Wednesday, August 1, 2018

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

Proteins/Enzymes

B-366

Diagnostic Performance of Enhanced Liver Fibrosis (ELF) test in Predicting Liver Stiffness

E. Nah[1], S. Cho[1], S. Kim[1], H. Cho[2]. *[1]Korea association of Heath Promotion, Health Promotion Research Institute, Seoul, Korea, Republic of, [2]Korea association of Heath Promotion, MEDIcheck LAB, Cheongju, Korea, Republic of*

Background: Fatty liver disease is not uncommon in general population. It represents a wide spectrum of pathologic findings, from simple steatosis to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. The diagnostic assessment of liver fibrosis is an important step not only in the management of patients with chronic liver diseases but also in the assessment of the true burden of liver disease in the general population. Liver biopsy is considered the gold standard for the assessment and quantification of liver fibrosis. But noninvasive techniques including serum biomarkers have been developed to circumvent the need for liver biopsy. Therefore, the aim of our study is to assess the diagnostic performance of enhanced liver fibrosis (ELF) test in predicting liver stiffness by using magnetic resonance elastography (MRE) as a reference standard in health checkups. **Methods:** This study included 89 health examinees who underwent MRE and ELF test at health promotion center in Korea, between July 2016 and December 2016. ELF score was compared with MRE results. Receiver operating characteristic (ROC) curve analysis was performed for ELF test as a predicting test for liver stiffness. **Results:** Area under ROC (AUROC) to predict mild liver stiffness, and moderate-to-severe liver stiffness were 0.613, and 0.891 for ELF test. Optimized cutoffs of ELF to maximize sum of sensitivity and specificity were 8.98, and 10.3 for mild stiffness, and moderate-to-severe stiffness, respectively. **Conclusions:** ELF test demonstrated considerable diagnostic value in predicting liver stiffness in health checkups.

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Targeting TMAO Biosynthesis. Discovery of New Novel TMA Lyase Inhibitors to Protect Atherosclerosis lesion, MI and Stroke

A. S. Duzan. *Cleveland state university, Westlake, OH*

Background: Recent clinical research evidence has marked TMAO as a biomarker molecule associated with several homeostasis disruptions, such as myocardial infarction, atherosclerosis, secondary hypertension, irritable bowel syndrome, chronic kidney disease, strokes, and heart failure. The priority is to stop the biosynthesis pathway of TMAO through inhibition gut microbial TMA lysate CutC/D. Trimethyl amine (TMA) formed in the gut from choline after breaking by anaerobic gut bacteria CutC/D lyase. TMA affects the microbiota environment and human health. TMA is transported to the liver and then is metabolized by flavin monooxygenase 3 (FMO3) to trimethylamine-N-oxide (TMAO). TMAO works as an independent atherogenic factor via increasing macrophages hyperactivity and lowering high density lipoprotein (HDL) level that leads to myocardial infarction, stroke and death. In fact, structure activity relationships (QSAR) provide the ability to predict potent inhibitors. **Methods**: Our analogue synthesis was designed by reacting different electrophilic groups to simple analogous. The synthesis was conducted through a sequence of reactions by nucleophilic substitution reactions, and sometimes de-esterification, amination, alkylation, and sulfonation. After completion the synthesis, characterization and purification process using HRMS, NMR (^{13}C,^{1}H,^{15}N) and FTIR. The biological specific activity measurements of CutC/D enzyme have done for each compound in vitro and in vivo screens using different gut bacterial species and female mice, and quantified it by using AB Sciex QTRAP 5500 LCMS-MS. The leading and the potent inhibitor will be used further for pharmacokinatic measurements. The mechanism of inhibition of the leading compounding was confirmed by time dependent inhibition assay, irreversibility assay, and the measured change in Km & Vmax over using different concentrations. **Results**: We describe the design, synthesis, chemical characterization, and the biological activity of inhibitors derived CutC inhibitor give an IC_{50} in Nano molar range (3 orders of magnitude lower than 3,3-dimethyl-1-butanol, "1stgeneration") (Wang.Z. Cell.2013) in vitro screen, and it shows a high potency in vivo screen. Moreover, our data shows potential results; (1). Synthesis of potent inhibitors that has highly efficacy in vitro and in vivo study; (2). Non-lethal effect on gut microbial community; (3). The leading inhibitor works as irreversible non-competitive inhibitor to get high potency and fewer side effects. (4) Our inhibitor possesses physic-chemical pharmaceutical properties as necessary for drug effect; (5). The leading compounds do show acceptable pharmacokinetic/ pharmacodynamics properties. **Conclusion**: This study shows that inhibitor like-substrate analogous gives a single digit Nano molar inhibition concentration in vitro screen and works irreversible noncompetitive inhibition of TMA lysate CutC/D without any cytotoxicity on gut microbiota or on mice. Docking helps to design and synthesis universal inhibitor that can stop cleaving choline from other species of gut microbiota. Complementary docking studies of this inhibitor to the crystal structure of the CutC enzyme point to specific inhibitory effects of this type of compound and pave the way for further optimization of the chemical structure to increase further the inhibitory potency of this class of compounds.

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Interference of daratumumab in the measurement of the monoclonal peak in patients with multiple myeloma

F. Barrero Alor, Á. Gragera-Martínez, A. León-Justel, I. Vázquez Rico. *Juan Ramon Jimenez Hospital, Huelva, Spain*

Background:
New therapies with monoclonal antibodies are being used more and more in the face of different pathologies. In the case of multiple myeloma, the use of Daratumumab constitutes a therapeutic advance and it is expected that in the future more and more drugs based on monoclonal antibodies will be commercialized. Daratumumab is a human monoclonal antibody IgG1k against the antigen CD38 produced in a Chinese hamster cell line that is being used in the treatment of multiple myeloma. We have a first patient in treatment with this drug in which we consider how to follow up during the treatment. The quantification of the monoclonal peak by capillary electrophoresis is useful for the clinician to see the tendency of the patient's response to treatment, increase and decrease. In IgG myeloma we can not know if the monoclonal peak we are seeing is due to myeloma or daratumumab. In addition, when quantifying the monoclonal component we can not know how much of that peak is due to the monoclonal component and how much to the IgG of daratumumab, making this measurement difficult.
Methods:
We use capillary electrophoresis (Capilarys®, Sebia) for the detection and quantification of the monoclonal component. The identification of the monoclonal component is done by immunofixation (Hydrasys®, Sebia). This is a patient in which there is no monoclonal peak in the graph of capillary electrophoresis and therefore an immunofixation is carried out. In the immunofixation a monoclonal band is observed, we can not differentiate if it is a band due to the drug or because the patient continues to have a monoclonal band due to multiple myeloma. The use of immunocomplex daratumumab / anti-daratumumab (Hydrashift®, Sebia) is proposed to perform immunofixation allowing the differentiation of the monoclonal component and the band due to the drug.
Results:
Once immunofixation was performed using the daratumumab / anti-daratumumab immunocomplex, the disappearance of the monoclonal band was observed, due to the drug and not to the progression of the disease. Therefore, the patient remained in complete remission, did not present a monoclonal component and serum free light chains were normal.
Conclusion:
The use of Hydrashift® (Sebia), although expensive, may be a solution at this time. We consider what will happen when other drugs based on monoclonal antibodies come out. It would be very expensive to have kits for each drug. At the moment we only have one patient in treatment with daratumumab. The option would be to send the sample to a centralized external laboratory that is in charge of immunofixing when these types of drugs are used. This is still necessary because, according to clinical guidelines, immunofixation is a necessary test for the assessment of response to treatment. On the other hand, the use of free light chains could be a more versatile alternative in the case of patients with multiple myeloma treated with monoclonal antibodies.

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Comparisons of Serum Albumin Measurement by Chemistry Analyzer and Electrophoresis

C. Ponchiardi, C. Drinka, G. Haddad, S. Fan. *Boston Medical Center, Boston, MA*

Background: Serum protein electrophoresis (SPEP) is a widely available and inexpensive laboratory test that examines specific serum proteins based on their physical properties. Albumin and five major globulin fractions are identified. In clinical practice, SPEP is indicated when multiple myeloma, macroglobulinemia, amyloidosis, or other protein disorders are suspected. After the SPEP is completed, a densitometric scan of the electrophoresis pattern is performed. Numeric values for each fraction can be obtained based on the percentage of serum total protein, including albumin. Some patients may have an albumin that resulted from the chemistry analyzer (bromcresol green dye) as well. This study is to compare the albumin results from two different methodologies. The findings may prevent duplicate orders and promote cost saving. **Methods:** A total of 214 serum samples were measured for albumin by the bromcresol green dye (Abbott Architect) and amidoblack dye (Sebia Hydrasys 2), ranging from 1.25 to 5.33 g/dL. Comparison analysis was perform in Microsoft Excel. **Results:** Comparison of results from both methods showed high correlation (R^2 = 0.923) with electrophoresis results 20% higher than the chemistry analyzer results. The mean of the chemistry analyzer results was 3.66 g/dL (n = 214), compared to the mean of the electrophoresis results at 3.69 g/dL, demonstrating no clinically significant difference. **Conclusion:** In summary, the albumin results from SPEP, although an estimation and analyzed in an indirect way, are comparable clinically to those from a standard chemistry analyzer. Clinicians should be informed with this finding and be mindful about ordering lab tests. Preventing duplicate albumin orders could save the cost in a long run.

B-370

Evaluation of the reference methods of alkaline phosphatase

Q. Guo, J. Wang, T. Qi, C. Zhang. *National Center for Clinical Laboratories,Beijing Hospital,National Center of Gerontology,Beijing, P.R. China, Beijing, China*

Background: Alkaline phosphatase (ALP or AKP) is widely distributed in human liver, bone, intestine, kidney and placenta and other tissues, and it's of great significance in the clinical diagnosis.And ALP is the very important routine test in serum enzymes determination. Diagnosis,treatment and prognosis of diseases are subject to the accuracy of the test. In consideration of it,this research bases on the reference method recommended by IFCC,to evaluate the performance of the reference methods of alkaline phosphatase (ALP). **Methods:** The reference method was established according to the primary reference procedure for the measurement of catalytic activity concentration of ALP(37°C),which had been published by IFCC. Furthermore, the EP5-A2 and EP6-A protocols were used for evaluation of the precision and linearity range of the methods. Because there is no ALP reference materials in current JCTLM(Joint Committee for Traceability in Laboratory Medicine) list, the samples of RELA[International Federation of Clinical Chemistry and Laboratory Medicine(IFCC) external quality assessment scheme for reference laboratories in laboratory medicine] were used for verification of the equivalence. **Results:** Ultraviolet spectrophotometer, analytic balance, pH meter, electronic thermometer,pipettes and volumetirc flask have been verified and its uncertainty fit to method's requirements.When the temperature controller showed 37.6°C,the temperature in cuvette was equal to 37.0°C. The temperature in cuvette reaches the set temperature after 180 seconds.The CV_r%(The imprecision within run) and CV_T %(Total imprecision) were less than 1%. The results of the RELA within the limits of equivalence provided by IFCC. They can verified the precision and trueness of the reference method. The upper limits of the measurement ranges of ALP were 610U/L. **Conclusion:** The reference method has been established, which can be used widely for reference measurement service and correlative standardization research.

