Wednesday, August 2, 2017

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

Proteins/Enzymes

B-327

The effects of protein supplements on liver enzymes activity

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Background: Training and diet are closely related, because intensive training causes increased metabolic, physical and mental activity, so the energy requirement of athletes is greater than the requirement of people who does not have sport activity. The input of protein supplements increases the influx of amino acids in the liver tissue and that increases the catabolic conversion of amino acids in terminal nitrogen products which are excreted from the body. This is caused by increasing the activity of liver enzymes involved in these processes. In our study we determine the activity of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LD) in athletes with high intensity and low intensity training. To establish whether there is a significant difference between the activity of the enzymes ALT, AST, GGT and LD in athletes after a seven-day break of using the supplementation.

Methods: The research included 180 male athletes. It contained three groups of subjects, athletes with high intensity training, athletes with low intensity training and the control group. In the first group it was professional athletes that take a 4000 and 5000 kcal a day, second group athletes who have low intensive training and daily take from 3,000 to 4,000 kcal. The control group was males who are not involved in sports and do not use protein supplements. The athlets take protein suplements Whey protein, Gainer, Isoactive, BCAA (branched-chain amino acids). The activity of enzymes (ALT, AST, GGT and LD) was analysed using BS-200 Mindray autoanalyzer. The method of determining these parameters is on the autoanalyzer modified and adapted according to the IFCC.

Results: The study showed that the mean value of the examined parameters is significantly lower in the serum of athletes who do not use supplements before, during or after training (ALT - 56.68 %, AST - 48.78 %, GGT - 14.17 and LD i 9.71 %) in comparison to athletes who use supplements. In the investigation of the differences between the parameters ALT, AST, GGT and LD between the two groups (athletes who use supplements and athletes who do not use supplements), we concluded that there was a statistically significant differences between the parameters ($p < 0.05$) subjects who use supplements and those who do not use supplements. The Man Whitney U test showed that between the two groups (subjects with high intensity training and subjects with low intensity training) there is a statistically significant difference between the examined parameters ALT, AST and GGT ($p < 0.05$), while the LD did not show a statistically significance difference ($p > 0.05$).

Conclusion: The results confirmed that the increased activity of the enzyme ALT, AST, GGT and LD in athletes decreases in the serum after a seven-day break of using the protein supplements.

B-331

Role of RNase L in Kidney

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Background: Renal diseases is continuing to be a prevalent problem. Current data indicate that 1% of patients admitted to hospitals are diagnosed initially with acute kidney injury(AKI), while 2-5% of hospitalized patients develop AKI secondarily. It has been reported that epidermal growth factors(EGF)/EGFR activation contributes to 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. In this study, we showed that RibonucleaseL(RNaseL), enzyme playing a role in interferon functions, mediated EGF/EGFR activation. Interestingly, we found that kidneys from aged RNaseL deficient mice were significantly smaller than that from wild type mice under the same condition. Histological staining revealed that there were remarkably more vacuoles in the kidney of KO RNaseL mice than WT mice. Proteomic analyses of urine discovered that lack of RNaseL exclusively block EGF excretion to urine. In this study, we will determine the role of RNaseL in the pathogenesis and elucidate how RNase L regulates the level of EGF in the kidney.

Methods: Investigate the role of RNaseL in the pathogenesis of AKI: Wild type(WT) &RNaseL null mice(KO) treated w/wo folic acid stained with PAS. Podocyte count and density analysis performed. Frozen kidney slides subjected to Oil Red O. Creatinine level in plasma and urine measured using HPLC MS. Investigate the mechanism by which RNaseL regulates the EGF: Total protein extracted from WT and KO kidneys and other organs. The level of EGF in blood investigated by qRT-PCR and ELIS under. The signaling of EGF/EGFR investigated by immunoblotting assays and the target molecules identified by using HPLC MS/MS. Investigate the corresponding enzyme of EGF cleavage: A Disintegrin and Metalloproteinase10(ADAM10). Renal cells isolated from WT and RNaseL KO mice. The cells treated w/wo PMA, total RNA and cell extracted. The expression of ADAM10 is examined by qPCR and Western blot. The release of ADAM10 to the media measured in the presence/absence of ADAM10 inhibotor Batimastat(BB-94).

Results: 1.Ratio of kidney weight/body for WT mice was 1.89-fold higher than KO mice. 2.kidney sections from KO RNaseL mice possess more vacuoles. 3.Two urinary protein bands in WT mice were missing in the urine of mice deficient RNaseL. The protein bands identified using LC-MS/MS &Western blot. Deficiency of RNaseL blocked the excretion of pro-EGF in urine. Determined expression at mRNA level in kidneys from 2-month-old WT &Ko RNaseL male mice. RT-PCR showed RNaseL deficiency doesn not affect the expression of EGF/EGF family at the transcriptional. 4.RNaseL has no protein-protein interaction with pro-EGF as the co-immunoprecipitation results show. 5.Western Blot analysis using p-Tyr and p-Thr antibodies showed that p-Protein profile is dramatically differs between WT &KO RNaseL kidney.

Conclusion: we should be able to obtain direct evidence that RNaseL contribute to kidney function. Data from this study will provide a better understanding of involvement of RNaseL in pathogenesis of AKI. The information about how RNaseL impacts ADAM10, subsequently regulating the EGF/EGFR activation, will reveal the molecular mechanism underlying the pathogenesis of AKI.

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Screening and Selection of Antibodies for the Detection of MIP-1 alpha and Its Application to the Study of Chronic Kidney Disease

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Background

The identification of the correct antibody pair is the most important step in ensuring the development of a highly specific and sensitive immunoassay. In order to do this, a high number of antibodies have to be screened as quickly as possible in order to find the best antibody pair for the development of an accurate immunoassay. Using Biolayer Interferometry (BLI) and Biochip Array Technology (BAT) the aim of this study was to develop a rapid, cost-effective screening and selection strategy for the establishment of a high sensitivity MIP-1alpha assay and evaluate its application to early detection and progression of Chronic Kidney Disease (CKD). CKD is defined as the progressive and irreversible decline in renal function and is classified into stages 1-5. Patients frequently present with chronic elevation of inflammatory biomarkers which appears to be exacerbated by disease progression. MIP-1alpha is known to have pro-inflammatory effects and has previously been shown to be significantly elevated in stages 1-3 of CKD patients as compared to control subjects. The effective detection of MIP-1 alpha with an optimal antibody pair, allows for the development of efficient immunoassay that can be used as an analytical tool in clinical studies.

