similar with LPL activity.
**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 immunosassay 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 tallied.

Results: Over 40,000 QC values for 70 chemistry, TDM and immunochromatography 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

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**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 NGS. Our laboratory personnel have a prejudice regards to assay HbA1c by turbidimetric inhibition immunosassay (TINA) and prefer to use high-performance liquid chromatography (HPLC). This study was aimed to assess the commutability between two methods certified by the NGS - TINA 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 Tinaquant 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: A total of 18,126 B12 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 B12 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 B12 and folate. Within the year studied, B12 or folate tests were ordered more than once on 1,104 (5.6%) patients. In 1029 patients with initial results indicating that B12 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 B12 or folate deficiency, and 12 (11%) patients had the same test ordered again within 30 days.

Conclusion: Vitamin B12 and folate tests are frequently ordered together despite the rarity of folate deficiency in the patient population. The prevalence of B12 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 B12 and folate testing.

**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:

$\text{HbA1c in cobas c501} = 0.142 + (1.022 \times \text{HbA1c in G8})$

Consequently, our laboratory will 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 testing order patterns for serum Vitamin B12 and folate

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Background: Serum Vitamin B12 (B12) and folate testing are commonly ordered as part of the assessment of macrocytic anemia or when patients present with physical symptoms suggesting deficiency. Although B12 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 B12 and folate test ordering in our institution and the prevalence of deficiency.

Methods: Serum B12 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 B12 deficiency, defined as <4.0 mcg/L and <150 ng/L, respectively, was determined. Data were also analyzed to identify how often B12 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 B12 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 B12 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 B12 and folate. Within the year studied, B12 or folate tests were ordered more than once on 1,104 (5.6%) patients. In 1029 patients with initial results indicating that B12 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 B12 or folate deficiency, and 12 (11%) patients had the same test ordered again within 30 days.

Conclusion: Vitamin B12 and folate tests are frequently ordered together despite the rarity of folate deficiency in the patient population. The prevalence of B12 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 B12 and folate testing.

**Summary and Analysis of Nine Years’ Point-of-care Glucose Meter External Quality Assessment Programs in China**

Y. Fei, W. Wang, F. He, K. Zhong, Z. Wang. Beijing Hospital, Beijing, China

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 is 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 analytic 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 ISO 13528 for each control material lot. Robust coefficient of variation (CV) based on ISO 15197. Robust criteria as well as the robust CV for each control material lot was shown in the picture below. The measurement performance was then evaluated based on the minimum system accuracy performance criteria in ISO 15197. Robust CV fluctuated largely among different lot, there was no obvious change trend among different years.

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. The measurement performance was then evaluated based on the minimum system accuracy performance criteria in ISO 15197. Robust criteria as well as the robust CV for each control material lot was shown in the picture below. The measurement performance was then evaluated based on the minimum system accuracy performance criteria in ISO 15197. 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.
Implementing Lean Six Sigma Methods to Improve Workflow in the Clinical Flow Cytometry Laboratory


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 for orders i.e. technical staff 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.

B-147
Implementation of six sigma as a quality control management tool

Y. Low, S. A. Rahman, H. Muhammed. Pantai Premier Pathology (M) Sdn Bhd, Kuala Lumpur, Malaysia

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.
Wednesday, July 29, 9:30 am – 5:00 pm

**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 at determining 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 redrawning.

**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 communication of non-critical, but clinically relevant, results to physicians by a medical team based outside the central lab.

M. D. C. Freire, D. M. V. Gomes, R. G. Fontes, Y. Schrank, P. B. M. C. Araújo, A. S. Santos. DASA, RIO DE JANEIRO, Brazil

**Background**

Quality and safety accreditors place increased emphasis on the effectiveness with which critical laboratory results are reported to caregivers. CAP Q-Profiles 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 prescribing 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 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.

**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 for physician telephone notification. The medical team successfully reported 1,258 results.

**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 sense 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


**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 homogeneous system, the analytical platform Architect ci8200 and Abbott reagents were used. The method used for the determination of creatinine was kinetic alkaline-picate. The theoretical MDPs (0.60, 1.60, 6.00 mg/dL) was selected from the tables in Statlab BE. Clinical Decision 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.

**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 redrawning.

**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-152**

Statistical analysis and management of quality control data in clinical toxicology laboratory

S. Y. Wu, F. Hassouna, G. Sweeney. Confirmatix Clinical Laboratory, Lawrenceville, GA

**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 analytic 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.

Evaluation of events involved in the pre-analytical phase and the financial impact: a case of risk management study.

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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 TAT results for 36 routine Chemistry and Immunology assays were calculated from the operational timestamp data in the Laboratory Information System (LIS). The TAT results for 36 routine Chemistry and Immunology assays were calculated from the operational timestamp data in the Laboratory Information System (LIS). The TAT results for 36 routine Chemistry and Immunology assays were calculated from the operational timestamp data in the Laboratory Information System (LIS). 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 specific one. 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.

Enhanced performance of a Clinical Laboratory with the use of Auto-verification

S. Vanavananan, P. Srisawadsi, N. Teerakanjana, N. Kumproua, K. Khupusup, P. Kiptoka, S. Wongwaisayawan. Pathology Department, Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand

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 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 specific one. 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.
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:

<table>
<thead>
<tr>
<th>Laboratory Process</th>
<th>Before</th>
<th>After</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total tests reviewed</td>
<td>49,310</td>
<td>48,273</td>
<td>-2.7%</td>
</tr>
<tr>
<td>Pre-Analytical Process</td>
<td>21 min</td>
<td>22 min</td>
<td>4.76%</td>
</tr>
<tr>
<td>Analytical Process</td>
<td>43 min</td>
<td>39 min</td>
<td>9.30%</td>
</tr>
<tr>
<td>Post-Analytical Process</td>
<td>11 min</td>
<td>6 min</td>
<td>45.45%</td>
</tr>
<tr>
<td>Average TAT</td>
<td>75 min</td>
<td>68 min</td>
<td>9.33%</td>
</tr>
<tr>
<td>Achievement of TAT goals</td>
<td>97.10%</td>
<td>98.90%</td>
<td>-1.80%</td>
</tr>
</tbody>
</table>

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-157

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 test for identifying individuals at risk for CD, replacing the EMA for this purpose. The longer and more expensive 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 234,551 inhabitants. From January 1st 2012 to December 31st 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 immunofluence 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, Upplands Väsby, 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>10x ULN 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.

B-158

A Prospective Assessment and Physician Satisfaction Survey of Repeated Chemistry and Hematology Critical Results

W. Q. Anani1, Q. Sun1, L. C. Contis1, H. C. Blair2, R. M. Amin3, O. M. Peck Palmer4, 1University of Pittsburgh Medical Center, Pittsburgh, PA, 2University of Pittsburgh School of Medicine, Dept Surgery, Pittsburgh, PA, 3University of Pittsburgh School of Medicine, Dept Pathology, Pittsburgh, PA

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 hemocrit, 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 randomly submitted to the clinical laboratory were analyzed on the following instruments: hematofen, DxH800 (Beckman Coulter, CA); chemistry, AU5822/AU680 (Beckman Coulter) or Dimension Vista 1500 (Siemens, DE); and immunohassay, Dxi800 (Beckman Coulter). All specimens with critical results were reanalyzed to confirm the initial result in the 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, medical education, radiology that participated in the survey. Overwhelmingly, 92% of the physicians were satisfied with the current critical result list. Eighty-nine percent of the 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 HIPPA-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.

B-159

Internal service culture and its influences on the quality of external customer service (investigation - SERVQUAL)

J. L. Silva, F. B. A. Ramos, O. Fernandes, O. V. P. Denardin, DASA, São Paulo, Brasil

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 ≥ 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.

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**B-159**  

**Is CV related to Productive Efficiency in the Clinical Laboratory Setting?**  

R. A. A. L. Cardoso, Laboratório Sabin, Brasília, Brazil  

**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 amininohidrolase/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 (Amininohidrolase/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 = \( \sqrt{\frac{CV^1 + CV^2}{2}} \). 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.  

**Results:** 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 product.  

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**B-160**  

**Follow up of Productive Efficiency on a Clinical Laborator**  

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**Background:** Productive Efficiency occurs when a productive model explores the full potential of its installed capacity. We aimed to determine the importance of periodic measurement of Productive Efficiency in production lines in a Clinical Laborator.  

**Methods:** We Adapted 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  

\[ \text{EP(%) = } \frac{C_t}{C_i} \times 100 \]  

Where EP(%) = productive input Efficiency; \( C_t \) = actual production capacity of the input; \( C_i \) = 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(%)\text{,} 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 fail.  

**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.  

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**B-161**  

**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 division 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 an 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 the test (more than 100 tests in last one year) were Neurosurgery/Neurology, Pediatric endocrinology, outpatient lab, Transplant division and gastroenterology divisions. The top five 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.

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 leading 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-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 Baldridge 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 leading 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.

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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-166
Utilizing Lean Six Sigma to Improve Processes in Hematology

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 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.

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 shifts from a time with few staff to a time with more staff. The two inpatient hem/onc units (HSUC 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 scans 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.

R-167
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 overdiasnosis 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?
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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.
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-Sigma. 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-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 and 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 18044 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.
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

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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


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.
**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.