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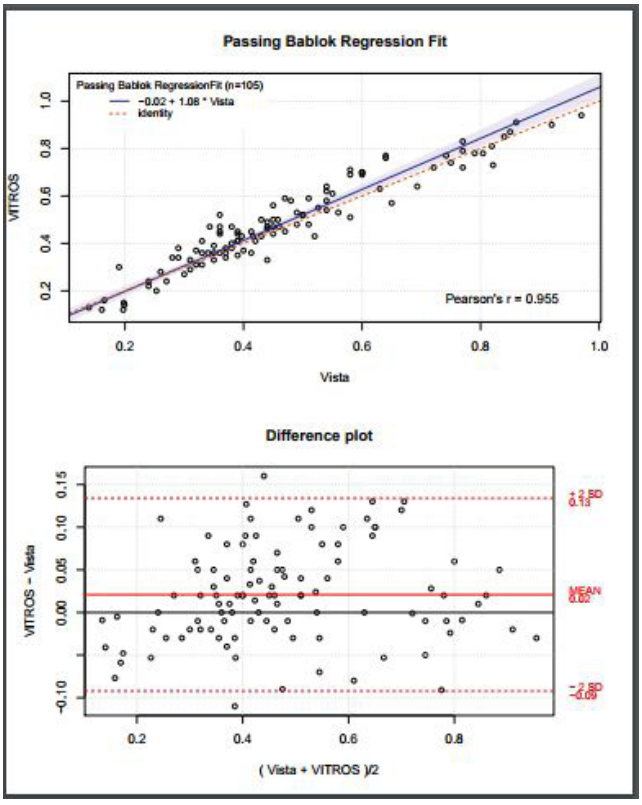
Transference of CSF Total Protein Reference Intervals from the Siemens Vista to the Ortho VITROS

C. McCudden[1], I. Blasutig[2], S. Bookalam[3], T. Ogilby[2], J. Brooks[4], P. R. Bourque[5]. *[1]The Ottawa Hospital, Ottawa, ON, Canada, [2]Children's Hospital of Eastern Ontario, Ottawa, ON, Canada, [3]The Eastern Ontario Laboratory Association, Ottawa, ON, Canada, [4]Dept. of Medicine, Division of Neurology, University of Ottawa, Ottawa, ON, Canada, [5]Dept. of Medicine, Division of Neurology, University of Ottawa; The Ottawa Hospital Research Institute, Ottawa, ON, Canada*

Background: Reference intervals are vital for interpretation of laboratory results. Many existing reference intervals for CSF total protein (CSF-TP) are derived from old literature because of the invasive nature of sampling. The objective of this study was to transfer reference intervals for CSF-TP from a previously validated instrument (Siemens Vista) to the Ortho Clinical VITROS. **Methods**: One hundred and thirty-three CSF samples ordered for CSF-TP testing were compared between the Siemens Vista 1500 and the Ortho Clinical VITROS 5,1; the Vista method uses pyrogallol red and sodium molybdate measured at 600 nm whereas the VITROS method uses a copper-azo dye complex measured at 670 nm. Samples were remaining waste from collection for clinical use and included 100 adults and 33 children aged 0-18 years of age. Values that were below the measuring limit of either method were excluded (n=3, <0.05 g/L Vista; <0.1 g/L VITROS). CSF-TP values >5 g/L were also excluded as they would all be 5x higher than the highest reference value (n=22). Analysis was performed with (n=115) and without (n=105) outliers defined as values >1.5 the interquartile range. Passing-Bablock regression and difference plots were used to compare methods. Bootstrap resampling was used to calculate confidence intervals for the slope and intercept estimates. **Results**: CSF-TP results were similar between instruments, with a slope of 1.09 (1.00-1.19) and y-intercept of -0.021 (-0.063-0.020; Pearson's R=0.85) for all samples; after removal of outliers, the slope was 1.08 (1.00-1.17) and y-intercept was 0.017 (-0.056-0.020) with the same intercept after removal of outliers (Pearson's R=0.96). There was mean bias of 0.02 g/L (-0.19-0.23) for the VITROS with and without outliers. **Conclusions**: The VITROS CSF-TP method agrees well with the Vista method. This indicates that the recently determined age and sex partitioned interval is application to the VITROS method.



B-372

Development of an Assay for Measurement of Transferrin (TRSF) in Urine Samples on Roche Clinical Chemistry Analyzers

C. Nowald[1], E. Sunnick[2], G. Papassotiriou[3], H. W. A. Klima[1]. *[1]Roche Diagnostics GmbH, Penzberg, Germany, [2]Roche Diagnostics GmbH, Mannheim, Germany, [3]Roche Diagnostics International Ltd., Rotkreuz, Switzerland*

Background:
Transferrin is a glycoprotein with a molecular weight of 79570 daltons. It consists of a polypeptide strand with two N-glycosidically linked oligosaccharide chains and exists in numerous isoforms.1,2 The rate of synthesis in the liver can be altered in accordance with the body's iron requirements and iron reserves.Urinary transferrin is considered as a biomarker of glomerular nephropathy with high association to albuminuria. Quantitation of urinary transferrin, when used in conjunction with albumin results, permits an estimation of the charge selectivity of glomerular defects as both proteins are of similar size but different charge. In diabetic patients, urinary transferrin is discussed as an earlier marker of glomerular damage than urinary albumin and increased urinary transferrin excretion might precede the development of microalbuminuria and more advanced tubulointerstitial lesions.
Methods:
Immunoturbidimetric assay.Human transferrin forms a precipitate with a specific antiserum which is determined turbidimetrically.
Development Goals:
-Development of an application for analysis of urine samples based on the existing Tina-quant® Transferrin Ver.2 assay
-Measuring range: ≥ 2.2 - 35.0 mg/L
-In use time Transferrin reagent: 8 weeks (cobas c pack), 4 weeks (cobas c pack large)
-Low sensitivity to drug interference.
Results:
The linear assay range of the TRSFU assay is 2.2 - 35.0 mg/L. Extended measuring range: 35.0 - 105 mg/L.
Limit of Blank: 1.0 mg/L, Limit of Detection: 1.5 mg/L, Limit of Quantitation: 2.2 mg/L with 20 % total error.
TRSFU assay has been standardized against the ERMDA470k/IFCC standard. Precision - CLSI EP5 - 21 days (Repeatability): SD ranging from 0.09 mg/L to 0.25 mg/L, CV ranging from 0.7% to 2.3%, sample concentration range: 4.4 mg/L to 33 mg/L. Method comparison study: Human urine samples obtained on a Roche/Hitachi cobas c 311 analyzer (y) were compared with those determined using the corresponding reagent on a Roche cobas c 501 analyzer (x) (n = 80). Passing/Bablok regression: y = 1.044x - 0.0461 mg/L. The sample concentrations were between 2.46 mg/L and 32.8 mg/L.
Conclusion:
All of the development goals for the urine application of Roche Tina-quant® Transferrin assay were met. The introduction of TRSF urine assay will complete the assay portfolio for screening and monitoring patients with kidney disease.

B-373

Validation of interleukin-5 (IL-5) and interleukin-9 (IL-9) multiplex electrochemiluminescence immunoassay

W. Obeid, D. G. Moledina, J. M. El-Khoury, C. R. Parikh. *Yale School of Medicine, New Haven, CT*

Background: Interleukin-5 (IL-5) and interleukin-9 (IL-9) are produced by type 2 immune responses, and regulate eosinophil migration into tissues, amplify allergic immune responses. IL-5 and IL-9 are being investigated as biomarkers of drug allergies affecting the kidneys. However, data on validation of these assays in the urine is currently not available. Our objective was to develop and validate a multiplex immunoassay panel for measuring IL-5 and IL-9 in urine
Methods: We developed an electrochemiluminescent (ECL) multiplexed assay to measure IL-5 and IL-9 in urine on the Meso Scale Discovery (MSD) U-Plex platform, which allows for flexible multiplexing of immunoassays while requiring a small volume (50uL) of sample. Briefly, the biotin congregated IL-5 and IL-9 capture antibodies were coupled to two different unique linkers. The linker coupled antibodies then assemble themselves on different spots in the U-Plex plate in each sample well. After analytes in the sample bind to their respective antibodies, detection antibodies coupled to SULFO-TAG ECL labels are added to complete the sandwich immunoassay. Once the binding is complete, the concentrations are measured by MESO QUICKPLEX SQ 120 instrument. To validate IL-5 and IL-9 assays in MSD, we performed dilution linearity and spike-recovery experiments. We investigated dilution linearity by performing serial dilutions at three levels (Neat, 1:2, 1:4) assayed in triplicate. We defined acceptance when %CV was less than 5% and recovery was between 80% - 120%. We performed spike and recovery experiment at three different concentration levels (High, Low, and assay diluent) by adding 5% spike volume of mixed IL-5, IL-9 calibrator or assay diluent to the samples. We defined acceptance when recovery was between 80% - 120%. We determined precision using two levels of QC over three days.
Results: IL-5 had a recovery of 83 - 94 %, intra-assay precision of 5.8 %, and a dynamic range of 0.10 to 2080 pg/mL. IL-9 had a recovery of 90 - 97 %, intra-assay CV of 4.1% and a dynamic range of 0.027 - 580 pg/mL. The samples did not require dilution to achieve acceptable recoveries.
Conclusion: We demonstrate that IL-5 and IL-9 can be routinely measured in urine samples using this duplex assay without the need for dilution and with minimal sample preparation.

B-374

Performance evaluation of the general chemistry panel on the Alinity c system

L. Ruvuna, A. Reeves, M. Berman. *Abbott Labs, Abbott Park, IL*

Background: Abbott General Chemistry solutions offer a broad menu of optimized Six Sigma quality chemistry assays that deliver consistent, comparable results across harmonized systems allowing evaluation of over one hundred analytes. The Alinity ci system is part of a unified family of systems that are engineered for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved workflow, and greater throughput with up to 1350 tests per hour. The Alinity ci system has an increased reagent load capacity, holding up to 70 Clinical Chemistry reagents, onboard QC and calibrators, clot and bubble detection ability, and smartwash technology to provide consistent and reliable results.
Objective: To demonstrate the analytical performance of representative assays from the General Chemistry Panel of the Alinity c system, which consists of assays that utilize photometric technology for the quantitative determination of analytes in human serum, plasma, or urine.
Methods: Key performance testing including precision, limit of quantitation (LoQ), linearity, and method comparison were assessed per Clinical and Laboratory Standards Institute (CLSI) protocols. The assay measuring interval was defined by the range for which acceptable performance for bias, imprecision, and linearity was met.
Results: The observed results for precision, LoQ, method comparison, and defined measuring intervals for representative assays in the General Chemistry Panel are shown in the table below.