Methods

A total of 22 purified MIP-1alpha sheep monoclonal antibodies (mABs) were screened using regenerable biosensors on the Octet RED96 instrument (ForteBio, USA). Capture and detector screening was performed by immobilisation of mABs and his-tagged antigen respectively. Top ranking MIP1-alpha pairs were then brought forward for assay development on the biochip platform using the Evidence Investigator. The final MIP-1alpha antibody pair was selected following sensitivity, reproducibility and cross-reactivity assessment. The clinical utility of the assay was then assessed by the analysis of 120 patient serum samples (normal n=60, CKD n=60; CKD stage 1 n=20, CKD stage 2 n=20 and CKD stage 3 n=20).

Results

Each MIP-1alpha mAB was ranked according to on- and off-rates using recombinant MIP-1alpha antigen. Top mABs exhibiting fastest on-rates and slowest off-rates were

used in a single epitope binning experiment resulting in the screening of 42 possible antibody pairs and the identification of three unique epitope bins. Using BAT six of the matched antibody pairs were used to successfully generate highly sensitive calibration curves (1pg/ml-500pg/ml). The assessment of serum samples using two sample Wilcoxon rank sum test showed significant separation of normal and stage 1 CKD patient samples (p-value<0.05), normal and stage 2 CKD patient samples (p-value<0.01) and normal and stage 3 CKD patient samples (p-value<0.001).

Conclusion

The data demonstrate the successful screening and selection of a suitable antibody pair that can be used for the development of efficient highly sensitive MIP-1alpha immunoassay using BLI and BAT. The strategy used shows how its resulting data can tailor the selection of mABs for their end-point assay platform such as BAT through the attention to antibody-antigen on- and off-rates. The resulting antibody pair was used to demonstrate the potential for MIP-1alpha as an early stage screening tool and disease monitoring biomarker for CKD.

B-333

Development of a Biochip Assay for the Detection of Thyroxine-Binding Globulin (TBG) on the New Random Access Fully Automated Evidence Evolution Analyser

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Background

Thyroxine Binding Globulin (TBG) is the main carrier protein for thyroid hormones in the blood. This 54 kDa protein reversibly binds thyroxine and tri-iodothyronine in circulation. Only free levels of these hormones are metabolically active, entering cells to regulate metabolism and body temperature. The normal reference range of TBG is 12-30 µg/ mL. Changes in TBG concentrations do not alter the metabolic state or cause thyroid disease but can produce changes in total thyroid hormone concentrations in serum. This may be mistaken for serious thyroid disorders such as hyper or hypothyroidism and if unrecognized can lead to inappropriate treatment. The aim of this assay was to develop a biochip based immunoassay for the quantification of TBG levels in serum applied to the new high throughput, fully automated random access Evidence Evolution analyser. The application provides a new analytical tool in the assessment of thyroid function.

Methods

A competitive chemiluminescent immunoassay was employed. The capture antibody was immobilised and stabilised on the biochip surface at a discrete test region. Calibrator material was prepared from purified human TBG antigen. The conjugate was also made from purified human TBG antigen labelled with HRP. The assay was applied to the Evidence Evolution analyser. The sample is diluted on-board the analyser at 1:10 and requires 8.3 µL of neat sample. Interference was tested for the following; haemaglobin 10 mg/ mL, triglycerides 10 mg/ mL, intralipids 40 mg/ mL and bilirubin 2 mg/ mL. The shelf life of key assay components was investigated. Serum patient samples (n=20) were assessed and the results compared with a commercially available method.

Results

A calibration range of 0-100 µg/ mL was established for the assay. Haemoglobin, triglycerides, intralipids and bilirubin were found to have no negative effects on performance. The stability of calibrators and conjugate were assessed and predicted to be stable for up to two years at 4°C. An r^2=0.96 and agreement of 69% were obtained based on the assessment of 20 patient serum samples with the biochip assay and another commercially available method.

Conclusion

The results indicate applicability of the developed biochip assay on the Evidence Evolution analyser for the detection of TBG. The assay presented a wide calibration range (0-100 µg/ mL) and the assessment of serum samples showed favourable correlation with another method. This new system represents a new analytical tool for a high throughput, assessment of samples and also incorporates random access and STAT sample capabilities. Moreover, the biochip platform offers flexibility to incorporate other tests on the biochip surface thus increasing the information to facilitate clinical understanding.

B-334

Development of a New Enzyme-Linked Immunosorbent Assay Kit to Detect NGAL in Human Serum and Its Application to Chronic Kidney Disease

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Background

Neutrophil gelatinase-associated lipocalin (NGAL) or Lipocalin-2 (LCN2) is a member of the lipocalin family of proteins which are known for the transportation of small hydrophobic ligands. NGAL was originally discovered in the granules of neutrophils but has since been found in many other human tissues including breast, kidney and liver. NGAL itself has many functions, for instance it's sequestering of iron, prevention of bacterial growth, chemoattraction of neutrophils, reduction of oxidative stress and regulation of cancer cell survival. It has been reported however that NGAL is highly upregulated upon kidney damage where levels can rise by ~ 10 fold in 3 hours depending on the type and severity of injury. The early detection of biomarkers for kidney injury may improve the diagnosis of conditions such as chronic kidney disease (CKD) or acute kidney injury (AKI) and allow timely determination of treatment which may ultimately slow progression. The availability of tests enabling the detection of this protein represents an advantage in clinical research settings. This study aimed to develop a new enzyme-linked immunosorbent assay (ELISA) for the detection of NGAL in human serum.

Methods

A colorimetric 2-step sandwich immunoassay was employed. The capture antibody was immobilised and stabilised on a 96-well microtitre plate surface. The analyte, if present in the sample, binds to the capture antibody and then a second antibody labelled with horseradish peroxidase binds to the analyte. Absorbances were read at 450nm. The signal is proportional to the concentration of the analyte in the sample. All assay kit reagents are ready to use. Recognition of NGAL was tested with analysis of 40 CKD samples (10 normal, 10 stage 1, 10 stage 2 and 10 stage 3). Statistical analyses were performed by Mann Witney test (with bon ferroni correction) (Medcalc version 16.4.3).These samples were also measured on another commercially available ELISA and the results compared by linear regression analysis.

Results

The assay exhibited a functional sensitivity of 20 ng/mL (measuring range of 0-2000 ng/mL, allowing for a 1 in 100 sample dilution). The intra assay precision value, expressed as %CV, was 8.9% and 6.6% based on 10 measurements at two different concentrations. Concentrations of native NGAL from Stage 3 CKD serum samples (Median 205ng/mL) were significantly elevated when compared to controls (Median 72ng/L), p=0.0030. Stage 1 and 2 samples were not significantly elevated when compared to controls. The correlation study showed, with 40 samples ranging from 32 to 302ng/ml, a correlation coefficient of 0.96 and a slope of 1.1.