**B-176**

**Melting Curve Analysis**

**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.**


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.
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. The variability of response to antiplatelet therapy in CAD patients and iposogen 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%CI 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 iposogen JAK2 MutaQuant kit was high (R=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.
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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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.

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 inhabitants. 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 validated 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%, M184V/I (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%.

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.
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, HEI 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 potential 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.


Intro/Background:

G6PD deficiency is the most common human enzyme defect, estimated to be present with low-level mutations.

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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 performed 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 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

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/critical/abnormal phenotyping results, carrier status in women, and classify patients experiencing CNSHA.
Whole-exome sequencing as a powerful diagnostic tool in clinical laboratories

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One of the main 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 and their relatives. 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 diagnosis 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 gastrointestinal syndrome (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 nonsense 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 failed to identify disease-causing mutations in 11 patients and this could be due to many factors, such as polygenic diseases, structural variants, epigenetics changes, insufficient coverage few exons in exome sequencing and intrinsic 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.
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 (XV) X1, (ISCN 2009). The heterogeneous 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, CXADR2P2, POTE2, NFIP1, LOC727924, OR4M2, OR4N4, OR4N3P, LOC646396, GOLGA9DP, GOLGA6L1.

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 by 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 exophialae 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 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 assay. The second methodology utilizes fragment detection of a single amplicon (~10kb) covering intron 2 through the 3-prime 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.

B-196
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 microdeletions at Yq11 may represent the etiology factor in as many as 10 to 20% cases of idiopathic azoospermia or severe oligozoospermia. 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 (QiAgen) from patients with infertility problems. Genotyping of the PKLR gene (Pyruvate Kinase, Liver, Red Blood Cell) was performed on samples that had low PK levels. 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) or digital PCR (dPCR), 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 cfDNA levels with higher precision than qPCR. However, both methods include a preamplification step 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. Fourplex 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 qPCR 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 HTx 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...
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Early Detection of Rejection after Heart Transplantation by a Universal Digital PCR Method.


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 cDNA per total cDNA 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 3.47cps/mL (IQR: 1.7-3.3,172,) 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 160cps/mL respectively (99th percentile).

In the initial 2 weeks distinct increases in individual courses are better suited to indicate a rejection, since the GcfDNA and total cDNA 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% (694cps/mL) and 11% GcfDNA (512cps/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 by 0.9% 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 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.

B-200

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 2015 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 N4b 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 CybB 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 CybB 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.

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 system for formalin-fixed paraffin-embedded tissue (FFPE) extraction.

The new DNAext protocol leverages the validated workflow of DNA standard QUANT-IT PICOGREEN dsDNA and QUANT-IT RIBOGREEN RNA RT-PCR assays. The DNA and RNA eluates were also qualified with in-house DNA and RNA surrogate gene real-time PCR, and the TPS protocols were qualified with the DNAext protocol. Nucleic acids extracted using 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 TPS system, 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 quantified with in-house hDNA 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 and RNA 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.

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 (22q11.2), Angelman and Prader-Willi (del 15q11) and Wolf-Hirschhorn(del 4p16) syndromes. The remaining cases are caused by other subtelomeric and balanced translocations, deletions, or mosaicisms. 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 abnormalities (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 kit P029 kit in the laboratory’s routine by comparing the results obtained in our verification and the results from 22 samples studied by the STR technique. The results were concordant with MLPA kit P029 in 4 patients. However, we found 3 discrepant results comparing the microsatellite markers 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 most important advantages of the MLPA kit is 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.
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


Background: Obesity is a 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 in 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. They 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: >30 kg/m2). CD40 (rs1883832) C>T, CX3CR1 (rs3732379) C>T, CX3CR1 (rs3732378) G>A, E-selectin (rs5368) C>T, ICAM-1 (rs7999696) G>A, ICAM-1 (rs281432) C>G, LIGHT (rs344560) G>A, LIGHT (rs2291668) C>T, RAGE (rs2070600) G>A, RAGE (rs2226495) C>T e VCAM (rs1576878) C>T gene polymorphisms and mRNA expression in peripheral blood leukocytes were analyzed by pyrosequencing and qRT-PCR, respectively. PAI-1, IL-6, TNF-a, 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-a 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 thrombotic mechanisms 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 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 possible 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 chromosones number and structure stability 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.

Molecular Pathology/Probes

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 3-8% of potential cause of delay development, dysmorphic features and 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 scanning. 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 MLPA kits to investigate CNV in subtelomeric regions of the 23 pairs of chromosomes in a clinical laboratory.

The samples of 3 patients 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 chromosome 1q 43.11 (8p23.3 and 22q11.21 (9.6.7%) and the kit P070 found two alterations, in the chromosomes 4pl16.3 and 8p23.3 (6.4%) in the samples of 31 patients. The difference in detection of chromosome 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.
**Wednesday, July 29, 2015**

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

**Pediatric/Fetal Clinical Chemistry**

**B-208**

**Effect of Multiple Transfusions on Lipid Peroxidation in Preterm Infants**


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 fluorescence 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 >450ng/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 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.

**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 test sample screening with our Care Plus II (CAP) assay 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 DxI800, 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; R²=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 DxHb 6.8 g/dL). The serial dilution curve plot showed an excellent fit with an expected log relationship (Hemocue = -11.5*ln(Dilution Factor) + 594.3). Precision studies showed CV% at seven levels ranging from 9.7 - 42 percent. LDH showed positive correlation with PfHb values obtained on the AA method (AA = 5.9*LDH + 290; R²=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 PbHb 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.
We collected 0.3 mL blood through an arterial catheter from adult inpatients in a 1 mL syringe. We evaluated minimum collection volume in the Smiths Medical Portex 1 mL Line 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 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 slowy 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild 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 ataxia, tremors and dyskinetic movements. He was able to speak short sentences with mild learning disabilities.
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 mEq/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 (>60mEq/mL) and 81 (10%) indeterminate (30-60mEq/mL). 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 5 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 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 c.844C>T of the L2HGDH gene). Our patients were started on Riboflavin and follow-up have shown improvement in the dystonia.

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 2014


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. umbilical cord samples from at-risk newborns submitted to our laboratory for forensic purposes.

Methods:
Due to ethical considerations, data of drug deposition and concentration in neonatal syndromes is rising. Meconium and umbilical cord are unique neonatal specimen types that provide longer detection window for drug testing than maternal/neonatal urine. Data of drug deposition and concentration in neonatal syndromes is rising. Meconium and umbilical cord are unique neonatal specimen types that provide longer detection window for drug testing than maternal/neonatal urine.

Results:
Cotinine (0.03% (414/8169)) 40 - 6,131 (156) Cotinine 12.2% (384/3186) 2.2 - 305 (58)

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 pediatric 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 1 year (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. 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 non-parametric 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


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 kit HPLC chemical 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 transferece 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 c8200 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 r² 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*RMSE. 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 (r² < 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


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±54 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±3.5 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 - 20.8) 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±6.5 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 reason for this effect but its use must be taken into account when calculating normal values.

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: Mass 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 chromatographic separation was performed on a Shimadzu Prominence UFLC. A 5 µL sample was injected into an Intakt 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.
CAPILLARY BLOOD SAMPLING KIT FOR HBA1C VERSUS VENOUS PUNCTURE ON CAPILLARYS 2 FLEX PERCING

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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 laborator.
**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 clinical 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 - 65.14). 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%).

**Conclusion:** 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.

**Using Point-of-Care Glucose Meters in the Critically Ill: Assessing Meter Performance in the Clinical Context**

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**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 Xceed 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, pO2) 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 r²=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) or 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 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.

**Glucose Connectivity Meter Evaluation in Intensive Care Unit**

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**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.

**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 6.1/3.4/4.5%, (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 R² =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 ±0.86 mmol/L.
Development of a Novel, Lab-on-Cartridge based Point-of-Care Device for the Measurement of Clinical Diagnostics Tests

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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 diagnostics 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 were run on 6, 7, 14 days respectively.

Results: Data analysis indicates that the assays have a CV < 3%, with R² > 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 specifica ions 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.

A comparison of values on point-of-care instruments in patients with EBOV

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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 (HE) 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.

Assessment of Interference from Hemolysis, Lipemia and Icterus on the Abaxis Piccolo Liver Panel Plus Reagent Disc

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Background: Interference due to hemolysis, lipemia or icterus in patient samples at the point-of-care can be problematic, especially in critically ill patients who tend to produce samples containing these endogenous interferences. 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 interference 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 used an interference 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 interferences are present, even at very high concentrations of interferent. Use of the
Clinical evaluation of mobiLab, a smartphone-enabled microfluidic NAAT platform for Chlamydia trachomatis screening


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.

References:

Viscoelastic Coagulation Monitoring: Current use of TEG, ROTEM, and Sonoclot

R. Dietrich, R. Christenson, S. Duh, M. Fontaine. University of Maryland Baltimore, Baltimore, MD

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.