Assay	Total %CV	LoQ	Method Comparison to ARCHITECT (Slope/r)	Measuring Interval
Pancreatic Amylase	≤ 3.6	2 U/L	0.99/1.00	2 to 2200 U/L
Total Bile Acids	≤ 1.2	0.2 µmol/L	1.01/1.00	1.0 to 180.0 µmol/L
Cholinesterase	≤ 0.9	115 U/L	0.99/1.00	164 to 25,000 U/L
Dibucaine	≤ 2.3	83 U/L	1.00/1.00	83 to 4,000 U/L
UIBC (Unsaturated Iron Binding Capacity)	≤ 3.3	25 µg/dL	1.00/1.00	25 to 500 µg/dL

Conclusion: Representative clinical chemistry assays utilizing photometric technology on the Alinity c system demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT clinical chemistry assays.

B-375

Development of a calibration verification Kit for Analytes in a Synthetic Body Fluid Matrix.

A. P. Nguyen, J. Pawlak, M. Sweatt, R. K. Ito. *LGC Maine Standards Company, Cumberland Foreside, ME*

Introduction: Body fluids are non-vascular liquids normally produced by the human body. Examples include cerebrospinal, peritoneal ascites, pleural and pericardial fluid. Accumulation of these fluids at abnormal levels can be an indication of serious pathological problems. Typically, samples of these body fluids are collected for analysis with methods that are validated for serum, plasma or urine matrices. In 2009-2010, validation of "alternate specimens," which includes body fluids, was implemented in

the CAP checklist. It is required to perform calibration verification for these analytes in a body fluid matrix or its equivalent. Our objective was to develop a liquid-stable, multi-constituent calibration verification and linearity test kit in a synthetic body fluid matrix that represented each individual assay's performance in native fluid in order to meet the clinical laboratory's need for method validation of assays used to test and report results for body fluid specimens. **Methods:** VALIDATE® Body Fluids was prepared with a synthetic body fluid matrix. Each of twelve analytes, albumin, amylase, CA19-9, CEA, cholesterol, creatinine jaffe, glucose, lactate, lactate dehydrogenase, total protein, triglycerides and urea nitrogen, were added to the matrix at individual, predefined concentrations. Recovery targets were optimized to align with the analytical measuring ranges (AMR) for the Roche cobas® 6000. Five levels, with equal-delta concentrations between consecutive levels, were formulated according to CLSI EP06-A. For each level, samples were tested in triplicate. Reported recoveries were evaluated for mean, SD and linearity using MSDRx® (LGC Maine Standards' proprietary linearity software). Limits were applied as 50% of the total allowable error (TAE) for the analyte. To establish product stability claims, samples were subjected to one freeze-thaw cycle, followed by a 72 hour, 30ºC stress condition and four subsequent open-vial events. Real time and stress stability testing is on-going. **Results:** All analytes were determined to be linear and within applied TAE limits through the validated range reported for the Roche cobas® 6000. Linear regression analysis of theoretical vs recovered, for example, glucose was $Y = 0.991\ X + 2.043$, $R^2 = 0.9997$. Other analytes demonstrate similar results. A comparison against the reportable range of the Roche cobas® 6000 demonstrates full coverage for each analyte in the body fluid matrix. All analytes were stable, recovering within 90% of the recovered values on date of manufacture for on-going stability studies. **Conclusion:** VALIDATE® Body Fluids is a liquid-stable, ready-to-use product stored at freezer temperatures. The product is fit-for-purpose as a calibration verification test kit that covers the full AMR for 12 analytes commonly requested for body fluid analysis and the product conforms to CLSI EP06-A guidelines for linearity testing. The VALIDATE® Body Fluids supports laboratorians' method validation for assays used for body fluid specimens. The Roche cobas® 6000 formulation is currently available and listed with the FDA.

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The New Dibucaine CHE (LN 4S21) Assay is Liquid and Ready-to-use

K. Landuyt[1], S. Williams[1], J. De Giorgio[2], R. Lucini[2], F. Vespasiani[2], M. Gramegna[2], K. Bachhawat[1], M. Beischer[1]. *[1]Abbott Laboratories, Irving, TX, [2]Sentinel Diagnostics, Milan, Italy*

OBJECTIVE: To present performance and interference test results of the new Abbott ARCHITECT Dibucaine CHE assay on the *c*System instrument. **RELEVANCE:** The new Dibucaine CHE assay (DIBCH, list number [LN] 4S21) is liquid, ready-to-use, and measures cholinesterase activity in plasma and serum in the presence of dibucaine (a cholinesterase inhibitor). This assay should be used, in combination with the Cholinesterase assay (ChE, LN 6K92), to determine if a patient is at risk for prolonged apnea and paralysis after administration of succinylcholine- or mivacurium-based general anesthesia. **METHODOLOGY:** The new DIBCH assay method utilizes butyrylthiocholine inhibition at 37°C. Cholinesterase metabolizes butyrylthiocholine to thiocholine and butyrate. Thiocholine reduces hexacyanoferrate (III), detectable by its absorbance at λ =404nm, to hexacyanoferrate (II). Dibucaine inhibits butyrylthiocholine metabolism (to thiocholine and butyrate) by cholinesterase. With low thiocholine concentrations, minimal reduction of hexacyanoferrate (III) to hexacyanoferrate (II) occurs and minimal change in absorbance at λ =404nm is observed. A patient's Dibucaine Number (DN) is calculated using DIBCH and ChE assays results (*DN = 100 * [1 – DIBCH_assay_result / ChE_assay_result]*). A patient's DN correlates with their plasma cholinesterase phenotype and speed of recovery from succinylcholine- or mivacurium-based general anesthesia. **VALIDATION:** Table 1 displays performance characteristics of the new DIBCH assay (LN 4S21) relative to the predicate (LN 6K92). The highest acceptable interferent levels for the new DIBCH assay, for low (391-516 U/L) and high (2162-2718 U/L) analyte concentrations respectively are: 7.6 and 64.7 mg/dL conjugated bilirubin, 7.4 and 57.3 mg/dL unconjugated bilirubin, 250 and 2000 mg/dL hemoglobin, 602 and 1205 mg/dL human triglycerides, 62 and 250 mg/dL intralipid, 13.0 and 14.0 g/L protein. **CONCLUSIONS:** The new DIBCH assay (LN 4S21) is liquid and ready-to-use. Like the predicate, it is used in combination with the ChE assay (LN 6K92) to determine a patient's DN.

Table 1. Comparison of performance characteristics of the new Dibucaine CHE assay (LN 4S21) and predicate (LN 6K92) and interference results for the new Dibucaine CHE assay (LN 4S21).

Characteristic	New Assay				Predicate Assay			
Configuration	Liquid, Ready-to-Use				Lyophilized			
Sample Type	Serum and plasma				Serum and plasma			
Sample Volume	4.0 µL				4.0 µL			
Measuring Interval (MI)	Analytical (AMI): 83 – 4000 U/L Extended (EMI): > 4000 – 16000 U/L				44 – 16000 U/L			
Verified Auto-dilution Application	1:4				none			
Imprecision	N	Mean (U/L)	Within-lab SD (U/L)*	Within-lab %CV*	N	Mean (U/L)	Within-lab SD (U/L)*	Within-lab %CV*
	84	109	14	12.9	48	938	54.3	5.8
	84	495	25	5.0	48	1573	83.0	5.3
	84	1244	18	1.5	48	1915	92.2	4.8
	84	1332	28	2.1	-	-	-	-
	84	1971	25	1.3	-	-	-	-
	84	3574	46	1.3	-	-	-	-
Stability: On-Board Calibration	28 days 168 hours				42 days 720 hours			

Study		New (y) vs. Predicate (x)	Abbott ARCHITECT Intraplatform *c*System**
Method Comparison – Dibucaine CHE Assay Result	N	130	127
	R	0.998	0.998
	Equation	y = 1.02x + 30.79	y = 0.97x + 33.68
	Range (U/L)	89 – 3949	99 – 3710
Method Comparison – Dibucaine Number	N	130	-
	R	0.986	-
	Equation	Y = 1.01x – 1.85	-
	Range (U/L)	70 - 91	-

* Per CLSI EP05-A2, the term *total-precision* was replaced with *within-laboratory (within-lab) precision.*
**Intraplatform comparison (ARCHITECT *c*16000 vs. *c*8000) for Dibucaine CHE (LN 4S21).

B-377

Development of a biochip array for the rapid, simultaneous detection of Pepsinogen I, Pepsinogen II and Gastrin 17, on the new random access, fully automated Evidence Evolution analyser

A. M. Flanagan[1], L. Kelly[1], C. McGee[1], S. Ward[1], L. Paloheimo[2], C. Richardson[1], R. I. McConnell[3], J. V. Lamont[3], S. P. FitzGerald[3]. *[1]Randox Teoranta, Dungloe, Ireland, [2]Biohit Oyj, Helsinki, Finland, [3]Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom*

Background: Atrophic gastritis (AG) is associated with a significantly higher risk of developing gastric cancer; the fifth most common cancer worldwide, in addition to enhancing the risk of malabsorption of vitamin B_{12}, iron, magnesium and zinc. AG involves a loss of gastric glands, affecting the secretion of Pepsinogen II (PGII) from all areas of the stomach and Pepsinogen I (PGI) and Gastrin 17 (G17) more specifically from the corpus and antrum respectively. During atrophic corpus gastritis, the levels of PGI in circulation are decreased and the ratio of PGI:PGII is also lowered. G17 is a crucial peptide hormone of the gastrointestinal tract and is secreted by the G cells in the antrum. During antral atrophy the levels of G17 are ultimately decreased. These three biomarkers are therefore valuable in the screening of AG and can provide a comprehensive diagnosis on the condition of the gastric mucosa. Individual enzyme-linked immunosorbent assays (ELISAs) have been developed for the single detection of PGI, PGII and G17 in plasma (Biohit Oyj, Helsinki, Finland). The objective of this study is to utilize Randox's patented Biochip Array Technology (BAT) to develop a multiplex product, which enables the simultaneous detection of PGI, PGII and G17 in a single plasma sample. **Methods:** Simultaneous chemiluminescent sandwich immunoassays were employed, with analyte-specific capture antibodies immobilised on the biochip surface. The immunoassay was applied to the Evidence Evolution analyser, Randox's newest, high throughput analyser. The Evidence Evolution can produce the first set of results in 36 minutes, and one set of results per minute thereafter, enabling rapid sample screening. Functional and analytical sensitivity were assessed to confirm assay performance characteristics. Assay performance was further evaluated through precision and cross reactivity assessments in accordance with Clinical and Laboratory Standards Institution (CLSI) guidelines. **Results:** Nine-point calibration curves for each individual analyte were simultaneously generated with assay ranges 0-300ng/mL for PGI, 0-50ng/mL for PGII and 0-40pmol/L for G17. Functional sensitivity was recorded as 4.26ng/mL for PGI; 0.56ng/ mL for PGII and 0.42pmol/L for G17. Total assay precision (%CV) was determined as 8.2%-10.1% for PGI, 5.9% - 6.6% for PGII and 9.3% - 10.0% for G17. Cross reactivity testing demonstrated that each individual assay was specific for its target analyte (<1% cross reactivity with the other analytes). The array demonstrated no interference with common interferents tested (haemoglobin, bilirubin, triglycerides and intralipids). Good agreement was observed in correlation studies between the new, fully automated analyser system and individual reference ELISAs. **Conclusion:** The results of this collaborative study indicate applicability of the Evidence Evolution for the rapid and simultaneous measurement of PGI, PGII and G17 from a single plasma sample. The use of this biochip array facilitates rapid screening and diagnosis of patients at risk of developing gastric cancer

with a time to first result of 36 minutes. This offers advantages over current methods such as gastroscopy, which can be highly invasive, time consuming and costly.