Conclusion

The results show applicability of the developed ELISA for the sensitive detection of NGAL in serum. NGAL is shown to be a useful biomarker for patients at stage 3 CKD. The assay presents all kit reagents ready to use and 48 samples can be measured in less than 3 hours. This assay is a useful analytical tool for clinical research studies.

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Development of a Highly Specific Monoclonal Antibody Pair for the Detection of Glutathione S-transferase Pi (GST Pi)

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Background Renal pathologies that drive distal tubular damage may cause increased release of GST Pi into the urine. Elevated urinary GST Pi levels are indicative of renal tubule damage in transplant rejection, nephrotoxicity, infection, diabetes and chronic renal injury. Increased plasma concentrations of GST Pi are associated with chronic cholestatic diseases, cholangiocarcinoma and a range of malignancies, including colorectal cancers and lung cancer. Furthermore, the concentration of GST Pi in plasma is significantly elevated following stroke and some reports indicate that measurement of GST Pi in the early stages post-symptoms onset could indicate time of stroke. The aim of this study was to develop a highly specific monoclonal antibody pair (capture antibody and detector antibody), which can be employed for the development of a robust, quantitative immunoassay for the detection of GST Pi. Methods Sheep were immunized with E. coli-derived, recombinant human GST

Pi. Lymphocytes were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of specific antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies generated were purified and evaluated by direct binding ELISA to determine their specificity for GST Pi and cross-reactivity with other GST family members, including GST A1, GST T1, GST mu, GST M1. Sandwich pairs were evaluated by employing biochip based immunoassays on the Evidence Investigator Analyser and the optimal combination was identified for assay development. The resulting assay was employed for analysis of plasma samples from stroke patients (n=32) on admission (within 6 hours of onset of neurological symptoms) and healthy control samples (n=9). The Mann-Whitney t-Test was applied to determine statistical significance ($p<0.05$) of the median from each group. Results The selected monoclonal antibody pair exhibited specificity for GST Pi (%cross-reactivity to other family members was <1%). It was employed in development of a biochip immunoassay with a calibration range of 0-200ng/mL, sensitivity <1.0ng/mL and within-run precision, expressed as CV (%), <10%. Results from analysis of stroke patients revealed an increased median concentration of GST Pi in ischaemic stroke (12.93ng/mL, p=0.0005) and haemorrhagic stroke (3.94ng/mL; p=ns) when compared to controls (1.17ng/mL). Conclusion Data indicate optimal analytical performance of the monoclonal antibody pair for the specific detection of GST Pi and its suitability for application to the development of robust, quantitative immunoassays. The determination of GST-Pi will contribute to the study of its role in various disease states, including renal pathologies, a range of malignancies and stroke

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Development of a Highly Specific Monoclonal Antibody Pair for the Detection of DJ-1

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Background The protein DJ-1 regulates redox signalling kinase pathways and acts as a transcriptional regulator of many anti-oxidative genes, consequently it is an important redox-reactive signalling intermediate controlling oxidative ischemic stress and neuroinflammation during age-related neurodegenerative processes. DJ-1 mutations are associated with rare forms of autosomal recessive early-onset Parkinson's disease, therefore augmenting DJ-1 activity could potentially offer novel approaches to treat such chronic neurodegenerative illnesses. DJ-1 is readily detected in tau inclusions in brain tissue from patients with neurodegenerative diseases such as Alzheimer's disease and other related tauopathies. Analysis of DJ-1 expression in 3 independent cohorts of stroke patients demonstrated significant early elevation following a stroke event, in some patients within 30 minutes of stroke onset. This early detection may offer meaningful thrombolytic therapy intervention. The aim of this study was to develop a highly specific monoclonal antibody pair (capture antibody and detector antibody), which can be employed for the development of a robust, quantitative immunoassay for the detection of DJ-1. Methods Sheep were immunized with E. coli expressed recombinant human DJ-1. Lymphocytes were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of specific antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies generated were purified and evaluated by direct binding ELISA to determine their specificity for DJ-1. Sandwich pairs were evaluated by employing biochip based immunoassays on the Evidence Investigator Analyser and the optimal combination was identified for assay development. The resulting assay was employed for analysis of plasma samples from stroke patients (n=32) on admission (within 6 hours of onset of neurological symptoms) and healthy control samples (n=9). The Mann-Whitney t-Test was applied to determine statistical significance ($p<0.05$) of the median from each group. Results The selected monoclonal antibody pair exhibited specificity for DJ-1 (%CR to other family members was <1%). It was employed in development of a biochip immunoassay with a calibration range of 0-100ng/mL, sensitivity <1.0ng/mL and within-run precision, expressed as CV (%), <10%. Results from analysis of stroke patients revealed a significantly increased median concentration of DJ-1 (9.55ng/mL; p=0.0004) in ischaemic stroke when compared to controls (1.5ng/mL). Conclusion Data indicate optimal analytical performance of the monoclonal antibody pair for the specific detection of DJ-1 and its suitability for application to the development of robust, quantitative immunoassays. The determination of

DJ-1 will contribute to the study of its role in various disease states, including neurodegenerative conditions, malignancies and stroke.

B-338

Development of a Biochip Array for the Simultaneous Measurement of Distinct Fatty Acid-Binding Proteins (FABPs)