<table>
<thead>
<tr>
<th>Hospital Area</th>
<th>% Responding institutions (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac Surgery</td>
<td>65%(20/31)</td>
</tr>
<tr>
<td>Emergency Room</td>
<td>23%(7/31)</td>
</tr>
<tr>
<td>Hepatic Surgery</td>
<td>48%(15/31)</td>
</tr>
<tr>
<td>Intensive Care Unit</td>
<td>35%(11/31)</td>
</tr>
<tr>
<td>Liver Transplantation</td>
<td>Liver Transplantation65% (19/31)</td>
</tr>
<tr>
<td>Obstetrics</td>
<td>19%(6/31)</td>
</tr>
<tr>
<td>General Surgery</td>
<td>29%(9/31)</td>
</tr>
<tr>
<td>Trauma</td>
<td>42%(13/31)</td>
</tr>
</tbody>
</table>

Viscoelastic Coagulation assays in hospital areas

**B-231** 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


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 were defined as follow: for CC, D = -0.35 ±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 >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 such as trauma will increase.
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 e502 and e702 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 programed 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 (~1.6 ± 14.8%; p<0.002), 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 whole blood. Serum whole blood 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.
Conclusions: Preliminary validation studies have demonstrated that it is possible to quantify Glycated Hemoglobin A1c from DBS specimens for the purposes of screening patients.

Patient identification errors in 20 point-of-care blood gas analyzers


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 to 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 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.

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:

<table>
<thead>
<tr>
<th>Setting (Number of analyzers)</th>
<th>Samples (n)</th>
<th>No patient ID (%)</th>
<th>Patient ID with operator number (%)</th>
<th>Patient ID with patient name (%)</th>
<th>Patient ID with unknown number (%)</th>
<th>Total errors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery room (3)</td>
<td>3,724</td>
<td>11.7</td>
<td>2.0</td>
<td>0.5</td>
<td>2.0</td>
<td>16.1</td>
</tr>
<tr>
<td>Pediatric intensive care unit (1)</td>
<td>3,090</td>
<td>2.4</td>
<td>0.2</td>
<td>0.6</td>
<td>1.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Pediatric reanimation unit (1)</td>
<td>2,252</td>
<td>2.6</td>
<td>0.2</td>
<td>0.1</td>
<td>0.7</td>
<td>3.6</td>
</tr>
<tr>
<td>Pediatric hemodynamics unit (1)</td>
<td>212</td>
<td>5.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>6.1</td>
</tr>
<tr>
<td>Neonatal intensive care unit (3)</td>
<td>5,955</td>
<td>6.4</td>
<td>0.6</td>
<td>7.1</td>
<td>3.1</td>
<td>17.3</td>
</tr>
<tr>
<td>Coronary intensive care unit (1)</td>
<td>913</td>
<td>9.7</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>10.7</td>
</tr>
<tr>
<td>Reanimation unit (3)</td>
<td>7,492</td>
<td>4.2</td>
<td>0.2</td>
<td>0.1</td>
<td>1.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Intensive care unit (1)</td>
<td>3,850</td>
<td>3.6</td>
<td>0.8</td>
<td>2.1</td>
<td>0.2</td>
<td>6.6</td>
</tr>
<tr>
<td>Burn unit (1)</td>
<td>1,793</td>
<td>3.5</td>
<td>0.4</td>
<td>0.2</td>
<td>1.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Surgical suites (3)</td>
<td>1,199</td>
<td>3.6</td>
<td>2.0</td>
<td>0.0</td>
<td>0.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Pulmonary offices (2)</td>
<td>649</td>
<td>0.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Total (20)</td>
<td>31,129</td>
<td>5.2</td>
<td>0.7</td>
<td>1.8</td>
<td>1.5</td>
<td>9.2</td>
</tr>
</tbody>
</table>

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.

Evaluation of a POCT Glucose Meter for High Complexity Testing in the ICU and NICU for Critically Ill Patients at Centura Health Network of Hospitals

S. Clark. Centura Health, Aurora, CO

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 hypoglycemic 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 hypoglycemia and hyperglycemia. Hypoglycemia was defined as a lab value of <40mg/dL and <70mg/dL for NICU and ICU respectively. Hyperglycemia was defined as any lab value between 71-180mg/dL, and hyperglycemia was defined as any lab glucose >180mg/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 hypoglycemic events (PPV=95%, NPV=98%). Two method comparison studies showed that 98% of values across the AMR were within a TE 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.

Acellinis Automated Flow Cytometry

H. Kasdan, A. Reiter, S. Keren, R. Caspi, J. Meissonnier. LeukoDx, Jerusalem, Israel

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 technicians. 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 Accelinis 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.

B-241
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 TC02, 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 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.

B-242
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 POCT 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

| PATHFAST imprecision data and results of method comparison |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| WB samples Mean(n=20) | Intra-assay CV (%) | Inter-assay CV (%) | Comparison | Intercept | Slope | r |
| CKBMB (µg/L) | 2.31 | 6.2 | 6.2 | E-170 | -1.278 | 0.9221 | 0.998 |
| cTnl (µg/L) | 0.19 | 6.1 | 5.6 | E-170 | 0.991 | 0.998 |
| D-Dimer(µg/L) | 0.69 | 4.8 | 5.5 | Integra | 0.3133 | 1.005 | 0.977 |
| hsCRP(pg/mL) | 0.63 | 6.4 | 6.6 | Integra | 0.3334 | 0.883 | 0.998 |
| NTproBNP(pg/mL) | 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%
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/mL compared to 11 and 1407 ng/mL (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. 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**

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>SENS (%)</th>
<th>SPEC (%)</th>
<th>Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDS</td>
<td>0.851</td>
<td>81.0</td>
<td>85.0</td>
<td>&gt;12</td>
</tr>
<tr>
<td>PSEP</td>
<td>0.810</td>
<td>80.0</td>
<td>70.0</td>
<td>&gt;1179 ng/mL</td>
</tr>
<tr>
<td>MEDS+PSEP</td>
<td>0.860</td>
<td>97.1</td>
<td>84.5</td>
<td>&gt;608 ng/mL</td>
</tr>
</tbody>
</table>

**B-243**

**Development of a new rapid assay for quantitative measurement of H-FABP in whole blood and plasma.**


**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 immunochromato-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 measurement of heparinized plasma and whole blood (r = 0.996, Y = 1.01X + 0.601).

**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.

**B-244**

**Point of Care Testing Expansion in Public Health; One County’s Experience**

B. L. Van, S. K. Mohamed, J. L. Mikell. Marion County Public Health Department, Indianapolis, IN

**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 particular. 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.

**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 suggestions 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:**


**B-245**


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**Background:** Available in a range of sizes, latex microspheres are versatile detector particles which can, depending on their composition, be colorimetry, fluorometer, 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® carbosyl-modified dyed microspheres and Hi-Flow™ Plus membranes.

**Methods:**
Lateral flow test strips for the detection of human chorionic gonadotropin (hCG) were assembled using blue Estapor carbosyl-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/6cm. Microspheres were conjugated to anti-hCG antibodies using a two-step EDC/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 10.8ug of microspheres per test. Hi-Flow Plus membranes were stripped with 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-248**

**Using urine dipstick as a presumptive result for urinary tract infection.**


**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 when applicable, identification and sensitivity were obtained through Vitek 2 (Biomerieux®). For positive cultures when applicable, identification and sensitivity were obtained through Vitek 2 (Biomerieux®).

**Results:**
When 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 positive 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.
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, pCO₂, pO₂, 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, pCO₂, potassium, glucose, and lactate. The performance of pO₂, sodium, ionized calcium, and hematocrit was more variable. The figure shows a user-dependent variation in accuracy for hematocrit and pO₂; 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.

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 (r=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 significantl , 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.

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 epidemiac strain of C. difficile producing a third toxin called the binary toxin, referred to either NAP1, B1, 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 CDI suspected were tested by Clorstridium 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 (cdtB), the binary toxin gene (cdt), and the cdA/C gene deletion at nt 117.

Results: A total of 63 stool samples from 63 patients were studied; 13 samples were Xpert C. difficile assay positive and 1 sample was also positive for toxigenic C. difficile 027-NAP1-B1. A total of 34 fecal specimens were tested by both methods PCR and Immunocassay; only 2 out of 13 positive samples by Xpert C. difficile were positive by immunocassay. The turnaround time for Gene 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.
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 different 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 specimen types 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 8000 analyzers measure glucose amperometrically by oxidation of glucose using glucose oxidase. Venous whole blood (WB) 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 WB vs plasma comparison employing Dxc as a reference. For WB vs AWB and for AWB vs WB, 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 WB 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 WB vs WB 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 i-STAT. For AWB vs WB 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 i-STAT.

**Conclusion:** Overall for WB vs plasma comparison, FreeStyle shows the most significant negative bias (-12%). FreeStyle also has significant negative bias in WB when compared to Radiometer (-12%) and i-STAT (-8%). However, in AWB, the negative bias of FreeStyle decreases to an insignificantly 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 in venous and arterial blood 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-check Inform II vs the Roche Accu-check 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-check Inform I, Roche Accu-check 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 61 mg/dL to 307 mg/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) + (-1.2909), with an R² of 0.9900 and a standard error of 0.099 of 0.099. 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 R² 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-check 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®Dx 8000 (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 employing linear regression. Total error of each Piccolo assay was determined by 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.
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. 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 measured 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.

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


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 AUCs of correlations in the ABL800 were 0.90 to 0.95, and 0.65 to 0.68, respectively, for adult and pediatric populations, and 0.85 for the StatStrip. Conclusion: Statistical performance of the StatStrip Lactate Xpress Hospital meter is acceptable for the intended use.

Demonstration of a Urine Dipstick Control in a Revolutionary Single-Use Pouch with Extended 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 thermostable 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 25°C for up to 60 days and up to 160 days at 2-8°C. 160 days at 2-8°C.