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Comparison of vitamin D assays ability to detect 25-hydroxyvitamin D in healthy volunteers, dialysis patients, and subjects taking vitamin D2 supplements

K. E. Mullins, R. Christenson, S. Duh. *University of Maryland, Baltimore, Baltimore, MD*

Background: 25-hydroxyvitamin D (25(OH)D) testing and subsequent assay development has rapidly increased in recent years due to increased awareness of the clinical consequences of vitamin D deficiency. Accurate determination of 25(OH)D has proven to be difficult due to the tight association of 25(OH)D to vitamin D binding protein, unequal measurement of 25(OH)D2 and 25(OH)D3, and cross-reactivity of assays to 25(OH)D3 epimers. In this study, we compared the Fujirebio and Abbott 25-hydroxyvitamin D immunoassays to a VDSCP certified LC-MS/MS method. **Methods:** Serum samples from 50 healthy African American (n=25) and Caucasian (n=25) volunteers, 50 African American hemodialyzed patients, and 236 subjects taking varying concentrations of vitamin D2 supplements were assayed for 25-hydroxyvitamin D. Passing-Bablok and Bland-Altman analyses were used to determine Lumipulse and Architect assay correlation to the LC- MS/MS reference method. Agreement between the two immunoassays and the LC-MS/MS method was evaluated by calculating the concordance correlation coefficient (CCC). **Results:** The overall CCC between the two assays and the LC-MS/MS method were 0.6419 (Fujirebio) and 0.465 (Abbott). The CCC ranged from 0.936 (healthy volunteers) to 0.458 (vitamin D2 subjects) for the Abbott assay and from 0.919 (hemodialysis patients) to 0.586 (D2 subjects) for the Fujirebio assay. (See Figure) The overall mean bias (SD) for the two assays were -12.31 (12.5) and -13.51 (16.39) for the Fujirebio and Abbott assays, respectively. The mean bias ranged from -17.57 (17.25) for the vitamin D2 group to 0.56 (3.79) for healthy volunteers when assayed on the Abbott instrument and from -15.59 (13.39) to -4.04 (3.01) for these same groups using the Fujirebio assay. **Conclusions:** Correlation between the two immunoassays and the LC-MS/MS method was poor for the vitamin D2 supplementation group and overall, the Fujirebio and Abbott assays under-recovered 25(OH)D when compared to the LC-MS/MS reference method in all groups.

Table1: Mean 25-hydroxyvitamin D values, Passing Bablok regression and concordance correlation analysis of Fujirebio and Abbott immunoassays against an LC-MS/MS reference method for healthy volunteers, hemodialysis patients, and subjects taking varying concentrations of vitamin D2 supplements.

Population	Method	Mean 25-hydroxyvitamin D values (ng/ml)			Passing Bablok regression		Concordance correlation		
		Mean (95% CI)	*SD*	*Range*	*Slope (95% CI)*	*Intercept (95% CI)*	*CCC (95% CI)*	*r*	*Cb*
Overall N=336	Fujirebio	24.9 (23.4-26.4)	13.8	4-114.1	0.64 (0.588-0.687)	2.20 (1.003-3.125)	0.642 (0.598-0.682)	0.879	0.730
	Abbott	23.7 (22.4-25.0)	12.0	<3.4-119.6	0.52 (0.463-0.588)	4.66 (2.961-5.981)	0.465 (0.412-0.516)	0.721	0.645
	LC-MS/MS	37.2 (34.8-39.6)	22.7	<4-163.4	*Reference method*				
Healthy Volunteers N=50	Fujirebio	20.5 (18.7-22.3)	8.8	5.6-39.2	0.88 (0.777-0.949)	-0.39 (-2.439-0.956)	0.867 (0.798-0.914)	0.958	0.905
	Abbott	25.1 (26.6-29.6)	11.2	7.7-48.3	0.92 (0.805-1.036)	1.38 (-1.043-4.146)	0.936 (0.893-0.963)	0.942	0.994
	LC-MS/MS	24.5 (21.4-27.6)	10.2	7.0-52.0	*Reference method*				
Hemodialysis Patients N=50	Fujirebio	24.9 (22.5-27.3)	15.1	4-89.3	0.78 (0.748-0.816)	1.47 (0.1845-2.642)	0.919 (0.883-0.944)	0.990	0.928
	Abbott	21.8 (18.7-24.9)	13.5	<3.4-91.4	0.60 (0.519-0.715)	4.45 (1.797-5.667)	0.760 (0.656-0.835)	0.909	0.836
	LC-MS/MS	30.1 (27.3-32.9)	19.3	<4-111.8	*Reference method*				
Vitamin D2 Supplementation N=236	Fujirebio	25.8 (21.6-30)	14.3	4-114.1	0.60 (0.538-0.652)	1.34 (-0.3988- 3.18)	0.586 (0.532-0.636)	0.875	0.670
	Abbott	23.9 (20.1-27.7)	11.7	<3.4-119.6	0.47 (0.410-0.546)	4.37 (2.427-6.006)	0.406 (0.346-0.462)	0.737	0.551
	LC-MS/MS	41.4 (35.7-46.5)	24.0	<4-163.4	*Reference method*				

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Performance Evaluation of the Atellica CH AAT, C3, C4, CRP_2 and Hapt Assays

J. T. Snyder, K. Estock, J. Parker, K. Hay, J. Cheek. *Siemens Healthcare Diagnostics Inc, Newark, DE*

Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH AAT, C3, C4, CRP_2, and Hapt Assays on the Atellica CH Analyzer. Measurement of AAT is used in diagnosing juvenile and adult cirrhosis of the liver. Measurements of C3 and C4 are used in determining inherited or acquired diseases, as well as diagnosing inflammatory and necrotic disorders. Measurement of CRP_2 is used in evaluating infection, tissue injury, and inflammatory diseases. Measurement of Hapt is used to aid in evaluating hemolytic disorders. The AAT, C3, C4, CRP_2 and Hapt assays all use antibody reactions that increase turbidity. The turbidity is proportional to the amount of analyte in the sample. **Method:** Performance testing included precision and accuracy. Assay precision was evaluated using the Clinical and Laboratory Standards Institute (CLSI) guideline EP05-A3. Each sample was assayed in duplicate twice a day for 20 days. Method comparison studies were conducted according to CLSI EP09-A3, with patient sample results compared to results from the ADVIA® 1800 Clinical Chemistry System. **Results:** For AAT, within-lab precision ranged from 1.8-2.7% CV in serum samples. For C3, within-lab precision ranged from 1.4-2.0% CV in serum samples. For C4, within-lab precision ranged from 1.3-1.8% CV in serum samples. For CRP_2, within-lab precision ranged from 0.8-2.3% CV in serum/plasma samples. For Hapt, within-lab precision ranged from 2.2-2.9% CV in serum samples. The AAT serum method comparison study yielded a regression equation of y = 0.99x 4 mg/dL with r = 0.994, versus the ADVIA Chemistry 1800 AAT Assay. The C3 serum method comparison study yielded a regression equation of y = 0.99x + 0.6 mg/dL with r = 0.999, versus the ADVIA Chemistry 1800 C3 Assay. The C4 serum method comparison study yielded a regression equation of y = 0.96x + 0.4 mg/dL with r = 0.999, versus the ADVIA Chemistry 1800 C4 Assay. The CRP_2 serum method comparison study yielded a regression equation of y = 0.95x + 0.0 mg/dL with r = 0.989, versus the ADVIA Chemistry 1800 CRP_2 Assay. The Hapt serum method comparison study yielded a regression equation of y = 1.06x + 0 mg/dL with r = 0.997, versus the ADVIA Chemistry 1800 HAPT Assay. **Conclusions:** The Atellica CH AAT, C3, C4, CRP_2, and Hapt Assays tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement with an on-market comparative analyzer.

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Cardiac troponin T degradation - is blood matrix of importance?

W. H. M. Vroemen, D. de Boer, O. Bekers, W. K. W. H. Wodzig. *Maastricht UMC+, Maastricht, Netherlands*

Background: Cardiac troponin T (cTnT) is a preferred cardiac biomarker for acute myocardial infarction (AMI) diagnosis. Recent studies demonstrated immunoreactive cTnT-derived degradation products in multiple pathologies (1-3). However, the cause of cTnT degradation was allocated to a pre-analytical effect caused by serum production (4-6). In this study, we investigated pre-analytical and *in vivo* cTnT degradation in multiple blood matrices and examined its impact on high-sensitivity cTnT (hs-cTnT) immunoassay results. **Methods**: The pre-analytical blood matrix influence on cTnT fragmentation was studied by adding intact cTnT to different blood tubes prior to blood withdrawal from a healthy volunteer. Subsequently, cTnT fragmentation was studied by immunoblotting. In addition, cTnT fragmentation was also investigated in simultaneously collected residual routine blood samples from patients with AMI. cTnT concentrations on residual routine blood samples collected at identical time points were determined by the hs-cTnT immunoassay. **Results**: When supplementing intact cTnT (40 kDa) in blood matrices of interest prior to blood withdrawal, it became apparent that cTnT is immediately and completely degraded to its primary fragment (29 kDa) in serum. Only minor cTnT degradation was observed in EDTA-, citrate- and hirudin-plasma. Li-heparin also showed minor cTnT fragmentation, though a thus far unseen cTnT fragment (26 kDa) was formed in this matrix. Residual blood samples of patients with AMI showed identical results. hs-cTnT immunoassay results on residual routine blood samples collected at identical time points (n=68) revealed no median significant difference between manufacturer approved blood matrices (p>0.05), though clinically significant individual differences were observed. **Conclusion**: This study revealed that cTnT degradation occurs *in vivo* and due to pre-analytical influence that significantly differs between peripheral blood matrices and impacts hs-cTnT immunoassay results at an individual level.