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Background Fatty Acid Binding Proteins (FABPs) are small 14-15kDa proteins which belong to a family of intracellular transport proteins. They are involved in the transport of long chain fatty acids and other hydrophobic ligands across cell and nuclear membranes. The family share moderate sequence homology of 20-70% and their tertiary structure is virtually superimposable. To date nine different FABPs, each with tissue specific distribution have been identified. Each FABP is named according to its tissue localisation; Liver-FABP, Intestinal-FABP, Heart-FABP, Adipose-FABP, Epidermal-FABP, Ileal-FABP, Brain-FABP, Myelin-FABP and Testis-FABP. These proteins have shown to be valuable biomarkers for a wide variety of conditions, including, but not limited to cancer, metabolic syndrome, irritable bowel disease, diabetes, acute myocardial infarction and renal failure. Biochip Array Technology enables the simultaneous detection of multiple analytes from a single sample. The development of a biochip array for the simultaneous measurement of multiple FABP proteins, will give a holistic profile of various organs and identify anomalies in certain tissues that may warrant further investigation. **Methods** The biochip incorporates multiple discrete test sites on its surface and allows quantification of specific FABPs using a chemiluminescent sandwich technique. Sixteen specific monoclonal antibodies were developed to bind a unique epitope on eight of the nine FABP family members. This facilitated the multiplexing of FABP proteins applied to the biochip analyser Evidence Investigator and analytical performance was evaluated. A study was conducted to measure the FABP serum levels in diseased cardiac patient samples (n=26) and a healthy patient cohort (n=36). A significant difference of FABP serum levels measured between healthy and diseased patients' samples was evaluated using the Kruskal-Wallis test (P value ≤ 0.05). **Results** The multiplex biochip immunoassay developed for FABP family members were specific for the targets with cross reactivity ≤ 1% observed when cross reactants were tested at excessive concentrations. The FABP multiplex array has shown sensitivity of 1.2ng/mL (Liver-FABP), 1.21ng/mL (Intestinal-FABP), 0.56ng/mL (Heart-FABP), 0.78ng/mL (Adipose-FABP), 1.95ng/mL (Epidermal-FABP), 1.46ng/mL (Ileal-FABP), 0.08ng/mL (Brain-FABP) and 0.72pg/mL (Testis-FABP). A cohort of cardiac disease samples were compared to normal healthy patient samples. Significantly elevated levels of Heart-FABP (P= <0.0001) and Adipose-FABP (P=0.0001) were observed in cardiac samples compared to healthy control patients, whereas other FABP family members such as Brain-FABP did not show significant differences. Average FABP levels measured in healthy individuals for Heart-FABP, Adipose-FABP and Brain-FABP were 3.18ng/mL, 1.92ng/mL and 0.02ng/mL respectively. For cardiac disease samples the average Heart-FABP, Adipose-FABP and Brain-FABP levels measured were 15.56ng/mL, 13.885ng/mL and 0.01ng/mL respectively. **Conclusion** The results indicate the applicability of the developed biochip array for the simultaneous detection of FABP proteins from a single serum sample. The simultaneous immunoassays are specific and sensitive to each FABP protein, and capable of discriminating sample types. This novel multi-analytical test allows for the initial screening of tissue damage which can then be a foundation for further patient investigations.

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Evaluation and Performance of a New and Novel Visible Method for the Measurement of ALT on VITROS® Systems

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VITROS Chemistry Products ALTV Slides quantitatively measure alanine aminotransferase (ALT) activity in serum and plasma using VITROS 250/350/5,1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System in the visible spectrum at 670nm. Alanine amino transferase measurements are used in the diagnosis and treatment of certain liver diseases (e.g., viral hepatitis and cirrhosis) and heart diseases. The VITROS ALTV Slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. The spreading layer contains the ALT substrates L-alanine and sodium α-ketoglutarate. Alanine aminotransferase catalyzes the transfer of the amino group of L-alanine to α-ketoglutarate in the presence of pyridoxyl-5-phosphate (P-5-P) to produce pyruvate and glutamate. Pyruvate is oxidized to acetylphosphate and hydrogen peroxide by

pyruvate oxidase. The final reaction step involves the peroxidase-catalyzed oxidation of a leuco dye to produce a colored dye. The rate of oxidation of the leuco dye is monitored by reflectance spectrophotometry. The rate of change in reflectance density is proportional to enzyme activity in the sample. Accuracy was evaluated for 124 patient serum samples (6 - 728 U/L) on the VITROS 350 and VITROS 5600 Systems compared to the IFCC comparative method adapted to a centrifugal analyzer at 37°C. The VITROS ALTV Slides assay showed excellent correlation with the IFCC method. VITROS 350 System = 1.00 * IFCC +2.0; (r) = 0.999. VITROS 5600 System = 1.00 * IFCC +1.9; (r) = 0.999. The 20-day precision studies conducted on the VITROS 350 and 5600 Systems showed excellent precision. Mean ALT concentrations of 33 U/L and 172 U/L resulted in within-laboratory percent coefficient of variation (%CV) of 1.9% and 1.7% respectively and within day %CV of 1.3% and 1.1% respectively on the VITROS 350 System. Mean ALT concentrations of 32 U/L and 171 U/L resulted in within-laboratory %CV of 1.3% and 1.6% respectively and within day %CV of 0.9% and 1.2% respectively on the VITROS 5600 System. The within run precision studies (n=44) also showed excellent precision. Mean ALT concentrations of 70.1 U/L, 324.0 U/L and 634.7 U/L resulted in within run %CV of 0.9%, 0.9% and 1.0% respectively on the VITROS 350 System. Mean ALT concentrations of 68.3 U/L, 318.2 U/L and 611.4 U/L resulted in within run %CV of 0.7%, 0.6% and 0.8% respectively on the VITROS 5600 system. The VITROS ALTV Slides assay exhibits higher sensitivity with a Limit of Detection (LoD) of 1.0 U/L based on 350 determinations with 5 low-level samples. The Limit of Blank (LoB) is 0.7 U/L based on 70 determinations with 5 blank samples. The VITROS ALTV Slides assay has exhibited good correlation with serum samples across a broad measuring range compared to the IFCC comparative method. In addition excellent precision and low end sensitivity has been observed on the VITROS 350 and 5600 Systems.

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Results of a time and motion study of special protein analyzers

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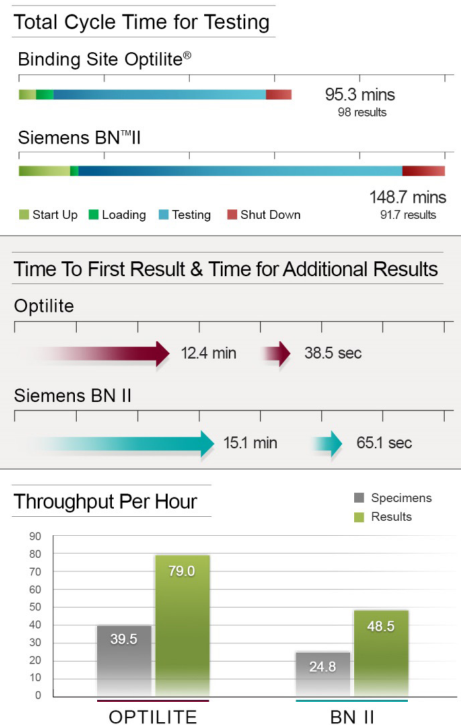
Background: The objective of this independent, observational time and motion study was to compare performance, labor and time requirements of the Binding Site Optilite® system and the Siemens BN™II system for special protein assays.