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 (IG/WBC) and procalcitonin had OR that did not differ significantly from 1.00 (p>0.26). Lactate, WBC, and neutrophil number/percent did not differ significantly (IG/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. OR

Conclusion: Lactate was the best biomarker for prediction of severe sepsis or septic shock, with an odds ratio (95% CI) of 3.07 (2.92-4.11) 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.

**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 I 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 the 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 sensitivities.

**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 analysers used in main laboratory. Now a new generation hematological analyzer (based on volumetric impedance and microfluidics) is available: the aim of the study was to compare the analytical performance of the new hematological analyzer Icon to the XE-2100 Sysmex analysers used in our laboratories.

**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, 95% 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 significantly 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.
**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 the patient’s 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 based on electrochemical enzymatic creatinine and urea sensors for the GEM Premier analyzer (Instrumentation Laboratory) is currently in development. This is an addition.

**METHODS:** A total of 60 blood 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.

**RESULTS:** Twenty samples were analyzed at each site immediately upon collection. Correlation for blood gases (pH, partial pressure of carbon dioxide (pCO2), 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 analyzers at three concentration levels. CVs of level 1 for pH, pO2, pCO2, Na+, K+, Cl- and ionized Ca2+ were less than or equal to 0.08, 2.72, 8.20, 0.93, 0.95, 0.96, 0.98 (level 2 were less than or equal to 0.06, 1.74, 5.38, 0.55, 0.50, 0.30, 0.70 and level 3 were less than or equal to 0.07, 2.74, 5.15, 0.53, 0.74, 0.97, 1.43 respectively. 

**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>
<thead>
<tr>
<th>Analyte</th>
<th>Slope Intercept</th>
<th>R2</th>
<th>Sample Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCO2</td>
<td>0.8622</td>
<td>3.1333</td>
<td>0.920</td>
</tr>
<tr>
<td>Crea (a)</td>
<td>0.9349</td>
<td>0.0896</td>
<td>0.937</td>
</tr>
<tr>
<td>BUN</td>
<td>0.9002</td>
<td>1.4842</td>
<td>0.989</td>
</tr>
<tr>
<td>Na+</td>
<td>1.1145</td>
<td>-0.9611</td>
<td>0.935</td>
</tr>
<tr>
<td>K+</td>
<td>0.9499</td>
<td>0.1763</td>
<td>0.984</td>
</tr>
<tr>
<td>Cl-</td>
<td>0.8347</td>
<td>17.752</td>
<td>0.941</td>
</tr>
<tr>
<td>Ca++</td>
<td>0.894</td>
<td>0.0497</td>
<td>0.963</td>
</tr>
<tr>
<td>Hct</td>
<td>1.0431</td>
<td>-0.1532</td>
<td>0.985</td>
</tr>
<tr>
<td>Glu (b)</td>
<td>0.9673</td>
<td>3.1615</td>
<td>0.996</td>
</tr>
<tr>
<td>Lac (b)</td>
<td>1.0312</td>
<td>-0.01512</td>
<td>0.995</td>
</tr>
</tbody>
</table>

**B-262**

**Performance Evaluation Of Roche Cobas b 221 Point Of Care Blood Gas Analyzers Used In Intensive Care Units**

N. M. AlHumaidan, S. Sobki. prince saltan military medical city hospital, Riyadh, Saudi Arabia

**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 (pCO2), 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 analyzers at three concentration levels. CVs of level 1 for pCO2, pO2, Na+, K+, Cl- and ionized Ca2+ were less than or equal to 0.08, 2.72, 8.20, 0.93, 0.95, 0.96, 0.98 (level 2 were less than or equal to 0.06, 1.74, 5.38, 0.55, 0.50, 0.30, 0.70 and level 3 were less than or equal to 0.07, 2.74, 5.15, 0.53, 0.74, 0.97, 1.43 respectively. 

**Conclusions:** Overall performance of cobas b 221 system was acceptable, it provided reliable results for all tests in all ICUs.

**B-263**

**Harmonization Verification of the 2 Platforms for Assaying Patient Blood Glucose. A Practical Example Using Polynomial Regression Analysis.**

V. M. Genta1, S. S. Churchi1, M. Ferguson1, L. Weyer2, Y. Shen1, 2Sentara Virginia Beach General Hospital, virginia Beach, VA, 3Sentara Healthcare, Norfolk, VA

**Background:** In our hospital patient blood glucose is assayed with three different platforms, namely cobas c-501® the laboratory method (LM), i-STAT® cartridges and ACCU-CHEK® InformII, 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...

**Table 1. Correlation of the GEM BMP vs. References (a) ARCHITECT (N=665), (b) GEM 4000 (N=121)**
the results for patient care. We employed polynomial regression analysis to evaluate bias, linearity, and equality of the regression lines. Methods: 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 (independent variables) were regressed against those obtained with the LM (dependent 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 autocorrelation. 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 subset (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 ±5 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® (sampling), 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.

B-264

Evaluation of Liver Function Tests on Piccolo Xpress Chemistry Analyzer

N. Akbas1, G. Gonzalez2, S. Devaraj3. 1Baylor College of Medicine and Texas Children’s Hospital, Houston, TX, 2Texas Children’s Hospital, Houston, TX

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. Results of each of the measurement studies were compared using two clinical chemistry methods having error 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 of 100 µL whole blood sample made the analyzer a good alternative for bedside analysis in infectious patients in the pediatriic setting, as well as in remote areas.

B-265

Telehealth and Glucose Monitoring in Nursing Homes and Patient’s Residence


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.
Point-of-Care Testing

Wednesday, July 29, 9:30 am – 5:00 pm

B-268

Roche Accu-Check® Inform II Blood Glucose Meter Reduces Maltose Interference and Risk to Patient Safety

M. Kozelkova, R. Kushlak, V. Luzzi, F. Meiet. Henry Ford Medical Laboratories, Detroit, MI

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:

<table>
<thead>
<tr>
<th>Initial Glucose concentrations with Inform I, Inform II, and DxC800, mg/dL</th>
<th>Maltose with Inform I, mg/dL</th>
<th>Maltose with Inform II, mg/dL</th>
<th>Maltose with DxC800, mg/dL</th>
<th>Δ Glucose with Inform I, mg/dL</th>
<th>Δ Glucose with Inform II, mg/dL</th>
<th>Δ Glucose with DxC800, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A (44, 51, 46)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A = 400</td>
<td>44</td>
<td>143</td>
<td>76</td>
<td>2 (3%)</td>
<td>99 (6%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>A = 610</td>
<td>45</td>
<td>268</td>
<td>71</td>
<td>3</td>
<td>224</td>
<td>20</td>
</tr>
<tr>
<td>A = 900</td>
<td>44</td>
<td>451</td>
<td>80</td>
<td>2</td>
<td>387</td>
<td>29</td>
</tr>
<tr>
<td>Patient B (147, 159, 145)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B = 280</td>
<td>149</td>
<td>205</td>
<td>163</td>
<td>4 (3%)</td>
<td>116 (44%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>B = 610</td>
<td>149</td>
<td>398</td>
<td>174</td>
<td>4</td>
<td>241</td>
<td>11</td>
</tr>
<tr>
<td>B = 900</td>
<td>150</td>
<td>529</td>
<td>182</td>
<td>3</td>
<td>682</td>
<td>25</td>
</tr>
<tr>
<td>Patient C (219, 227, 248)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C = 280</td>
<td>250</td>
<td>349</td>
<td>218</td>
<td>7 (3%)</td>
<td>130 (33%)</td>
<td>9 (6%)</td>
</tr>
<tr>
<td>C = 610</td>
<td>255</td>
<td>316</td>
<td>233</td>
<td>12</td>
<td>297</td>
<td>6</td>
</tr>
<tr>
<td>C = 900</td>
<td>252</td>
<td>393</td>
<td>253</td>
<td>8</td>
<td>592</td>
<td>36</td>
</tr>
<tr>
<td>Patient D (359, 417, 364)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D = 280</td>
<td>567</td>
<td>489</td>
<td>423</td>
<td>3 (3%)</td>
<td>110 (23%)</td>
<td>6 (2%)</td>
</tr>
<tr>
<td>D = 610</td>
<td>571</td>
<td>quit</td>
<td>322</td>
<td>quit</td>
<td>quit</td>
<td>quit</td>
</tr>
<tr>
<td>D = 900</td>
<td>570</td>
<td>quit</td>
<td>441</td>
<td>quit</td>
<td>quit</td>
<td>quit</td>
</tr>
</tbody>
</table>

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).
As per EP7-A, no interference was observed in the RAMP D-dimer test as the result on three lots of RAMP® D-dimer tests. Repeatability and total precision CVs were determined for whole blood samples by testing three levels in triplicate, in five runs over three days. The % interference compliance rate using “disinfection” comment enty of 50/100. Discussion on thoroughness (%) = (f devices with both fluorescent markers removed/ devices with ≥ 1 marker removed)*100. The % interference compliance rate using “disinfection” comment enty of 50/100.

Results: 98% compliance with glucose meter disinfection was captured using the “disinfection” comment method. Using the fluorescent marker approach identified 64% interference 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 rates. 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.

**Determination of Analytical Performance Characteristics of the RAMP® D-dimer Test**

J. F. Wilson, S. Bie, P. Tan, V. Ng, E. Siu, M. L. Zaharak, Response Biomedical Corp., Vancouver, BC, Canada

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 2735 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 (200 mg/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.