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B-381

Abbott Alinity c System Sigma Metrics for Clinical Chemistry Assays

V. Petrides, S. Schneider, M. Berman, A. Orzechowski. *Abbott, Abbott Park, IL*

Background: Assay performance is dependent on the accuracy and precision of a given method. These two attributes can be combined into a sigma metric, providing a simple value for laboratorians to use in evaluating test methods. In addition to sigma metrics, precision profile charts can be used to visually assess the precision performance of a product across a concentration range. Sigma metrics were determined for more than 17 clinical chemistry assays tested on the Alinity c system. In 2017, a separate and distinct subset of 40 CC assays were analyzed and presented in two AACC posters using similar methods of analysis. **Methods:** A sigma metric was estimated for each assay and was plotted on a method decision chart. The sigma metric was calculated using the equation: *sigma = (%TEa - |%bias|) / %CV*. A precision study was conducted at Abbott on each assay using the Alinity c system per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, >100 samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity c and ARCHITECT *c*8000 systems. The 1st replicate from the Alinity c system was regressed versus the mean ARCHITECT *c*8000 concentration and a Passing-Bablok or weighted Deming regression model was fit. Using the regression model, the %bias was estimated near a critical concentration level. For a subset of assays, a precision profile chart was created by plotting the within-laboratory %CV values versus the mean concentration values for both the Alinity c system and the ARCHITECT *c*8000 system, where the ARCHITECT *c* system within-laboratory %CV and mean concentration values were obtained from the assay package insert. **Results:** The method decision chart showed that a majority of the assays demonstrated at least 5 sigma performance at or near a critical concentration level. The precision profile charts of the within-laboratory %CV results for the Alinity c system overlaid with the ARCHITECT *c* system showed similar performance across the subset of assays evaluated. **Conclusion:** Sigma metrics, method decision charts, and precision profile charts can be valuable tools for evaluating and comparing product performance by providing a comprehensive understanding of expected assay performance. The majority of Alinity c system assays had sigma metrics greater than 5. The precision performance on the Alinity c and ARCHITECT *c* systems was comparable for the subset of assays for which a precision profile was created. Laboratorians can use these tools as aids in choosing high-quality products, further contributing to the delivery of excellent quality healthcare for patients.

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Performance of the Sentinel Diagnostics C-Reactive Protein Ultra (MP)® Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System.

G. Snodgrass[1], K. Ackles[1], A. Cugini[2], R. Lucini[2], D. Muzzini[2], A. Zeberl[1]. *[1]Ortho Clinical Diagnostics, Rochester, NY, [2]Sentinel CH. SpA., Milan, Italy*

Introduction: The Sentinel Diagnostics C-Reactive Protein (CRP) Ultra (MP) is an immunoturbidimetric assay which quantitatively determines the concentration of C-reactive protein in serum or plasma. C-reactive protein is an acute phase protein synthesized in the liver in response to proinflammatory cytokines. CRP binding to ligands exposed during cell death and CRP binding to bacterial surfaces activates the complement cascade and stimulates phagocytosis. Therefore, CRP levels increase in infection, inflammation, tissue infarction, and trauma. CRP measurement may also be used for monitoring response to treatment and screening for infection postoperatively. **Method:** The CRP Ultra MP Assay reagent contains latex particles with adsorbed anti-CRP polyclonal antibody. The antibody binds to CRP in the patient sample resulting in agglutination. The increased turbidity in the reaction solution is detected as an absorbance change at 575nm. The rate of change in absorbance is directly proportional to the concentration of CRP in the sample. The assay is conducted using 2.0 uL of patient sample and the two CRP Ultra reagents. Two-point rate is calculated and converted to a concentration using a cubic spline calibration model. **Results:** The performance of the CRP Ultra (MP) Assay was assessed on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System. We evaluated accuracy with 95 serum samples (0.057 – 33.710 mg/dL) on the VITROS 4600 and VITROS 5600 Systems compared to the VITROS® 5,1 FS Chemistry System. The VITROS 4600 and VITROS 5600 Systems showed excellent correlation with the VITROS 5,1 FS System. VITROS 4600 = 0.99 * VITROS 5,1 FS + 0.07; (r) = 0.995. VITROS 5600 = 1.03 * VITROS 5,1 FS + 0.09; (r) = 0.995. A 22-day precision study conducted on the VITROS 4600 and VITROS 5600 Systems demonstrated optimal precision: CRP patient pools targeted at ≤ 0.5 mg/dL, ~0.5 mg/dL and > 0.5mg/dL resulted in within-laboratory percent coefficient of variation (%CV) of 2.55%, 2.44% and 1.77% respectively, for the VITROS 4600 System and 2.47%, 1.79% and 2.03% respectively, for the VITROS 5600 System. Linearity was evaluated using a 14 level admixture series. The observed linear range for the VITROS 4600 and VITROS 5600 Systems was 0.494 – 33.869 mg/dL and 0.475 – 34.332 mg/dL, respectively. The Limit of Quantitation (LoQ) for the VITROS 4600 and VITROS 5600 Systems was 0.024 mg/dL and 0.023 mg/dL, respectively based on 120 determinations with 10 low-level CRP serum samples. The Limit of Detection (LoD) for the VITROS 4600 and VITROS 5600 Systems was 0.027 mg/dL and 0.026 mg/dL, respectively based on 120 determinations with 2 CRP serum samples. The Limit of Blank (LoB) for the VITROS 4600 and VITROS 5600 Systems was 0.017 mg/dL and 0.014 mg/dL, respectively based on 120 determinations with 2 blank samples. **Conclusions:** The CRP Ultra (MP) assay evaluated on the VITROS 4600 and VITROS 5600 Systems exhibited excellent correlation with VITROS 5,1 FS System, optimal precision, linearity and low end sensitivity.

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High Levels of Serum Lipase without evidence of Pancreatitis in Emergency Room patients: case reports.

C. Villela[1], M. F. M. C. Pinheiro[2], R. Iglezias[1], M. H. Pizarro[1], R. Goes[1], G. A. Campana[2]. *[1]Hospital Samaritano, Rio de Janeiro, Brazil, [2]DASA, Rio de Janeiro, Brazil*

Background: Several illnesses other than pancreatitis can result in increased serum lipase levels, such as reduction of renal clearance, neoplasia, critical illness, non-inflammatory pancreatic disease, diabetes, drugs and infections. Elevation of lipase is also described in patients with acute gastroenteritis, especially younger patient but it is not considered as a marker of poor prognosis. The objective of this study was to describe three case-reports of patients seen in the Emergency Room with increased serum levels of lipase in the absence of pancreatitis. **Methods:** Case reports, with retrospective analysis of medical records. **Results:** CASE 1. A 17-year old male presented with recurrent vomiting and epigastric abdominal pain. Abdomen was tender on palpation. Serum lipase was 1090 U/L. Abdominal ultrasonography was normal. Abdominal CT showed bowel distension and normal pancreas. Four hours later, serum lipase had dropped to 347 U/L. The patient was febrile and developed vomiting, requiring hospitalization. The next day, lipase levels decreased to 117 U/L and the patient was discharged uneventfully. CASE 2. A 37-year old woman presented with a 36-hour history of persistent nausea, with no relief on antiemetic medications. The physical examination was normal. Serum lipase was 1263 U/L. A CT scan of abdomen showed no pathological changes. Patient remained under observation, with intravenous hydration and symptomatic medications. On the morning of the following day, lipase dropped to 421 U/L, and the patient was discharged shortly thereafter. CASE 3. A 19-year old man presented with a 48 hour-history of vomiting and diarrhea. The abdomen was diffusely painful, with no signs of peritoneal irritation. Abdominal ultrasound and CT scan were normal. Serum lipase was 1329 U/L. The patient was admitted to the hospital for intravenous hydration and symptomatic medication. The next day, serum lipase dropped to 143 U/L. Patient was discharged uneventfully shortly thereafter. The same laboratory methodology was used in all cases, with reference values for lipase = 73 to 393 U/L. In all three cases, a rapid decline in serum lipase levels was seen. Serum amylase levels remained normal at all times. In all three cases, all the laboratory results were confirmed with another assay and documented consistent results. **Conclusion:** We identified three cases of elevation of lipase without evidence of pancreatitis, indicating the existence of other causes for such elevation. The elevated lipase levels were transitory and did not seem to influence evolution or outcome. The differential diagnosis with pancreatitis continues to be relevant for decision analysis in an emergency room environment.

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Development of an Assay for Measurement of β2-Microglobulin (B2MG) in Urine Samples on Roche Clinical Chemistry Analyzers

C. Nowald[1], J. Kaufmann[1], G. Papassotiriou[2], H. W. A. Klima[1]. *[1]Roche Diagnostics GmbH, Penzberg, Germany, [2]Roche Diagnostics International Ltd., Rotkreuz, Switzerland*

Background:
B2MG is a low-molecular-weight protein with approximately 12 kDa. It occurs on the cell-membrane of all nucleus-containing cells except trophoblasts. Due to its low molecular weight, it is rapidly filtered through the renal glomeruli. Thereafter, up to 99.9 % is reabsorbed by the proximal tubules.Acute changes in tubular reabsorption and progressive renal diseases causing irreversible structural tubular defects impair tubular reabsorption of numerous smaller proteins including B2MG. Thus, urinary B2MG is discussed as a marker for the diagnosis and monitoring of tubulointerstitial renal damage. Elevated B2MG levels may identify patients at higher risk of glomerular filtration rate (GFR) decline in other kidney diseases such as membranous nephropathy. Furthermore, there is evidence that B2MG excretion is associated with acute allograft rejection in renal transplant recipients.
Methods:
Immunoturbidimetric assay.Latex-bound anti-β2-microglobulin antibodies react with antigen from the sample to form antigen/antibody complexes which are determined turbidimetrically after agglutination.
Development Goals:
-Development of an application for analysis of urine samples based on the existing Tina-quant® β2-Microglobulin assay
-Measuring range: 0.2-5.8 mg/L (16.9-491.3 nmol/L)
-In use time β2-Microglobulin reagent: 12 weeks (cobas c pack and cobas c pack large)
-Low sensitivity to drug interference-Improved in use time for β2-Microglobulin calibrator and controls
Results:
The linear assay range of the B2MGU assay is 0.2-5.8 mg/L (16.9-491.3 nmol/L). Extended measuring range: Extended measuring range: 63.8 mg/L (5404.3 nmol/L). Limit of Blank: 0.1 mg/L (8.5 nmol/L), Limit of Detection: 0.15 mg/L (12.7 nmol/L), Limit of Quantitation: 0.2 mg/L (16.9 nmol/L) with 20% total error. B2MGU assay has been standardized against the WHO standard 1st WHO standard 1985, NIBSC code B2M). No interference of Albumin (5000 mg/L), Calcium 12.0 (mmol/L), Creatinine (10.0 mg/ml), Glucose (70.0 mg/ml) and Hemolysis (1100 mg/dL) was observed (Acceptance criteria: Recovery of β2-microglobulin concentrations ≤ 1.0 mg/L: ≤ ± 0.1 mg/L and > 1.0 mg/L: ≤ ± 10 % of initial value). Precision - CLSI EP5 - 21 days (Repeatability): SD ranging from 2.54 nmol/L (0.03 mg/L) to (0.13 mg/L), CV ranging from 2.3% to 9.4%, sample concentration range: 25.4 nmol/L (0.3 mg/L) to Precision - CLSI EP5 - 21 days (Repeatability): SD ranging from 0.09 mg/L to 0.25 mg/L, CV ranging from 0.7% to 2.3%, sample concentration range: 25.41 nmol/L (0.3 mg/L) to 465.85 nmol/L (5.5 mg/L). Human urine samples obtained on a Roche/Hitachi cobas c 701 analyzer (y) were compared with those determined using the corresponding reagent on a Roche cobas c 501 analyzer (x) (n = 118). Passing/Bablok regression: y = 0.967x - 0.0005 mg/L (0.04 nmol/L). The sample concentrations were between 0.21 and 5.76 mg/L (17.79 and 487.87 nmol/L). Comparison to B2MG urine method on Siemens ProSpec: y = 0.989x - 0.0539 mg/L (4.57 nmol/L).
Conclusion:
All of the development goals for the urine application of Roche Tina-quant® β2-Microglobulin assay were met. The introduction of the B2MG urine assay will complete the assay portfolio for screening and monitoring patients with kidney disease.