Methods: A before and after study was performed by observing all activities related to system operations. Observations were conducted and data was collected for four days on each system in the same location with the same layout, processes, volumes, schedules and staffing. The test menus were identical and daily average volumes were very similar. Testing was performed once per day with batch loading. The data is presented as daily weighted averages.

Results: The study revealed that the Optilite required significantly less time to complete all testing. On average, the Optilite required 95.3 minutes to produce 98 results and the BNII needed 148.7 minutes to complete 91.7 results. The time to first result on the Optilite was 12.4 minutes compared to 15.1 minutes for the BNII; and the time for each additional result was nearly twice as fast at 38.5 seconds for the Optilite compared to 65.1 seconds for the BNII. The Optilite produced higher throughput of both specimens and results at 39.5 specimens and 79.0 results per hour compared to the 24.8 specimens and 48.5 results per hour for the BNII. The total labor and cycle time for daily, weekly and monthly maintenance needed for the Optilite was less than the BNII (see figure).

Conclusions: The study design provided an ideal comparison of the two systems. The Optilite demonstrated faster turnaround times, faster time to first result, increased throughput and reduced maintenance requirements when compared to the BNII.



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Multisite study of analytical performance reveals disparities in global performance specifications

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Background:

Analytical Performance specifications for AST are not harmonized throughout the world. Allowable total error specifications range from 12% to 21% depending on the country or EQA/PT program. Since the use and interpretation of the AST test is standardized, the goals to judge method acceptability should also be standarized

Methods: 30 instruments in 19 laboratories in 8 countries participated in a Sigma Verification program. Imprecision was estimated from routine controls, with 1 to 3 months of data. Bias or inaccuracy was estimated from EQA/PT programs, peer group comparisons, or comparisons vs. the assayed/target values of the controls.

Allowable total errors from Rilibak, "Ricos Goals", CLIA Goals, and the Australian RCPA goals were compared. These range from 12% to 21% allowable total error.

Performance was evaluated using analytical Sigma-metrics. The standard Sigma-metric equation was used (TEa - bias) / CV

The percentage of laboratories able to achieve 5 Sigma (excellent) performance or better based on these goals was determined. A target of achieving 80% or better was considered success.

Results:

100% of labs and instruments can achieve CLIA and Rilibak goals, indicating that these performance specifications may be too lenient. These goals may be more like a rubber stamp than a performance standard.

In contrast, only 1/3 of instruments and labs are able to achieve an excellent level of quality using the RCPA goal, and nearly 1 in 6 labs would be considered unacceptable (less than 3 Sigma performance). This is a high level of failure and would represent a true crisis in the laboratory diagnostics market, if the goal was clinically appropriate. However, we do not see such problems, which is further evidence that this goal may not match up with the clinical use of the test.

For the Ricos goal, nearly 80% of the labs can achieve 5 Sigma or higher performance at the critical decision level, with less than 4% receiving unacceptable grades. This is

a much better spread of evaluation. It does not show that too many labs are going to fail, nor is it so low a bar than all labs are passing it.

Conclusion:

For the AST method, instrumentation quality has improved since the CLIA goals were established in 1992, and tighter goals are now achievable by a significant majority of the methods. This is demonstrated by the ability of multiple instruments and laboratories operating under a wide variety of circumstances reflective of typical routine operation. Data of this type can help inform the global debate on which analytical performance specifications to adopt during the harmonization efforts.

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Production of recombinant Streptolysin O from *Streptococcus pyogenes*

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Background: The ASO test is a method to determine a recent infection with group A *streptococcus*. Steptolysin O (SLO) is an essential raw material for ASO *in vitro* diagnostic (IVD) reagents. Recently several groups are studying the recombinant expression of SLO in *Escherichia coli*. On the other hand, it has been reported that low molecular form of SLO is also produced due to degradation by protease from *E. coli* (Pinkey et al. 1995). Hence, it is difficult to produce highly purified SLO cost-effectively in industrial scale. In addition, SLO has originally hemolytic activity which is an undesirable property from a viewpoint of the safety of production workers.

Methods: We attempted to produce a truncated SLO to avoid the degradation and eliminate hemolytic activity. The SLO gene without C-terminal region was cloned for the construction of a recombinant expression system in *E. coli* based on the fact that C-terminal region is involved with the degradation as well as hemolytic activity (Pinkey et al. 1995 and Yamamoto et al. 2001). The truncated SLO was overexpressed in *E. coli*, and the SLO was purified to homogeneity by general purification systems. Subsequently, we evaluated hemolytic activity, heat-stability and application using the highly purified SLO.

Results: As a result of the hemolytic activity test, it was elucidated that the hemolytic activity of the truncated SLO was completely eliminated. Besides, it was shown that the heat-stability of the truncated SLO was much higher than that of full-length form. In addition, a competitive assay using 50 serum samples was performed showing that no clinically relevant epitopes are lost in this truncated SLO compared with native SLO. Interestingly, ASO-latex IVD reagents were successfully prepared with this new raw material. The details will be discussed at the conference.

Conclusion: This novel truncated SLO will be useful for the manufacturing of ASO IVD reagents.

B-343

It's Complex: A Multistep Process to Determine a Clinical Cutoff for Macroenzyme Determination