**Analytical validation of MRSA detection test through GeneXpert®**


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 an infinite 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 *mecA*, 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.

**Linearity Standards for Automated Urinalysis Systems**

A. T. Schaeffer, P. Lenichek, M. Ban, M. Ghadessi. Quantimetrix Corporation, Redondo Beach, CA

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 Quantimetrics Corporation from human urine with added reagents to simulate positive human samples for each of the analytes on the Multistix® 10 SG strips; leukocytes, 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. 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
S. A. SAKYI1, D. K. YEBOAH-MANU2, S. ABOAGYE2, I. OTCHERE- DARO3, A. LIAO4, R. E. DADZIE5. 1KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, Ghana, 2NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH, ACCRA, Ghana, 3APTAGEN LLC, YORK, PA, 4UNIVERSITY OF CAPE COAST, CAPE COAST, Ghana

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 morbidity 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 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-276
Discrepancies in Hematocrit and Hemoglobin Measurements by Point-of-Care (POC) Devices and Laboratory Instruments in Patients with Hemodilution

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/ CPT (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 compared in surgical Intensive Care Unit (ICU) 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 PO2.

Laboratory glucose was measured in venous plasma samples on the Vitros® 6600 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 PO2, 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 antihypertensive (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 PO2 ranged from 53 to 230 mmHg without correlation between PO2 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-275
Inaccurate Point-of-Care Glucose in a Critically Ill Patient due to Norepinephrine Administration
C. B. Vona1, M. Blank2, R. J. Elin1, J. J. Miller1. 1University of Louisville, Louisville, KY, 2University of Louisville Hospital, Louisville, KY

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 PO2.

Laboratory glucose was measured in venous plasma samples on the Vitros® 6600 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 PO2, 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 antihypertensive (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 PO2 ranged from 53 to 230 mmHg without correlation between PO2 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.

Wednesday, July 29, 9:30 am – 5:00 pm
calculated based on HGB, which was measured by osimetry 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 = 0.84X + 4.39 (r = 0.92); i-STAT (Y) vs Sysmex (X): Y = 0.99X + 0.09 (r = 0.92); Radiometer (Y) vs Sysmex (X): Y = 1.14X - 3.70 (r = 0.98).

**HGB**
i-STAT (Y) vs Radiometer (X): Y = 0.86X + 1.68 (r = 0.91); i-STAT (Y) vs Sysmex (X): Y = 0.89X + 1.29 (r = 0.90); Radiometer (Y) vs Sysmex (X): Y = 1.04X + 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 mostly 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**

V. Parrillas, D. Prieto, P. Oliver, P. Fernández-Calle, M. Alcaide, A. Buño, La Paz University Hospital, Madrid, Spain

**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 monitoring lactate levels in real-time monitoring of lactate at the patient’s bedside. Similarly, 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:**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>No Sample Mixing</th>
<th>Rock and Roll Mixing</th>
<th>Vortex Shaking</th>
<th>8-3 Axis Mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺</td>
<td>p = 0.551</td>
<td>p = 1.000</td>
<td>p = 0.263</td>
<td>p = 1.000</td>
</tr>
<tr>
<td>Hct</td>
<td>p = 0.343</td>
<td>p = 0.667</td>
<td>p = 0.83</td>
<td>p = 0.070</td>
</tr>
<tr>
<td>tHb</td>
<td>p = 0.403</td>
<td>p = 0.895</td>
<td>p = 0.652</td>
<td></td>
</tr>
</tbody>
</table>

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**

G. J. Berry⁴, O. O. Oladipo², D. Wittnebert², P. Mann¹, M. Loeffelholz¹, J. R. Petersen¹, UTMB, Galveston, TX, ¹Memorial Medical Center Hospital, Port Lavaca, TX

**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 and 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™.

| Categories | Alere™ I | BD Veritor™ | % Agreement
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>7</td>
<td>6</td>
<td>85.7</td>
</tr>
<tr>
<td>Influenza</td>
<td>3</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>14</td>
<td>92.9</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

**B-280**

Evaluation of Siemens Immulite and CLINITEST hCG Assays for Recognition of hCG Variants in Urine


**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, hCGα, hCGß, hCGßcf 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 hCGßcf 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


**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 e Dx 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 association 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 e Dx automated system is fully developed as a programmable automated system for sample to results for all assays. Clinical data presented here for hsTnT, hsTnl 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 hs-CRP). 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 hs-CRP 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.
### B-283

**A Comprehensive Database of Pediatric and Adult Reference Intervals for Biochemical Markers based on the Canadian Health Measures Survey (CHMS)**

**V. Higgins**\(^1\), M. Nieuwuesteeg\(^2\), J. E. Raizman\(^1\), Y. Chen\(^1\), S. L. Wong\(^1\), D. Blais\(^1\), K. Adeli\(^1\).

\(^1\)CALIPER Program, Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada, \(^2\)Health Analysis Division, Statistics Canada, Ottawa, ON, Canada

**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, ALP, 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.

**Methods:**
Ortho Vitros 5600 analyzer was used to measure 23 chemistry based analytes on two ARCHITECT c8000 systems using different reagent lots. Some marked differences.

### B-284

**Assessing Analytical Quality of Hb A1c Assays Using Accuracy Based Grading and Sigma Metrics**

D. Armbruster\(^1\), G. Maina\(^1\), J. Litten\(^1\), S. Jortani\(^2\), S. Pickering\(^1\).

\(^1\)Abbott Laboratories, Lake Villa, IL, \(^2\)Winchester Medical Center, Winchester, VA, \(^3\)Kentucky Clinical Trials Laboratory, Louisville, KY

**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 Glycosylated hemoglobin 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 (\(\text{Sigma} = (\text{Tea} - \text{bias})/\% \text{ CV}\)).

**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:

<table>
<thead>
<tr>
<th>Assay</th>
<th>Target Value (% A1C)</th>
<th>Architect Bias (%)</th>
<th>Architect Precision (%CV)</th>
<th>Roche Bias (%)</th>
<th>Roche Precision (%CV)</th>
<th>Tosoh Bias (%)</th>
<th>Tosoh Precision (%CV)</th>
<th>Bio-Rad Bias (%)</th>
<th>Bio-Rad Precision (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott Next 6/8</td>
<td>4.99</td>
<td>1.4</td>
<td>1.0</td>
<td>5.0</td>
<td>4.6</td>
<td>1.8</td>
<td>3.4</td>
<td>3.8</td>
<td>3.3</td>
</tr>
<tr>
<td>Abbott G8/8</td>
<td>5.70</td>
<td>0.9</td>
<td>0.9</td>
<td>6.9</td>
<td>1.8</td>
<td>1.6</td>
<td>2.9</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Roche G8/8</td>
<td>6.72</td>
<td>0.3</td>
<td>0.8</td>
<td>3.7</td>
<td>2.9</td>
<td>4.0</td>
<td>3.4</td>
<td>2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Tosoh G8/8</td>
<td>7.55</td>
<td>1.1</td>
<td>0.6</td>
<td>0.8</td>
<td>1.9</td>
<td>6.5</td>
<td>1.2</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Bio-Rad G8/8</td>
<td>8.44</td>
<td>1.7</td>
<td>0.5</td>
<td>0.8</td>
<td>1.1</td>
<td>5.6</td>
<td>1.3</td>
<td>2.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Abbott G8/8</td>
<td>9.33</td>
<td>2.9</td>
<td>0.0</td>
<td>0.9</td>
<td>1.5</td>
<td>6.5</td>
<td>1.2</td>
<td>2.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Abbott Next 5/8</td>
<td>10.36</td>
<td>1.4</td>
<td>0.3</td>
<td>2.0</td>
<td>1.2</td>
<td>3.9</td>
<td>4.9</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Abbott G8/8</td>
<td>11.26</td>
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<td>1.5</td>
<td>4.4</td>
<td>1.6</td>
<td>1.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**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**

D. Armbruster\(^1\), D. Yahalom\(^2\), L. Lennartz\(^1\), M. Orth\(^1\).

\(^1\)Abbott Laboratories, Lake Villa, IL, \(^2\)ABBOT Laboratories, Dallas, TX, \(^3\)ABBOT Laboratories, Wiesbaden, Germany, \(^4\)VINZENZ von Paul Kliniken, Institute for Laboratory Medicine, Stuttgart, Germany

**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 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 \(-75^\circ\)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.

<table>
<thead>
<tr>
<th>Assay</th>
<th>N</th>
<th>Correlation Coefficient (r)</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline Phosphatase</td>
<td>57</td>
<td>0.9695</td>
<td>1.02 (1.01, 1.04)</td>
<td>-1.09 (-1.33, -0.85)</td>
</tr>
<tr>
<td>Amylase</td>
<td>51</td>
<td>0.9973</td>
<td>0.99 (0.96, 1.01)</td>
<td>2.35 (2.05, 2.65)</td>
</tr>
</tbody>
</table>

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 allowed 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.

Development of New Antibodies for the Specific Detection of Cleaved SLPI to Facilitate More Rapid Testing of Bacterial Infection Status in Chronic Lung Disease

M. S. Twigg1, S. Brockbank1, P. A. Lowry1, C. Taggart1, S. Weldon1, R. Duncan2, S. P. FitzGerald3. 1Centre for Infection and Immunity, School of Medicine, Dentistry and Biomedical Sciences, Queen’s University, Belfast, United Kingdom, 2Random Laboratories Limited, Crumlin, United Kingdom

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 cleared 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 hybridomas 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 were 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: Respective, 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 mg/mL to 2000 mg/mL. 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.