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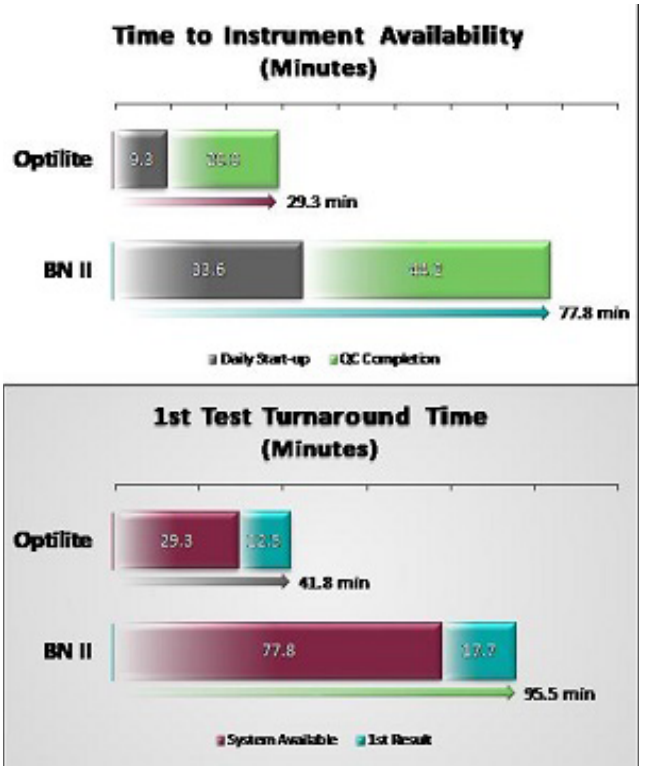
Laboratory workflow analysis in special protein testing

S. Stone[1], E. Olgaard[2], K. Sanderson[2], S. LeSourd[2]. *[1]Argent Global Services, Oklahoma City, OK, [2]University of Arkansas for Medical Sciences, Little Rock, AR*

Background: Dedicated special protein analyzers play an important role in the clinical laboratory. Historically the Siemens BN™II system has been one of the most commonly utilized protein analyzers. The Binding Site Optilite® system is a new protein analyzer that has been recently introduced to the clinical laboratory market. The objective of this study was to compare workflow and time requirements for daily startup and test performance for both systems in an actual clinical diagnostic laboratory setting. **Methods**: A before and after time and motion study was performed by observing the daily start-up and testing activities for both systems. Observation data was collected for four days on each system with no variation in location, layout, workflow, scheduling and staffing. The special protein test menus were identical and average daily volumes were very similar. Testing was performed daily through two shifts which required a daily startup and shut down. The data is presented as weighted averages. **Results**: On average, the Optilite required 9.3 minutes to perform daily start-up while the BNII needed 33.6 minutes. Additionally, the Optilite required 20.0 minutes for daily QC, compared to 44.2 minutes for the BNII. Cumulative time to instrument availability was 29.3 minutes for the Optilite and 77.8 minutes for the BNII. The average time to first result on the Optilite was 12.5 minutes, compared to 17.7 minutes for the BNII. Total time to first result was 41.8 minutes for the Optilite and 95.5 minutes for BNII. **Conclusions**: The Optilite required significantly less time for start-up. This allows testing to begin sooner and contribute more staff time availability to perform other clinical testing. The Optilite special protein analyzer provides rapid start-up times, reduced QC times and improved result reporting which has a substantial impact on laboratory workflow, technician time and reporting speed.



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Development of a multiplex biochip array for early detection of Chronic Kidney Disease on the new fully automated Evidence Evolution analyser

A. M. Flanagan[1], M. Foley[1], E. McCole[1], J. McFarlane[1], C. Richardson[1], R. I. McConnell[2], J. V. Lamont[2], S. P. FitzGerald[2]. *[1]Randox Teoranta, Dungloe, Ireland, [2]Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom*

Background: Chronic Kidney Disease (CKD) defines the progressive loss of kidney function, over a period of time. The early stages of CKD progress asymptomatically, and are therefore difficult to diagnose. However, late diagnosis of CKD can ultimately lead to end-stage renal disease requiring kidney dialysis or transplantation. The use of a screening test for the detection of early stage CKD biomarkers has the potential to identify individuals at risk of developing progressive renal disease. Biochip array technology (BAT) facilitates the detection of multiple analytes from a single sample, allowing comprehensive sample screening. The objective of this study is to develop a multiplex biochip array for the simultaneous measurement of Fatty Acid-Binding Protein 1 (FABP1), soluble Tumour Necrosis Factor Receptors 1 and 2 (sTNF-R1 and sTNF-R2) and Macrophage Inflammatory Protein 1 alpha (MIP-1α), with utility for the early detection of CKD on a fully automated platform. **Methods:** Simultaneous

chemiluminescent sandwich immunoassays were employed. Capture antibodies were immobilised on discrete test regions on the biochip surface and applied to the fully automated Evidence Evolution biochip analyser. Sensitivity, repeatability, within lab precision, assay specificity and interference were evaluated in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, in order to confirm assay performance characteristics. A comparative study was conducted with plasma patient samples (n=40) from controls and defined CKD stages, classified by estimated Glomerular Filtration Rate (eGFR) using the CKD Epidemiology Collaboration (CKD-EPI) equation. **Results:** Each analyte was tested simultaneously and utilised assay ranges of 0-200ng/mL for FABP1; 0-10ng/mL for sTNF-R1; 0-130pg/mL for MIP-1α and 0-20ng/mL for sTNF-R2. Assay sensitivity was recorded as 1.56ng/mL for FABP1; 0.04ng/mL for sTNF-R1; 0.50pg/mL for MIP-1α and 0.16ng/mL for sTNF-R2. Average repeatability (%CV) was determined to be 9.62% for FABP1, 8.78% for sTNF-R1, 8.07% for MIP-1α and 10.61% for sTNF-R2. Average within-lab precision was determined to be 16.75% for FABP1, 11.13% for sTNF-R1, 10.74% for MIP-1α and 15.93% for sTNF-R2. Cross reactivity analysis determined that each individual assay was specific for its target analyte. Additionally, no cross reactivity was observed with non-panel, homologous proteins (cross reactivity <1%). The assay demonstrated no significant interference with common interferents tested (triglycerides, haemoglobin, intralipids and bilirubin). A cohort of 40 samples assessing normal plasma samples compared to Stage 1, 2 and 3 CKD plasma samples (n = 10 each), yielded AUC values for individual biomarkers in the range of 0.705-0.905, differentiating normal controls from early CKD stages. **Conclusions:** The findings of this study highlight the utility of a multiplex immunoassay array for the early detection of CKD, through rapid, fully-automated, simultaneous measurement of FABP1, sTNF-R1, sTNF-R2 and MIP-1α on the Evidence Evolution analyser platform. This biochip array provides a valuable and reliable multi-analytical tool for the identification of early CKD.

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Development of a biochip array for the detection of Adhesion Molecules on the new random access fully automated Evidence Evolution analyser

N. Cutliffe[1], M. Summers[1], L. McClafferty[1], C. Richardson[1], R. I. McConnell[2], J. V. Lamont[2], S. P. FitzGerald[2]. *[1]Randox Teoranta, Dungloe, Ireland, [2]Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom*

Background: Cell adhesion molecules are complex membrane proteins which mediate cell-to-cell interactions and subsequently influence a wide range of intracellular signalling cascades. Adhesion molecules can also be detected in soluble forms in the circulation. These molecules are implicated in a diverse range of physiological processes such as cell proliferation, migration, differentiation, apoptosis, and the mediation of inflammatory processes. Altered circulating levels of adhesion molecules have been reported in a wide range of physiological conditions, such as cardiovascular disease, stroke, cancer, chronic kidney disease (CKD) and diabetes. Consequently, the measurement of circulating adhesion molecules has importance for identifying and monitoring disease. The objective of this study is to utilize Randox's proprietary, multiplexing biochip array technology to develop an Adhesion Molecule array. The array, encompassing five adhesion molecules - Vascular Cell Adhesion Molecule 1 (VCAM-1), Intracellular Adhesion Molecule 1 (ICAM-1), E-selectin (ESEL), P-selectin (PSEL) and L-selectin (LSEL) - was applied to the new, fully-automated Randox Evolution Analyser. **Methods:** Antibodies specific to VCAM-1, ICAM-1, ESEL, PSEL and LSEL were immobilised to discrete testing regions within a biochip surface and a chemiluminescent sandwich immunoassay format was used for this array. The array has been developed on the Evidence Evolution analyser which requires minimal user input and provides rapid results, with the first test read after 36 minutes and a test per minute thereafter. Assay sensitivity, precision, cross reactivity and interference were evaluated to define assay characteristics. Clinical utility was also evaluated using a cohort of 41 samples (22 healthy controls and 19 CKD). **Results:** The assays were simultaneously evaluated and yielded the following ranges and sensitivities - VCAM-1, range 0-6600 ng/ml and sensitivity 34ng/ml; ICAM-1, range 0-2000 ng/ml and sensitivity 13ng/ml; ESEL, range 0-500 ng/ml and sensitivity 4.0ng/ml; PSEL, range 0-2400 ng/ml and sensitivity 29ng/ml, and LSEL, range 0-7000 ng/ml and sensitivity 36ng/ml. Average repeatability was recorded as 4.5% for VCAM-1; 5.6% for ICAM-1; 12.8% for ESEL; 3.5% for PSEL and 5.7% for LSEL. Average within lab precision of 6.8% was observed for VCAM-1; 8.8% for ICAM-1; 16.6% for ESEL; 4.7% for PSEL and 8.2% for LSEL. Cross reactivity analysis demonstrated that each individual assay was specific for its target analyte (cross reactivity <1%) and that no cross reactivity was observed with non-panel homologous proteins (cross reactivity <1%). Common blood interferents - haemoglobin, triglycerides, intralipids and bilirubin - demonstrated no interference with assay performance. Significant differences in biomarker concentrations were observed when CKD samples were compared to controls - VCAM-1 (AUC = 0.706; p = 0.010), ICAM-1 (AUC = 0.789; p = 0.006) and LSEL (AUC = 0.610; p = 0.007). **Conclusion:** This study reports on the development of a multiplexed array for the simultaneous measurement of VCAM-1, ICAM-1, ESEL, PSEL and LSEL. This array offers a rapid, fully-automated alternative to traditional ELISA methods, with minimal sample volume requirements. This newly developed Adhesion Molecule array can be applied to a diverse range of pathologies.