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Background: Macroenzymes are antigen-antibody complexes formed between a specific autoantibody and enzyme. Their formation increases enzyme half-life and therefore inhibits in vivo clearance from circulation. Lack of clearance may cause abnormal elevations in serum enzyme activity, independent of pathology. Detection of macroenzyme forms is therefore crucial to avoid unnecessary clinical intervention. Macroenzyme detection methods range from complex gel filtration chromatography (GFC) and electrophoresis methods to less labor-intensive ultrafiltration and polyethylene glycol (PEG) precipitation techniques. **Objective:** Validate a clinical cutoff for the detection of a macroenzyme complex, macro-aspartate aminotransferase (AST), using PEG precipitation of serum. **Design and Methods:** PEG precipitation was performed by vortexing, incubating, and centrifuging equal amounts of PEG (250g/L) and patient sample. Neat samples and supernatant were analyzed using Roche cobas 602. Monomeric recoveries (measurement of unbound enzyme after complex removal) were determined by dividing the activity of the supernatant (following dilution correction) by the neat activity and converting to percent. To determine a clinical cutoff for macro-AST, central 95% reference intervals (RIs) were first established in well-characterized, healthy adults and children. Serum was collected from 124 adults (age 19-62 years, 58 males, median 33 years) and 74 children (age 6 months-17 years, 36 boys, median 7 years). Second, newly established RIs were verified using 252 residual specimens with elevated AST concentrations (> 31 U/L). AST concentrations ranged from 33-10,923 U/L, median 115 U/L. Third, a group of clinically suspect samples (n=6; 4 adults, 2 children) were analyzed. All suspect cases had elevated AST concentrations and clinical suspicion of macro-AST presence. Last, a subset of the above groups was evaluated using confirmatory GFC (n=8; 2 healthy, 2 verification, 4 suspect samples). **Results:** Nonparametric central 95% RI for the monomeric recovery in healthy adults was 33-83%. Parametric central 95% RI for healthy children was 26-71%. Using this statistically derived RI, 15% (37/252) of the verification samples (AST >31 U/L) would be considered positive for macro-AST. This likely exceeds the prevalence of macro-AST in the general population, thus the lowest monomeric recovery observed in the healthy populations (19% adults, 16% children) was evaluated as alternative positive cutoffs. This reduced positivity in the verification group to 0.8% (2/252). Four of six clinically suspect samples had monomeric recoveries ≤12%, 2 had recoveries ≥33%. Confirmatory testing of healthy (recoveries ≥33%) and verification (recoveries 25%, 29%) samples were negative by GFC. The 4 clinically suspect samples (with recoveries ≤12%) were positive by GFC. Based on these results, samples from adults with calculated monomeric recoveries ≤12% were considered positive for macro-AST, recoveries 13-18% were considered indeterminate, ≥19% was considered negative. In children, samples with calculated monomeric recoveries ≤12% were considered positive for macro-AST, recoveries 13-15% were considered indeterminate, ≥16% was considered negative. **Conclusions:** Macroenzymes are relatively rare, however, identification and diagnosis of these primarily benign anomalies is important. Extensive evaluation of the clinical cutoffs used for these assays is necessary. When empirically-derived RI do not align with clinical expectations, further evaluation using well-characterized samples is essential for proper clinical interpretation.

B-344

IFCC Traceable Calibration Factors for Abbott ARCHITECT non activated ALT and non activated AST assays

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IFCC Traceable Calibration Factors for Abbott ARCHITECT non-activated ALT and non-activated AST assays

Objective: Determine metrological traceability by optimizing calibration factors (k-factors) for Abbott ARCHITECT non-activated ALT (ALT, 7D56) and non-activated AST (AST, 7D81).

Method: The study was performed in three phases: 1) Estimation of IFCC traceable k-factors for ALT/AST using IFCC traceable activated ALT (AALT, 8L92) and activated AST (AAST, 8L91) method means and non-activated method means with the current k-factors ; 2) Verification of the new adjusted k-factors for ALT/AST by a method comparison with AALT/AAST; 3) Validation of IFCC traceable k-factor for ALT/AST by comparing the results to AALT/AAST in 31 laboratories across China.

Results: Sixty serum samples were used for estimating the IFCC traceable k factors, the comparison between current factor and IFCC traceable factor. Fifty serum samples were used for verification of IFCC traceable k-factors for ALT/AST. Method comparison results between ALT/AST with the optimized k- factor and AALT/AAST. See table below:

En-zyme	Estimation of IFCC traceable k-factors				Verification of IFCC traceable k-factor		
	ARCHI-TECT	Current factor	IFCC traceable factor	%diff current vs adjusted factor	R	Slope	Intercept
AST	C8000	8141	9860	21.12	0.9962	1.00	-5.76
	C16000	8492	10077	18.66	0.9959	1.00	-6.41
ALT	C8000	8141	9810	20.50	0.9937	0.95	2.39
	C16000	8492	10024	18.04	0.9935	0.94	2.88

The correlations for validation studies using 8 pooled serum samples across 31 laboratories were: ALT (R2: 1.00; Slope: 0.98) and AST (R2: 1.00; Slope: 0.92). The Bland Altman analysis showed a mean % difference of 0.22% for ALT and -7.78% for AST.

Conclusion: The study demonstrated comparable performance between AALT/AAST (IFCC traceable methods) and ALT /AST assays with newly optimized k-factors. The use of new optimized k-factor for ALT /AST assays may provide results that are comparable to IFCC traceable methods for patient samples in the clinical setting. However, AALT and AAST reagents contain the coenzyme pyridoxal -5-phosphate

(P-5-P) whereas ALT and AST reagents do not, so the use of the IFCC traceable k-factor for ALT and AST may result in either over or underestimation of true values for some patient or external proficiency survey samples.

B-345

Unnecessary Repeat Gamma Glutamyl Transferase Requesting

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Background: There is presently much interest in reducing waste in health care. In laboratory medicine, unnecessary repeat testing is such a focus. Various Minimum Retesting Intervals (MRIs) for gamma glutamyl transferase (GGT) have been proposed: 24 hr, 36 hr, 3 days and 1 week. This study examined the pattern of repeat GGT testing in adult patients at a 1400 bed general hospital in Singapore. **Methods:** Anonymised details of all GGT testing (Beckman-Coulter DxC-800; glutamyl-p-nitroaniline and glycylglycine; 410 nm) for Jan-June 2016 were extracted from the laboratory information system for analysis in Excel. Repeat testing was calculated for the above time cut-offs (with a generous 2 hour buffer subtracted for day-to-day variation in ward round timing, phlebotomy rounds etc.). **Results:** There were 30446 requests in 6 months. 9776 (32 %) were repeat samples with up to 50 repeats in a single patient in a single admission. The cumulative distribution of repeat testing was: 1.0% within 10 hr (12 hr minus 2 hr) of the initial test, 4.0% within 22 hr, 10.8% within 34 hr, 13.3% within 46 hr, 19.7% within 70 hr, 27.7% within 166 hr. **Conclusion:** Using conservative MRIs of 36 hr and 72 hr, 11-20% of all GGT requests on inpatients are inappropriate repeats. This represents 19-34 tests per day. Poor understanding by clinicians of the timeframe for GGT change may contribute to this practice - better education and/or introduction of computerized minimum retest interval guidelines could reduce such over-requesting.