Evaluation of a new Urinary and CSF Albumin Assay on the Beckman Coulter AU5800® Clinical Chemistry System

B. Godber1, H. Parsch2, A. Schmid3, R. Haase1, J. Reynolds1, R. FitzGerald1, S. Jordan3. 1Beckman Coulter Ireland, Co. Clare, Ireland, 2Zentrallabor des Universitätsklinikums, Erlangen, Germany, 3Beckman Coulter, Brea, CA

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 treatment strategies for Alzheimer’s disease, as well as the monitoring of changes in expression levels.
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 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; 50 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 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.

L. E. Kilpatrick, L. E. Kilpatrick. National Institute of Standards and Technology, Gaithersburg, MD

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 could 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 pentamer 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 6420). SEC was conducted by HPLC (Thermo Scientific 3000 UHPLC) 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.

R. Hasanato, W. Tamimi, Z. Shakoor, King Khalid University Hospital, Riyadh, Saudi Arabia. King Abdulaziz Medical City, Riyadh, Saudi Arabia

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-albuminuria and macro-albuminuria 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 macroalbuminuria. 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 (161±104vs1416±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-albuminuria 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.

B-292

Development of a New Rapid Enzyme-Linked Immunosorbent Assay Kit to Detect Gfibrillary Acidic Protein in Human Serum

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Background: Gfibrillary 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 dysfunction or depletion is associated with injury. GFAP is a 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 dysfunction or depletion is associated with injury.

Methods: This method was compared with existing Beckman Coulter Urine and CSF assay demonstrating that the assay is accurate, precise and reliable. Method comparison results show good agreement with existing assays.

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 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; 50 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 Urine/CSF assay demonstrating that the assay is accurate, precise and reliable. Method comparison results show good agreement with existing assays.
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 horseshadish 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. Wieneck, 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 (meizothrombin 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-dependent 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 into 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 carbosylated rPro. The rPro molecules were analyzed for their activity to be activated by both fXa-alone or the prothrombinase complex by SDS-PAGE. Gel electrophoresis revealed fXa-alone exhibited slightly inhibited catalytic activity toward rProWT and rProΔ478-482/W2 when compared 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 prothrombin times for plasma-derived Pro, 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 prothrombin times for plasma-derived Pro, whereas prothrombinase activity towards both rPro molecules was severely impaired.

### B-294

**Evaluation of Seven Commercially Available Clinical Chemistry Assays on the ARCHITECT® c System**

H. Guo1, S. Ning1, G. Sumerdon2, S. Tomazic-Allen1, E. Yang1, P. Yin1, 1Abbott Diagnostics, Shanghai, China, 2Abbott Diagnostics, Abbott Park, IL

**Evaluation of Seven Commercially Available Clinical Chemistry Assays on the ARCHITECT® c System**

Background: Expansion of instrument analyte menu 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:

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Precision: Within-run CV</th>
<th>Accuracy: Bias from Target Value</th>
<th>Linearity (correlation factor r)</th>
<th>LoQ</th>
<th>Correlation (correlation factor r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDP</td>
<td>1.8%</td>
<td>-0.42%</td>
<td>0.9987</td>
<td>0.85 μg/mL</td>
<td>0.9921</td>
</tr>
<tr>
<td>FN</td>
<td>0.8%</td>
<td>-2.99%</td>
<td>0.9993</td>
<td>40 ng/mL</td>
<td>N/A</td>
</tr>
<tr>
<td>GLDH</td>
<td>2.0%</td>
<td>4.7%</td>
<td>0.9991</td>
<td>10.66 U/L</td>
<td>0.9995</td>
</tr>
<tr>
<td>GA</td>
<td>0.8%</td>
<td>-4.69%</td>
<td>0.9998</td>
<td>7.13 μg/dL</td>
<td>0.9917</td>
</tr>
<tr>
<td>H-FABP</td>
<td>6.9%</td>
<td>5.69%</td>
<td>0.9999</td>
<td>2.5 μg/mL</td>
<td>0.9838</td>
</tr>
<tr>
<td>MPO</td>
<td>3.2%</td>
<td>0.75%</td>
<td>0.9975</td>
<td>13.97 μg/mL</td>
<td>0.9937</td>
</tr>
<tr>
<td>SA</td>
<td>1.3%</td>
<td>5.4%</td>
<td>0.9999</td>
<td>2.89 μg/dL</td>
<td>0.9918</td>
</tr>
</tbody>
</table>

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 Leadmamba, 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 response®920**


**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 responses®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.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Precision: Within-run CV</th>
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<td>0.85 μg/mL</td>
<td>0.9921</td>
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<tr>
<td>FN</td>
<td>0.8%</td>
<td>-2.99%</td>
<td>0.9993</td>
<td>40 ng/mL</td>
<td>N/A</td>
</tr>
<tr>
<td>GLDH</td>
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<td>0.9998</td>
<td>7.13 μg/dL</td>
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</tr>
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<td>H-FABP</td>
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<td>0.9999</td>
<td>2.5 μg/mL</td>
<td>0.9838</td>
</tr>
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<td>MPO</td>
<td>3.2%</td>
<td>0.75%</td>
<td>0.9975</td>
<td>13.97 μg/mL</td>
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</tr>
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<td>0.9999</td>
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<td>0.9918</td>
</tr>
</tbody>
</table>
Development of a latex-enhanced immunoturbidimetric assay for the measurement of L-FABP levels on automated clinical chemistry analyzers

K. Kobayashi1, A. Kamijo-Ikemori2, T. Sugaya2, T. Matsumoto1, M. Asami2, M. Ikegami1, S. Kitahara1, H. Takahashi1, M. Yamamoto1, H. Yago1, K. Kimura1, Y. Shibagaki1, M. Takahara1, SEKISUI MEDICAL CO., LTD., Ibaraki, Japan, 1Division of Nephrology & Hypertension, Department of Medicine, St., Kanagawa, Japan, 2Tokyo Takanawa Hospital, Tokyo, Japan

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 37°C. 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 CV (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 CV (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, 4000 mg/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.
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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 ≤ 10% for samples in which MTX levels ranged 0.040 to 15.000 µmol/L and a total CV ≤ 10% for samples in which MTX level > 12.500 µmol/L (n = 86). Deviations from linearity were ± 10% within the range of 0.040 to 15.000 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 DX/LX MS FTC MTX II (FTDx) generated a Passing Bablok correlation as [ARCHITECT] = 0.005 + 0.946x [FTDx] for samples ranging 0.040 - 0.993 µmol/L (n = 86) and [ARCHITECT] = -0.004 + 1.016x [FTDx] for the samples ranging 0.400 - 888,000 µmol/L (n = 119), respectively. Method comparison ARCHITECT Methotrexate to LC/MS/MS ACUTY 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. 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 Immunnoassay 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 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 yields 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+norpufrenorphine, 0.01 vs. 8.9%; diazepam+nordiazepam am+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


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 328.1-233.0, RT 3.91 min; and 6-MAM, MRM 328.1-316.1 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 quantitated 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 with the other RT 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 without detectable 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 µg/mL) to obtain concentrations of 0.5, 2.0, 5.0 and 10.0 µg/mL. 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, Validated linearity testing material (Maine Standards Company, Cumberland Foreside, ME, USA) was purchased, aliquoted and distributed for analysis. Target validation linearity samples are prepared gravimetrically and three intermediate concentrations by mixing appropriate proportions of low and high material. Tobramycin immunoassays 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 homogeneous enzyme immunoassay (HEIA) method. Analyses were carried out within 48% 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 µg/mL 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 µg/mL 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 overdose 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 Biphelyn 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 anxiolytics/ 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 MolanTm 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 was used 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 Biphelyn 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 LLLOQs 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.

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 the ADVIA® 1800 Clinical Chemistry System (Siemens, Malvern, PA). Eleven drug classes were tested including amphetamine(AMP), barbiturate(BAR); benzodiazepines(BZO), cocaine(COC), methamphetamin(MAMP), opiates(OP1) including methadone(MTD) and oxycodone, phenycyclidine(PCP), tricyclic(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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Total Discrepant</th>
<th>MEDTOX + Advia -</th>
<th>Advia + MEDTOX -</th>
<th>McNemar's Test</th>
<th>Chi-Square</th>
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<tr>
<td>Benzodiazepines</td>
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<td>0</td>
<td>P=0.13</td>
<td>P=0.22</td>
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<td>Oxycodeone</td>
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<td>0</td>
<td>P=0.25</td>
<td>P=0.34</td>
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<td>Tricyclics</td>
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<tr>
<td>Methamphetamine</td>
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<td>P=0.67</td>
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<tr>
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<tr>
<td>Total Discrepancies Individual Assays</td>
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<td>12</td>
<td>5</td>
<td>P=0.14</td>
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</table>

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.
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: Ilicit 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 1 g 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 temperature. 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 quadrupole mass spectrometer with an electrospray source monitored in positive and negative ion modes.

Results: Specificity was assessed by retention times and qualifier/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 agonists - 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 Phenomenex 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.

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 narcotic potentiating the toxicity of other solvents, particularly carbon tetrachloride hepatotoxicity and neurotoxicity hexane (inhibition of the biotransformation metabolite 2,5-cyclohexanediol).