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Development of a liquid Procalcitonin calibration verification set to verify the method's analytical range

A. P. Nguyen, J. Pawlak, M. Sweatt, R. K. Ito. *LGC Maine Standards Company, Cumberland Foreside, ME*

Introduction: Procalcitonin (PCT) is a 116 amino acid peptide precursor of the hormone calcitonin. It is a biomarker associated with the inflammatory response to bacterial infection. Localized infections, allergies, autoimmune diseases and transplant rejections do not usually induce a PCT response. PCT aids in the assessment of critically ill patients to determine if a patient has severe sepsis or is in septic shock. Healthy individuals will have a PCT of less than 0.1 ng/mL; a result above this can indicate a bacterial infection requiring treatment. If responsive to therapies, the PCT value will decrease and continue to decrease until reaching normal levels. PCT immunoassay methods have been cleared by the U.S. FDA for several automated platforms, including the bioMérieux VIDAS®, Roche Diagnostics cobas®, Abbott ARCHITECT and Beckman Coulter AU. As non-waived laboratory tests, calibration verification is required under CLIA '88. Our objective was to develop a liquid-stable, human serum based PCT calibration verification test kit for use by clinical laboratories' method validation. **Methods:** VALIDATE® Procalcitonin was formulated in a human-serum matrix according to CLSI EP06-A into five equal delta concentrations to cover the analytical measuring range (AMR), 0.02 to 100 ng/mL, of the Roche cobas® 6000's PCT reagent. In total, 5 individual lots were manufactured. For each level, samples were tested in triplicate on the Roche cobas® 6000 analyzer. Reported recoveries were evaluated for mean, SD and linearity using MSDRx® (LGC Maine Standards' proprietary linearity software). Limits were applied as 50% of the total allowable error (TAE) for the analyte. To establish product stability claims, samples were subjected to one freeze-thaw cycle, followed by a 24-hour, 22ºC stress condition and four subsequent open-vial events. Stability testing is on-going. **Results:** Procalcitonin is linear and within applied TAE limits through the validated reportable range of the Roche cobas® PCT assay. Linear regression analysis of theoretical vs recovered was Y = 1.007 X + 0.031, R^2 = 0.9984. Typical recovered values for level 1 and 5 are 0.08 ng/mL and 92 ng/mL respectively. All levels were stable, recovering within 90% of the recovered values on date of manufacture in on-going real time and stress stability studies. A comparison of the validated range against the Roche cobas® 6000 demonstrates full reportable range coverage for the analyte in a human-serum matrix. **Conclusion:** VALIDATE® Procalcitonin is a liquid-stable, ready-to-use product stored at freezer temperatures. The product is fit-for-purpose as a calibration verification test kit that covers the full AMR for the Roche cobas® 6000 PCT reagent. The product conforms to CLSI EP06-A guidelines for linearity testing. The Roche cobas® 6000 formulation is currently available and listed with the FDA. Formulations for the Abbott ARCHITECT, bioMérieux VIDAS® and Beckman Coulter AU are currently in development.

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Clinical utility of cystatin C as a biomarker of kidney function

A. C. Dias, K. D. F. Neves, K. F. V. Carrero, A. L. Barbosa, L. F. Abdalla. *Laboratório Sabin, Brasília, Brazil*

Background: Chronic kidney disease (CKD) is a public health problem. In Brazil, it is estimated that in 2014, 112,004 patients undergo dialysis as a consequence of hypertensive nephropathy and diabetes. Glomerular filtration rate (GFR) is the main indicator of renal function, correlating with the severity of CKD and reduced before the onset of clinical manifestations. Studies indicate that Cystatin C has a higher sensitivity than creatinine in detecting renal disease and in assessing risk for clinically relevant events such as heart failure, hypertension, and diabetes. Cystatin C is a non-glycosylated protein, produced at a constant rate and freely filtered by the kidneys, less influenced by variables such as age, sex, race, muscle mass and physical activity than serum creatinine and has the sensitivity of detecting small reductions in renal function in patients with GFR 60-90 mL/min/1.73m2, even with creatinine concentrations within normal range, providing high sensitivity and specificity in the evaluation of GFR. The KDIGO guideline, published in 2013, reports that the CKD-EPI creat/cyst equation that combines Cystatin C and serum creatinine has obtained better results in the estimation of GFR and classification of patients with renal disease. The purposes of this study were to evaluate the performance of Cystatin C against

serum creatinine when analyzed alone; the agreement between eGFR based on formulas derived from Cystatin C when compared with the equation derived from serum creatinine and the possible influence of some parameters on the magnitude of the differences between the different equations derived from Cystatin C and creatinine for eGFR and to analyze the reuptake of DRC based on these different equations. **Methods:** Population study with 164 adult patients at different stages of CKD. Serum and urinary creatinine analyzed by the enzyme method in Advia 2400 and cystatinC were analyzed in BN II by the immunonephelometric method. The CKD-EPI-derived cystatinC (CKD-EPI cyst and CKD-EPI creat/cyst) and creatinine-derived (CKD-EPI creat) equations were used to estimate GFR. **Results**: The study of correlation, trend and determination of cut-off value of DPU24h indicate that both cystatinC and serum creatinine results show the same results when we associate the isolated cystatin C and creatinine results to the DPU24h result. The results of creatinine and cystatin C begin to extrapolate the upper limit of the Reference Interval when the DPU24h presents values below the range of 79 to 80mL/min/1.73m2. The values of DPU24h lower than 79mL/min/1.73m2 show creatinine and cystatinC results above the upper limit of the reference range. The comparison study between the CystatinC-derived equations and the creatinine-derived equation showed that CKD-EPI creat/cyst is much more precise and accurate than CKD-EPI cyst. Proteinuria and albuminuria were statistically significant for the group of patients between stages 3b and 5 of CKD and 25% of patients presented a different staging when compared to CKD-EPI creat/cyst result with CKD-EPI creat. **Conclusions:** Isolated CystatinC and creatinine were more sensitive in detecting alterations in renal function, and the CKD-EPI creat/cyst equation, recommended by KDIGO in specific situations, presented excellent accuracy and precision when compared to CKD-EPIcreat, is recommended by Brazilian medical societies.

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Development of an enzymatic assay to measure lactate in perchloric acid-precipitated cerebrospinal fluid

J. Lu[1], J. R. Genzen[2], D. G. Grenache[3]. *[1]ARUP Laboratories, Salt Lake City, UT, [2]University of Utah School of Medicine, Salt Lake City, UT, [3]TriCore Reference Laboratories, Albuquerque, NM*

Background: Individuals with inherited deficiencies of the pyruvate dehydrogenase complex or the respiratory chain complex can have increased concentrations of cerebrospinal fluid (CSF) lactate. Such measurements are clinical useful when measured in conjunction with pyruvate in order to calculate the lactate:pyruvate (L:P) ratio, a useful surrogate of cytosolic redox status. CSF pyruvate is measured in a protein-free supernatant prepared by the addition of CSF to perchloric acid while lactate is measured in untreated CSF. Utilizing the same sample for both lactate and pyruvate measurements is desirable. The objective was to develop a method to measure lactate in perchloric-acid precipitated CSF and validate the L:P ratio as calculated from the analysis of both analytes in the same sample. **Methods:** Samples were prepared by the addition of 1 mL CSF to 2 mL 8% (w/v) cold perchloric acid, incubated on ice for 10 min, then centrifuged to obtain a protein-free supernatant. Lactate was measured by its oxidation to pyruvate and hydrogen peroxide using lactate oxidase and the absorbance of the resulting chromogen determined at 540 nm on a Roche cobas c501 chemistry analyzer. Method accuracy, linearity, imprecision and sensitivity were determined and a reference interval was verified. **Results:** To assess accuracy, this method was compared to lactate determined in unaltered CSF at another laboratory using 41 specimens with lactate concentrations from 0.6-11.9 mmol/L. Linear regression produced a slope of 1.09 and y-intercept of 0.26 (R^2 =1.00). Recovery was performed by ad-mixes of a high lactate standard and a CSF pool in different ratios to create a set of 19 samples prior to preparing protein-free supernatants. Recovery was 94.6-100% (mean±SD was 97.4±1.4%) at lactate concentrations of 2.68 to 12.63 mmol/L. Linearity was determined by combining two supernatants with low and high lactate concentrations in different ratios to create a set of six samples (0.15-12.70 mmol/L) that were tested in duplicate. Linear regression generated a slope of 1.01, y-intercept of -0.04 (R^2=1.00). Precision was verified by analyzing quality control materials (acid-treated lactate standard) in 3 replicates each day for 5 days. Within-laboratory imprecision was 2.3% at 1.5 mmol/L and 1.5% at 10.5 mmol/L. The limit of blank was 0.05 mmol/L as determined by the mean added to three standard deviations determined from 10 replicates of perchloric-acid treated saline pool. The limit of detection was determined to be 0.12 mmol/L calculated from 10 replicates of a patient sample treated with perchloric-acid. The manufacturer's reference interval of 1.1-2.4 mmol/L was verified using 20 residual patient CSF samples. **Conclusion:** CSF lactate can be measured with accuracy and precision using the same perchloric-acid treated sample that is used for pyruvate.

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Performance verification and assessment of the consistency of four Lp-PLA2 activity reagents

D. Wang, L. Hou, L. Qiu, X. Guo, L. Xia, Q. Wu, Y. Yin, H. Li, D. Li, Q. Liu, X. Gao, L. Lin. *Peking Union Medical College Hospital, Chinese Academic Medical Science and Peking Union Medical Col, Beijing, China*

Background: To validate the analytical performance and the consistency of four Lp-PLA2 activity reagents (Evermed, DiaSys, Hengxiao and Zhongyuan were labeled as A, B, C and D, respectively) on Beckman Au5800 automatic biochemical analyzer. **Methods:** Performance validation. The remaining serum samples of 214 patients and 140 apparently healthy individuals were collected during May to August 2017 in Peking Union Medical College Hospital (PUMCH) and used to method comparison and reference interval validation, respectively. According to the standard of CLSI EP15-A, EP6-A, EP-17and EP7-P,the precision, linearity range, sensitivity, common interference (free bilirubin, conjunct bilirubin , hemoglobin, chyle) were assessed. According to EP9-A2, method comparison was conducted and deviations of each reagent were compared in the medical decision level (328U/L, 391U/L, 485U/L). **Results:** The precision of four reagents were good and the repeatability *CV%* of A-D were 0.5%-1.7%, 0.7%-3.0%, 0.9%-2.0%, 0.5%-3.3%, and reproducibility *CV%* were 0.7%-2.9%, 1.4%-3.2%, 1.3%-1.9%, 0.8%-4.1%, Only B reagent is greater than the manufacturer's stated total *CV%*, but both are less than the 5% quality target in the laboratory .The linearity range of A to D were 44 -1992 U/L, 39 -1798 U/L, 13 -540 U/L and 75 -1717U/L, the regression coefficient R^2 was between 0.997 and 1.000, and the correlation coefficient (r) was between 0.998 and 1.000. The anti-interference of chyle were good among four reagents which met the manufacturer's claims or clinical needs. Bilirubin at low levels of Lp-PLA2 interference on C reagent obvious; B, C in the hemoglobin 4.5g / L was significantly negative interference, and D in hemoglobin 2.45g /L is showing interference. The regression coefficients R^2 of A, C, D compared with B were between 0.978 and 0.995, and the correlation coefficients (r) were between 0.989 and 0.998. The expected deviations at the medical decision level ranged from -240 U / L to 113 U / L. 131 (93.6%), 140 (100%), 82 (58.6%), and 128 (91.4%) of Lp-PLA2 activity test results were found to be within the manufacturer's stated reference intervals for A to D, respectively. **Conclusion:** The four Lp-PLA2 activity reagents, performed on automatic biochemistry analyzer, had good quality of precision and linearity range, but the anti-interference should be improved.