B-346

Evaluation of the Clinical Utility of Neutrophil Gelatinase Associated Lipocalin as a Biomarker of Kidney Injury in Patients with Monoclonal Gammopathies

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Background Monoclonal proteins are frequently associated with kidney injury in multiple myeloma (MM) and other plasma cell dyscrasias. We aimed to evaluate the clinical utility of neutrophil gelatinase associated lipocalin (NGAL), a sensitive marker of acute kidney injury, as a biomarker of renal impairment in patients with monoclonal gammopathies. ***Materials and Methods*** We studied 131 samples from 73 patients with monoclonal gammopaties (57 MM, 4 monoclonal gammopathy of undetermined significance [MGUS], 4 solitary plasmacytoma, 2 smoldering MM, 4 POEMS [polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes] syndrome, and 2 malignant lymphoma) and 30 healthy control samples. We measured serum creatinine (Cr), cystatin C, and NGAL (Bioporto Diagnostics, Denmark) on ADVIA Chemistry XPT System (Siemens Healthcare Diagnostics, USA). Estimated glomerular filtration rate (eGFR) was calculated using CKD-EPI cystatin C equation. Serum M protein and free light chain concentrations were also measured. ***Results*** There were significant correlation between serum NGAL level and Cr (r=0.68, p<0.001), cystatin C (r=0.73, p<0.001), and eGFR based on cystatin C (r=-0.43, p<0.001). Regarding the disease group, the median level (range) of NGAL were 122.8 ng/ml (29.0-1190.6 ng/ml) for MM, 234.7 ng/ml (137.0-428.9 ng/ml) for MGUS, 135.1 ng/ml (68.2-334.6 ng/ml) for solitary plasmacytoma, 132.1 ng/ml (73.5-190.7 ng/ml) for smoldering MM, 195.2 ng/ml (111.3-528.6 ng/ml) for POEMS syndrome, and 150.6 ng/ml (127.7-173.4 ng/ml) for malignant lymphoma. The median levels (range) of serum NGAL were 112.2 ng/ml (29.0-710.9 ng/ml), 162.7 ng/ml (64.2-563.3 ng/ml) and 380.5 ng/ml (173.4-1190.6 ng/ml) for patients with eGFR ≥60 ml/min, 30-59 ml/min and <30 ml/min, respectively (p-ANOVA<0.001). However, serum NGAL level did not correlate with serum M protein burden. Cystatin C levels were in significant relationship with M protein (r=0.33, p=0.005) and involved free light chain concentration (r=0.34, p=0.007). ***Conclusions*** Our data showed that serum NGAL levels were correlated with serum Cr and cystatin C, and measurement of serum NGAL could provide supportive information for monitoring of kidney function in patients with monoclonal gammopathy. **Keywords:** Cystatin C, Kidney injury, Monoclonal gammopathy, Neutrophil gelatinase associated lipocalin

B-347

CSF Total Protein Reference Intervals Determined from 20 years of Patient Data

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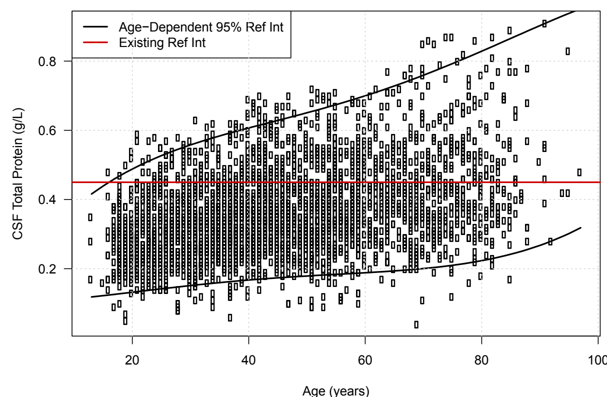
Background: Reference intervals are vital for interpretation of laboratory results. Many existing intervals employed for CSF total protein (CSF-TP) are from old literature, yet performing studies for such invasive samples is challenging. The objective of this study was to determine reference intervals for CSF-TP using patient data.

Methods: Twenty years of hospital database information was mined for previously reported CSF total protein results. Associated demographic, laboratory, and clinical diagnosis (ICD-9/10 codes) were extracted. CSF-TP results included three different analytical platforms, the Siemens Vista 1500, Beckman Lx20, and Roche Hitachi 917. From an initial data set of 18,119 samples, we removed cases with incomplete data and applied the following laboratory exclusion criteria: WBC>5×10^9/L, RBC>50×10^9/L, and glucose>2.5 mmol/L. Patient charts were also reviewed in detail to exclude 60 different conditions where elevated CSF-TP would be expected (e.g. Guillan Barre, brain tumors, MS); pediatric patients were also excluded. After exclusions, outliers were removed using Tukey's interquartile range method. A total of 3,186 samples were included (59% female, median age 44 years old). Continuous reference intervals were determined using quantile regression. Age and sex partitioned intervals were established using the quantile regression equation and splitting age groups into 5-year bins.

Results: CSF-TP showed a marked age dependence (Figure). Males had a significantly higher CSF-TP than females across all ages. CSF-TP results from the three different instruments and manufacturers showed no differences and values were consistent across two decades. CSF-TP showed weak, but statistically significant correlation with WBC, RBC, and creatinine (R^2<0.1), but was completely independent of serum total protein concentration.

Conclusion: CSF-TP reference intervals based on available literature and published in manufacturer's package inserts appear much lower than those determined in this study, particular with advancing age. This is the first report of a sex difference for CSF-TP. Improved diagnostic accuracy from age-partitioned reference intervals for CSF-TP may benefit patients.



B-348

Development of an Automated Enzymatic Method to Quantify Pyruvate Kinase in Red Blood Cells

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Background: Pyruvate kinase (PK) deficiency, an autosomal inherited disorder, is the most common cause of nonspherocytic hemolytic anemia due to defective glycolysis. The objective of this study was to develop and validate an automated method to measure PK activity in red blood cells (RBC).

Methods: PK catalyzes the reaction of phosphoenolpyruvate with ADP to form pyruvate and ATP. In the presence of lactate dehydrogenase and NADH, pyruvate is

reduced to lactate and NADH is oxidized to NAD^+. The rate of absorbance decrease at 340 nm is proportional to PK activity. PK and hemoglobin (Hb) measurements were performed on a Roche Cobas c501 analyzer using RBC hemolysates from residual patient samples sent to ARUP Laboratories. Hemolysates were prepared by combining RBCs with hemolyzing solution (5% Triton X-100, 0.27 M EDTA). After establishing a rate constant (k), accuracy, linearity, imprecision, analytical sensitivity, and analyte stability were validated and a PK reference interval was verified.