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 polyurethane 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 gas chromatography with flame ionization detection. The method consists in a simple extraction of MEK using 2-propanol as internal standard by evaporation and sampling the vapor above the fluid (blood, urine or others) after reaching thermal equilibrium gas in a closed vial. The volatilized components are aspirated by a syringe and injected into chromatograph. The extraction involves the addition of 5 ml diluent solution containing the internal standards. All stock standards were 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 Phenomenex 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.

The linear analytical range of the procedure was between 1.0 and 6.0 mg/L. Accuracy, precision, repeatability and reproducibility, detection limit, quantification limit and matrix effect. The calibration curves for all compounds were linear with r² > 0.9993, respectively. Correlation of this method with a reference lab had a regression of y=1.0642x +0.0827, and correlation coefficient (γ)=0.991. The AMR for posaconazole 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=0.03x +0.131, and R²=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 R²=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=0.9548x -0.0338, and R²=0.997. 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.

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 in a wide range of matrices, such as veterinary drugs in animal tissues, mycotoxins in grains, poylectic 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-extracts, 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 0.9983 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.

Measurement of synthetic cannabinoids in human urine by liquid chromatography-tandem mass spectrometry


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, ∆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 50°C for 30 minutes. The synthetic cannabinoids and internal standards were extracted by solid phase extraction using Bond Elut SPEC C18 3mL (Agilent Technologies, Santa Clara, CA). The samples underwent separation via liquid chromatography using a Kinetex™ 5μm C18 30x4.6mm 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 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 (R2) 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.
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 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 91.2% 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 bu fer.

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. Cocaethylene (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 cardiovascular 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 R²>0.998 and cut off value of 50 ng/mL. Linearity for EtS and EtG ranged from 200- 50,000 ng/mL with R²>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.

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**TDM/Toxicology/DAU**

**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. Competitive chemiluminescent immunoassays were employed. The capture antibodies are immobilised and stabilised on the biochip surface defining microarrays. The immunoassays 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, meperidine, synthetic cannabinoids, dextromethorphan, tricyclic antidepressants, meperidine, methylphenidate, mitragynine and ketamine. The Quantisal Oral Fluid Collection Device was used following manufacturer’s instructions.
**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 drugs are metabolized to 10-monoxykynine 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 essay 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 Precision Baccle 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.

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**B-317**

ARK™ Lacosamide Assay for the Beckman AU480 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. 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 (0.00, 1.50, 7.00 and 15.00 µg/mL) and 3 controls (3.00, 10.00 and 30.00 µg/mL). The performance of the ARK assay was evaluated on the 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 µg/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 Precision Baccle 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.

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**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 33.6-1432.9ng/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, hemocrit) and exogenous (including; insulin, intralipid, K2EDTA, Vitamin B12 and other immunosuppressant agents) potential interfering compounds were tested. Cyclosporine spiked recovery ranged from 91-99% for samples over the range 49.3-1317.1ng/mL. Tacrolimus spiked recovery ranged from 94-101% for samples over the range 3.0-25.1ng/mL.

**Conclusion:**
- 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

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**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, DiscoverTM Multi-Panel Drug Test Cup, MEDTOX® urine drug test cassette, BioRad TOX/SeeTM 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/SeeTM) do not necessarily outperform waived products (DiscoverTM). 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®, DiscoverTM and TOX/SeeTM showed the best performance. The advantage of MEDTOX® is that it uses a reader to read the results, thereby removing subjectivity from individual. DiscoverTM cup is a waived product, therefore is subjected to less regulatory requirements. This makes it simpler to implement as an alternative to performing immunoassay screen on the Chemistry analyzer.

<table>
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<th>Drug Class</th>
<th>Alere iScreen Dx</th>
<th>DiscoverTM</th>
<th>MEDTOX®</th>
<th>BioRad TOX/SeeTM</th>
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**B-320 Should mass spectrometry be used for urine drug screens in peripartum patients?**

Y. Xie, R. A. Dahal, A. O. Okorodudu, Anthony O. . University of Texas Medical Branch, Galveston, TX

**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 placenta, fetal death, preterm labor, and intraterine 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 opiates and metabolites (morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, noroxycodone, and 6-monooxyethy morphine) 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 (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 (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.

**Results:** Preliminary results are as follows: the mean age was 42.5 years (range: 1 day-93.2 years). Out of 405 samples, 32.4% were female, 31.4% were African Americans, and 60.3% were Caucasians. The enzyme immunoassay result distribution was as follows: 67.4% (>=50 ng/mL), 14.0% (30-50 ng/mL), 12.5% (20-30 ng/mL), 6.7% (50-300 ng/mL), 2.9% (>=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 88%, 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 employ 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 ml 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 (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 Bisod BiureiCheck 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 6.6 - 11.3% CV (L1 Biorad QC). Linearity was verified in the range -0.5 - 50 mmol/L for each analyze and the LOQ was calculated to be between 0.25 and 0.40 mmol/L for each analyze by calculating the noise in the lowest calibrator (10 µL) 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-end range-high) for all analytes, although EG exhibits more variable 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 tolune (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 derivatization to obtain adequate sensitivity for clinical specimens.

**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 Chromatography-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 is 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-327**

**Monitoring of micafungin use in clinical practice using high-performance liquid chromatography with fluo scence 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 98.8 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 side 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 immunosuppressants 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 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.
High-Throughput Urine Analysis to Detect Buprenorphine and Ethanol Use by Multi-channeling LC-MS/MS


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-D3 and Norbup-D3, 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-D3 and EtS-D3. 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 (r² > 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.

Milrinone therapeutic drug monitoring in pediatric cardiac surgery

J. E. Raizman, K. Adeli, D. Colantonio. Division of Clinical Biochemistry, Pediatric Laboratory Medicine, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

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. Calibrators 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 ng/L. The lower limit of quantification is 5.9 ng/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 ng/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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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 5%). 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.

**B-333**

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 (SA), 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 SA-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), SA (10 mg/kg), and SA 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 chemoattractant 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 SA group compared to control group. On the other hand, thymoquinone suppressed these laboratory signs, which are thought to be the characteristic signs of SA toxicity, most probably by its ameliorative effects including anti-inflammatory and antioxidant properties.

CONCLUSIONS: In conclusion, As causes tissue damage by disintegrating metabolic responsive's and regulations, vitiating antioxidant systems, decomposing immune competent cells, and finally inducing DNA damages. Therefore, it may be concluded that the results of this study reflect the characteristics of SAs toxicity, most probably by its ameliorative effects including anti-inflammatory and antioxidant properties.

**B-334**

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 aliquoted 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.

**B-336**

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 sample. 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 sample. The method is based on the ionization of the analyte with a flame in the gas chromatograph and the detection of the ionization products by a flame ionization detector.

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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.

The determination of methanol in urine and plasma by headspace gas chromatography autosampler and flame ionization detector was carried out with the aim of validating a fast, easy and cheap method for the determination of methanol in urine and plasma by headspace gas chromatography with flame ionization detection.

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The method is based on the ionization of the analyte with a flame in the gas chromatograph and the detection of the ionization products by a flame ionization detector.
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 immunosassay (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 immunomassay (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 weeks 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 concentration of 12.5 ng/mL 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.

A retrospective study of urine drugs of abuse screening positivity rates by immunomassay 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 immunomassay 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 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 halluciocinogen 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.

Improved, simple, accurate Tacrolimus assay without Manual Extraction for the Dimension RxL and EXL systems


Background: The new Dimension® Integrated Chemistry Systems Tacrolimus (TAC) assay provides evidence 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-fl-galactosidase conjugate is added next and allowed to react with tacrolimus from the patient sample. Finally, pre-decorated chrome particles coated with a tacrolimus monoantibody are added, 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 assay 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 (LCMS/MS)+0.30; r = 0.97 (n=515, 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 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 versus LCMS show excellent agreement with the new TAC assay. The new antibody and conjugate provide more sensitivity and better precision.

Conclusions: The percent of 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.
Studies on Profiling of Drugs in the Clinical Urine Samples using Automated ToxPrep Method on ToxPrep™ 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 hydrolyzing 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 ToxPrep1™ 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 R²=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 LCMS/MS 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 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 C50, 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 C50 (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 C50. However, normalizing of the data to the internal standard and establishing a new normalized area cut-off at the C50 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.

The Development of an LC-MS/MS Screening Method for 104 Targeted Compounds in Whole Blood, using Library Searching on a QTRAP Mass Spectrometer

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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.

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, 2.6µm) 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.
Validation of Peritoneal Dialysis Fluid Assays: Does Analytical Performance Meet Clinical Need?


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 hypertonic 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 Main 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), cefazidime (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(-20 to -24°C, n=8) temperatures in plain tubes and NaF/K-oxalate tubes for glucose. Transporter categories were determined using glucose (PD/4hr gluc/serum), and creatinine (PD/serum) ratios for a cohort of 25 patients, and Kt/V calculated as (24hrPD/ Serum*24hrDrainVol/7)/(VolUreaDistribution) where Serum varied from 40mg/dL to 80mg/dL. A bias in PD/4hr gluc and PD/serum was simulated by systematically adding -15 to +15% glucose, -0.2 to +0.2 mg/dL and oxalate tubes for glucose. Transporter categories were determined using glucose (PD/4hr gluc/serum), and creatinine (PD/serum) ratios for a cohort of 25 patients, and Kt/V (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.