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Comparison of monoclonal protein concentration in serum measured by Hevylite or by electrophoresis

M. C. Cárdenas[1], D. Pérez[2], J. Jiménez[3], R. Pérez[4], E. Zapico[5], E. Cruz[6], M. Fernández[7], A. Gella[8], S. Hermoso[9], G. Marcadia[10], C. Valldecabres[11]. *[1]Hospital Clínico San Carlos, Madrid, Spain, [2]Laboratori PASTEUR, Andorra La Vella, Andorra, [3]Hospital Severo Ochoa, Leganés, Spain, [4]Hospital de Cruces, Barakaldo, Spain, [5]Hospital de Sant Creu i Sant Pau, Barcelona, Spain, [6]Hospital Universitario de Basurto, Bilbao, Spain, [7]Hospital Comarcal Santiago Apóstol, Miranda, Spain, [8]Institut de Neurociències-UAB, Barcelona, Spain, [9]Complejo Hospitalario de Navarra, Pamplona, Spain, [10]Hospital General Universitario de Valencia, Valencia, Spain, [11]Hospital Universitario de La Ribera, Alcira, Spain*

Background: The measurement of the monoclonal protein (M-protein) is important for the diagnostic and follow-up of monoclonal gammopathies. The guidelines of clinical practices recommend that its concentration should be measured by serum protein electrophoresis (SPE) for the majority of cases. Immunochemical methods able to measure the isotype-specific heavy and light chain (HLC) are an alternative to measure the M-protein. Objective: Compare the HLC assay (Hevylite) with the electrophoretic methods of SPE, namely capillary electrophoresis (CE) and electrophoresis in agarose gel (AG), and estimate the systematic measurement error. **Methods:** The SPE was performed in a series of dilutions of 12 serum samples with M-protein (range 20-40 g/L, 6 isotypes with beta migration and 6 with gamma). Dilutions were performed with a pool of sera without M-protein and a pool of hipogammoglobulinemic sera. SPE was performed by CE (Capillarys 2 from Sebia) and AG (Hydrasys from Sebia). The integration of the M-protein peak in the electropherogram was performed by the perpendicular drop method. The measurement of the involved pair with the Hevylite assay was performed in the SPAplus analyzer (The Binding Site). The quantification methods for statistical comparison used were Pearson correlation, Passing-Bablok regression and Bland-Altman graphics. SPE was considered the reference method.

Results: For IgG M-proteins, Hevylite assay always retrieved a positive constant systematic error (SEc) ranging between 1.69 and 3.87 g/L, depending on the mobility and the SPE method. Hevylite showed proportional systematic error (SEp) only when it was compared to CE and M-protein migration was gamma. SEp underestimated the M-protein. For IgA M-proteins, Hevylite did not show SEc when comparing with AG. It showed SEc regarding CE, with a value ranging between -1.17 and -1.46, depending on the respective mobility. Hevylite did not show SEp when compared with the AG for M-protein IgA, except for beta migration patterns, and for CE when it has mobility gamma. SEp for all the other cases always overestimated the M-protein. For IgM M-proteins, Hevylite always presented SEc and SEp. SEc varied between -1.12 and -6.14 according to the mobility and the electrophoretic method used to compare. The SEp always overestimated the M-protein. **Conclusion:** Hevylite results show a better metrological comparability with the electrophoresis in agarose gel (although it presents a SEc < 3.23 g/L), especially for IgG and IgA M-proteins migrating in beta. Regarding capillarity electrophoresis, Hevylite is only comparable when the M-protein is IgG and it migrates in beta or is IgA migrating in gamma (SEc < 3.87 g/L, -0.94 g/L). Measurement results of IgM M-protein using Hevylite assay are not comparable with those from electrophoresis, since its SEp (slope >1.32) tends to overestimate the concentration. Nevertheless, Hevylite represents the advantage to be able to follow the uninvolved polyclonal pair, measuring the immunosuppression as a risk factor towards a shorter time to progression.

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The Performance of Diazyme PCT Assay on Beckman DxC 700 AU Analyzer

c. dou. *diazyme, poway, CA*

Background: Procalcitonin (PCT) is a specific biomarker for systemic sepsis and septic shock. In healthy subjects, the PCT levels in circulation are very low (<0.05 ng/ml). Elevated circulating levels of PCT are important indicators in response to microbial infections. Diazyme PCT Assay (k162297) is a latex particle enhanced immunoturbidimetric method for the quantitative determination of PCT in human serum, EDTA or lithium heparin plasma. Measurement of PCT in conjunction with other laboratory findings and clinical assessments aids in the risk assessment of critically ill patients on their first day of ICU admission for progression to severe sepsis and septic shock. In this study, the performance of the PCT Assay was evaluated on the Beckman DxC 700 AU analyzer. Method: Diazyme PCT Assay is based on a latex enhanced immunoturbidimetric assay. It contains two reagents: reaction buffer and specific anti-PCT antibody coated latex particles. PCT antigen in the sample binds to the antibody coated latex particles and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of PCT in the sample. In this study, analytical performance of the assay was evaluated on the Beckman DxC 700 AU analyzer according to CLSI guidelines. Results*:* The precision of the Diazyme PCT Assay was evaluated according to modified CLSI EP5-A2 guideline. In the study, two levels of the PCT serum controls and two patient serum samples were tested in duplicate per run, 2 runs per day for 5 days. The within-run, between-run, between-day, and total CV% were ≤ 10% when PCT > 1.0 ng/mL and SD ≤ 0.1 ng/mL when PCT ≤ 1.0 ng/mL. Method comparison study was performed following CLSI EP9-A2 protocol. A total of 120 serum samples were compared on the Beckman DxC 700 AU and the master analyzer Beckman AU 400 and results yielded R^2 value of 0.9886 with a slope of 0.9529 and y intercept of -0.1113. Clinical sensitivity studies were conducted by testing 90 serum samples from patients on their first day of ICU admission. With cut-off at 0.5 ng/mL, the sensitivity, specificity and total agreement were 98.0%, 45.0% and 74.4%, respectively. With cut-off at 2.0 ng/mL, the sensitivity, specificity and total agreement were 92.0%, 77.5% and 85.6%, respectively. The assay was linear from 0.20 to 52.0 ng/mL with limit of quantitation (LOQ) of 0.20 ng/mL. Twenty interfering substances were tested and showed < ± 10% interference. Calibration was stable for 14 days, and reagents on-board were stable for 4 weeks. Conclusion: the performance of Diazyme PCT Assay on Beckman DxC 700 AU is accurate, sensitive and significantly equivalent to that on the master analyzer Beckman AU 400.

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Performance Evaluation of the New ADVIA Chemistry Cystatin C_2 Assay*

P. Datta, P. Hickey, M. Chakrabarty, J. Dai. *Siemens Healthcare Diagnostics, Newark, DE*

Background: The ADVIA® Chemistry Cystatin C_2 assay* (CYSC_2 from Siemens Healthineers) is standardized to the IFCC international reference material, ERMDA-471. The assay measures the serum protein cystatin C in human serum or plasma and is useful in the diagnosis and treatment of renal insufficiency. Serum concentrations of cystatin C are almost totally dependent on the glomerular filtration rate (GFR) and not affected by factors that have been demonstrated to affect creatinine values such as muscle mass and nutrition. In addition, a rise in serum creatinine does not become evident until the GFR has fallen approximately by 50%. The ADVIA Chemistry Cystatin C_2 assay has been evaluated on the automated, random-access ADVIA® 1800, ADVIA® 2400, and ADVIA® Chemistry XPT Systems (Siemens). The evaluation of this assay included precision, linearity, interference, high dose hook, and method comparison studies. **Methods:** All ADVIA Chemistry Systems use the same CYSC_2 reagent packs, calibrators, and commercial controls. In this assay, a specimen containing human cystatin C is diluted and then reacted with antibody (rabbit) coupled to latex microparticles. The increased turbidity is measured at 571 nm. By constructing a six-level standard curve (water is used as reagent blank) from the absorbances of standards, the analyte concentration of the sample is determined. **Results:**
The repeatability and within-lab CVs (80 replicates per sample) of the new assay with three commercial controls (~0.5, 0.9, and 3.9 mg/L cystatin C) and two serum pools (1.1 and 8.8 mg/L cystatin C) on all ADVIA Chemistry Systems were <2.7% and <4.4%, respectively. The analytical range/linearity of the assay on all ADVIA Chemistry Systems was from 0.25 mg/L to the cystatin C concentration in the highest level of calibrator (8.96-9.65 mg/L). The assay on the ADVIA 1800 system (y) correlated well with the cystatin C assay on the BN ProSpec® System (x), also from Siemens (both assays are standardized to IFCC reference material): y = 1.01x + 0.10 (r = 0.99, n = 155, range: 0.56-6.93 mg/L). The ADVIA 2400 and ADVIA XPT CYSC_2 assays , in turn, agreed with the ADVIA 1800 CYSC_2 assay : ADVIA 2400 CYSC_2 = 1.00 (ADVIA 1800 CYSC_2) - 0.02 (r = 0.99, n = 155, range: 0.64-6.75 mg/L); and ADVIA XPT CYSC_2 = 1.01 (ADVIA 1800 CYSC_2) - 0.00 (r = 0.99, n = 155, range: 0.64-6.75 mg/L). The ADVIA CYSC_2 Assay showed <10% interference with bilirubin (conjugated or free) up to 60 mg/dL, with hemoglobin up to 1000 mg/dL, with lipids (INTRALIPID, Fresenius Kabi AB Corporation) up to 1000 mg/dL, and with rheumatoid factors up to 1200 IU/mL. The assay has a minimum of 60 days on-system and calibration stability on all ADVIA Chemistry Systems. No prozone was observed with the assay on any platform up to the highest cystatin C concentration tested in a sample (69.55 mg/L). **Conclusion:** We conclude that the Cystatin C_2 assay, standardized to ERMDA-471, when used on any ADVIA Chemistry System, can measure serum or plasma cystatin C concentrations precisely and accurately over a broad range in routine laboratory use.
*Currently under development.