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=96.7%(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 <=1%, and urea nitrogen <=1.3%. 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%(-4.8%-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%. PD fluid meets what is required for clinical use of the assays.

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.

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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 likelihood 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 test strips and particle analysis in urinary 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.

**Effectiveness of Urine Sediment Test Strips for Diabetic Status 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 glyceria 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 fructoseamine-3-kinase (F3K) (1 µg/ml; 37°C) was used to analyse effects of protein deglycation.

Nail clippings of 25 diabetic and 25 healthy subjects were collected and subjected to FT-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 of 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.

**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 in positive electrospray ionization mode, the panel of interest is usually split between positive 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, 2.7µm) 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 Prominance 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
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.

Step 1: Ac-DAA + DAA1 → Zinc ion + DAA + CH3COOH
Step 2: DAA + DAAO → α-keto acid + NH3 + H2O2
Step 3: H2O2 + Trinder + 4AA → POD Color + H2O
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 63 - 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 analytic 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.

Paper test card for quantifying iodate in fortified salt

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University of Notre Dame, Notre Dame, IN

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 of 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 µL 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 detection were 97.0 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.

Analytical evaluation of the 25-OH Vitamin D total assay on the BioPlex® 2200

M. Abou El Hassan, D. C. Lin, T. Earle, M. Millar, I. M. Blasutig.
University Health Network, University of Toronto, Toronto, ON, Canada

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 D3 and 25-OH D2 against a gold-standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) method and the DiaSorin LIASON® 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 LIASON (n=56) was assessed using patient samples with varying amounts of 25-OH D3 and/or 25-OH D2. 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 R2 of 0.9988. Method comparison revealed a strong correlation between the BioPlex assay and LC-MS/MS for samples containing 25-OH D3 alone (n=5; R2 = 0.999), 25-OH D2 alone (n=118; R2 = 0.953) and both (n=13; R2 = 0.919). A strong correlation with the LIASON assay was also observed (n=56; R2 = 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 LIASON assay. Importantly, the assay is capable of equivalent detection of both 25-OH D3 and 25-OH D2.

An Imaging Reflectometer for Measuring Dry Slides Technology

K. Ryan, J. Kelly, G. Denton.
Ortho Clinical Diagnostics, Rochester, NY

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 along 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 PV1 at 10µL: CREA 2.1%, BUN/UREA 1.17%, PROT 8.25%, ACET 0.75%, Ca 0.55% and ALKP 1.66%. For PVII, CV were: 6.06%, 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 PV1 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 PV1 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.
**Technology/Design Development**

**B-361**

**Evaluation of the Sebia Capillaries 2 FLEX PIERCING Instrument for Determination of HbA1c in a High Volume Laboratory**

J. Reddie, Greenville Health System, Greenville, SC

**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 Capillaries 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 Capillaries 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 HbA1e 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**

L. Nan, X. Zeng, M. Kong, Y. Qian, Y. Wu, D. Yu, Ningbo Health Gene Technologies, Ningbo, China

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 SNPdetection 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**

K. Rehfeld1, J. Stackawitz2, 1STRATEC Biomedical AG, Birkenfeld, Germany, 2Quotient Biodiagnostics, Newtown, PA

**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 dispens technology and reagent concept is realized on the MosaQ™ 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 on board 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 elagic acid with addition of cinnamaldehyde for the treatment of triple negative breast cancers**

Z. Lin1, S. Jiang2, X. Zhang1, C. Mohan1, T. Wu1, 1University of Houston, Houston, TX, 2University of Wuhun, Wuhun, China

**Background:**
Triple-negative breast cancer (TNBC), a highly aggressive malignancy, accounts for about 10%-20% of breast cancer, yet without effective molecularly targeted therapies.

**Methods:**
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 on board 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.

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**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**

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L. Nan, X. Zeng, M. Kong, Y. Qian, Y. Wu, D. Yu, Ningbo Health Gene Technologies, Ningbo, China

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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 efficient, 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.
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.

**Background:**

B. Medaugh, R. H. Brown. MedTest, Canton, MI

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.

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**Table 1** Inaccuracy of the proposed method for lyophilized and liquid serum specimens (nmol/L)

<table>
<thead>
<tr>
<th>Cations</th>
<th>Specimen</th>
<th>IC</th>
<th>CV(%)</th>
<th>Reference Value</th>
<th>Bias(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>Serum</td>
<td>132.79</td>
<td>0.36</td>
<td>133.00</td>
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<td>Na</td>
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<td>126.73</td>
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<td>0.62</td>
<td>129.88</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Mg</td>
<td>Serum</td>
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<tr>
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<tr>
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<td>Ca</td>
<td>2013 RELA-B</td>
<td>2.866</td>
<td>0.86</td>
<td>2.879</td>
<td>-0.45</td>
</tr>
</tbody>
</table>

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**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.
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 place/Mediterranean 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 ACOG guidelines.

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.

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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

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B-375

Initial Implementation of a Risk Based Quality Control Scheme Using Bio-Rad Mission: Control Software

M. A. Cervinski1, F. A. Polito1, L. Kuchipudi1, Dartmouth-Hitchcock Medical Center, Lebanon, NH, 2Bio-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 125 mmol/L (0.847) and 152 mmol/L (0.732). Using the running mean and SD in the QC rule, a SEC = 1+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 126 mmol/L (1.2) and 153 mmol/L (1.2) is predicted to detect a SEC = 2.9 mmol/L. 0.2 QC events with a false rejection rate of 0.4%. Narrowing the fixed SDs to 1.1 mmol/L at 126 mmol/L and 1.0 mmol/L at 153 mmol/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 risk 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 efficiency 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.

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**B-377**

**Enzymatic HbA1c assay on ARCHITECT c8000: Effect of extended wait time prior to sampling**

S. Reed1, L. Stubbs2, R. Wonderling2. 1St. Joseph Regional Health Systems, Bryan, TX, 2Abbott Laboratories, Abbott Park, IL

**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.

**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. 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.

**Results:** Table 1: HbA1c results for 14 different samples tested at different time points after mixing.

<table>
<thead>
<tr>
<th>Sample No.</th>
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<th>0.5 hr</th>
<th>1 hr</th>
<th>1.5 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>5 hr</th>
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**Conclusion:** 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.

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**B-378**

**New Solution to Patients Identification with Laboratory Information System**

L. Wang1, Y. Tsai1, Y. Yang1, H. Chou1, L. Wu1, J. Li2. 1Chi-Mei Medical Center, Tainan City, Taiwan, 2Chi-Mei Medical Center, Chiali, Tainan City, Taiwan

**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 mismixed 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 through a process that is electronically recorded, 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, 14 mislabeled incidents of 18322 samples were found; however, in 2014, no mislabeled case was reported (N=19016). Besides, the records indicated that 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 incidence of misidentified and miscollected transfusion samples were totally eliminated.
Enhancing Quality Control in Clinical and Point of Care Settings

S. Mansouri, M. Wright. Instrumentation Laboratory, Bedford, MA

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.

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 (r² ≥ 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) (r² = 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 (r² = 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.

Overall Equipment Effectiveness (OEE) as a Tool to Improve Productivity in a Clinical Laboratory (Dasa, Brazil)

L. C. M. Silva, F. Niglio, O. Fernandes. DASA, São Paulo, Brazil

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 endocrinology 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.

Implementation of equipment management and its impact on a Clinical Laboratory production

J.R. OLIVEIRA, L. NERY, L. LOPES, R. CARDOSO, R. JACOMO.
LABORATORIO SABIN, BRASILIA, Brazil

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. Engcompány, Itajai, 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 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: Implementing an electronic management process to the equipment led to higher delays and trustful results.

Development of an Automated Solid Phase Extraction of Procainamide in Serum Samples


Background: In determining patient compliance and assessing dosage of antiarrhythmic drugs like procainamide (C₉H₁₉N₃O), quantification in the patient serum or plasma samples is essential. As the demand on sample processing and analysis increases, ensuring that the processes were adequate. In essence, OEE worked as the missing link between capex implementation and production processes.

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 Plastisol ODS column.

Results: Positional biases were investigated on the VERSA 10 SPE by examining the recovery of 20ng 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.

Comparison of Two Automated Microscopy Image-Based Urine Sediment Analyzers

A. Yaman1, F. Gerin2, O. Baykan3, M. Arpa4, P. Vatansever1, O. Siricki5, G. Haklar1, 1Marmara University, Istanbul, Turkey, 2Central Laboratory of Public Health, Istanbul, Turkey, 3Ataturk State Hospital, Balikesir, Turkey, 4Rize Recep Tayyip Erdogan University, Rize, Turkey

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. Nevertheless, 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 (Diriü Industri, 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 show 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.

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Wednesday, July 29, 9:30 am – 5:00 pm

**B-385**

**On-line flagging monitoring - A new quality management tool for the analytical phase**

K. Geossens, K. Van Uytfanghe, L. Thiendonp. Ghent University, Gent, Belgium

**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.theflagge.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; lab enterprise consisting of 6 hospital labs 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**


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 skyrocketing cost of laboratory tests.
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 for 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.

Assessment of utility of daily patient results averages as adjunct quality control in a weekday-only satellite chemistry laboratory


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, ±0.07 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, ±0.24 mmol/L, 0.74; Na, ±0.48 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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