Results: The k-factor was established as -9477 by measuring PK activity in 10 patient samples on the c501. Accuracy was evaluated with replicate measurements of PK in 56 patient samples analyzed by the current laboratory-developed, manual PK assay and the c501 over 10 days. Linear regression produced a slope of 1.0, y-intercept of -0.57, and R^2 of 0.93. Linearity was determined by combining a high PK sample with hemolyzing solution in different ratios to create 6 samples that were tested in two replicates. Linear regression analysis produced a slope of 1.02, y-intercept of -2.68, and R^2 of 1.0. The assay was linear to 87 U/dL. Precision was evaluated by testing hemolysates stored at -70°C in 3 replicates once each day for 10 days. Within-run imprecision was 1.9 and 2.5% and total imprecision was 4.0 and 5.6% at 14.0 and 8.1 U/g Hb, respectively. The limit of blank (LOB) was 0.0 U/dL as calculated from the mean plus 3 SD of 10 replicates of saline. The limit of detection (LOD) was determined to be 1.0 U/dL calculated as the LOB added to 3 SD of 10 replicates of a hemolysate pool with a low PK activity (2.3 U/dL). Analyte stability was determined in 4 sample types, each at 2 PK activities. Compared to time zero, the changes of the PK activities were less than 7% when 1) whole blood specimens were stored at 4-8°C for 21 days; 2) hemolysates prepared from washed RBCs stored at -70°C for 1 month; 3) hemolysates in hemolyzing solution stored at -70°C for 2 weeks; and 4) hemolysates in hemolyzing solution stored at -20°C for 2 days. The current PK reference interval of 4.6-11.2 U/g Hb was verified by measuring PK activity in 20 healthy individuals (10 males and 10 females; 21-59 years old). The mean PK activity in paired EDTA and heparinized blood samples obtained from the same individuals was not significantly different (7.8 vs. 7.9 U/g Hb, respectively; p=0.94).

Conclusion: This automated assay for quantifying PK in RBCs has acceptable performance characteristics and is fit for intended use.

B-349

Performance Evaluation of the ADVIA Chemistry System Alanine Aminotransferase P5P (ALTPLc) and Aspartate Aminotransferase P5P (ASTPLc) Assays

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Background: The ADVIA® Clinical Chemistry (CC) Alanine Aminotransferase with liquid P5P (ALTPLc)* and Aspartate Aminotransferase with liquid P5P (ASTPLc)* liquid reagent assays are IFCC traceable. Each assay contains liquid, ready to use P5P (pyridoxal-5′-phosphate), an enhancement over the lyophilized P5P used in the Siemens Heathineers ADVIA ASTP_c and ALTP_c assays.

Methods: The ALTPLc and ASTPLc assays are adaptations of the IFCC Reference Methods. The reactions are initiated by the addition of α-ketoglutarate. The concentration of reduced nicotinamide adenine dinucleotide (NADH) is measured by its absorbance at 340/410 nm; the rate of absorbance decrease is proportional to the analyte concentration. Both the ADVIA ASTPLc and ALTPLc assays provide IFCC reference assay traceable results on both serum and plasma within the analytical range of 8-1000 U/L (ASTPLc) and 9 - 1000 U/L (ALTPLc) undiluted. Each assay can be extended up to 7800 U/L with dilution. Both assays accomplish this with a 25 µL sample size at a time to first result of 10 minutes.

Results: Observed patient sample agreement via method comparison studies for the ALTPLc assay is as follows: ADVIA CC ALTPLc = 0.96 * IFCC Reference Assay - 1.6 U/L (r = 1.00, n = 101, range 12 to 1016 U/L); ADVIA CC ALTPLc = 1.03 * ADVIA CC ALTP_c + 0.2 U/L (r = 1.00, n = 104, range 10 to 950 U/L) ADVIA CC ASTPLc = 1.03 * IFCC Reference Assay - 1.6 U/L (r = 1.00, n = 103, range = 9.9 to 910.9 U/L) ADVIA CC ASTPLc = 0.97 * ADVIA CC ASTP_c - 1.4 U/L (r = 1.00, n = 103, range = 11 to 970 U/L). Precision was evaluated across the assay range per CLSI EP05-A2 using serum/plasma pools and commercial quality control materials. Repeatability and within-lab precision were ≤ 3.6 % CV and ≤ 4.9 % CV for ALTPLc and ≤ 1.1 %CV and ≤ 2.1 %CV for ASTPLc, respectively. Limit of Blank, Limit of Detection and Limit of Quantitation were observed to be 2, 4 and 9 U/L for ALTPLc, and 2, 4 and 8 U/L for ASTPLc respectively. Minimal interference (≤ 10%) was observed for both assays for Bilirubin (20 mg/dL) and Lipemia (500 mg/dL). Agreement of serum and plasma in the ALTPLc assay is represented by: Plasma = 0.99 * Serum - 0.3 U/L (n = 50, range = 15 to 916 U/L) and for the ASTPLc assay by: Plasma = 1.02 * Serum - 1.0 U/L (n = 53, range = 21 to 961 U/L).

Conclusion: The ADVIA CC Alanine Aminotransferase (P5P; ALTPLc) and Aspartate Aminotransferase (P5P; ASTPLc) assays exhibit equivalent performance characteristics to the current Siemens ALTP_c / ASTP_c assays respectively and provide greater ease of use by virtue of ready to use liquid P5P. *Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements. ADVIA and all associated marks are trademarks of Siemens Healthcare Diagnostics, Inc. or its affiliates.

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Performance Evaluation of the Atellica CH Creatinine Kinase, Alanine Aminotransferase (with P5P), and Aspartate Aminotransferase (with P5P) Assays

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Background: This investigation evaluates the performance of clinical chemistry enzyme assays including creatine kinase (CK_L), alanine aminotransferase with P5P (ALTPLc), and aspartate aminotransferase with P5P (ASTPLc) on the Atellica Chemistry (CH) Analyzer.*

Methods: Performance testing, evaluated using Clinical and Laboratory Standards Institute (CLSI) guidelines, included precision (CLSI EP05-A3); method comparison (CLSI EP09-A3); limit of blank, detection, and quantitation (CLSI EP17-A2); interference (CLSI EP07-A2); and serum/plasma equivalence (CLSI EP09-A3).

Results: Assay range for the CK_L assay is 15 to 1300 U/L, the ASTPLc assay is 8 to 1000 U/L, and the ALTPLc assay is 9 to 1000 U/L. Observed agreement in patient sample method comparison studies using Deming regression: Atellica CH CK_L assay = 0.96 * ADVIA® Clinical Chemistry CK_L assay − 3.1 U/L (r = 1.00, n = 177, range: 17-1289 U/L); Atellica CH ALTPLc assay = 1.02 * ADVIA CC ALTPLc assay − 0 U/L (r = 0.999, n = 103, range: 10-995 U/L); and Atellica CH ASTPLc assay = 1.00 * ADVIA CC ASTPLc assay − 4 U/L (r = 0.999, n = 110, range: 13-984 U/L). Precision was evaluated across the assay range using serum/plasma pools and commercial quality control materials. Each sample was assayed in duplicate twice a day for 20 days. Creatine kinase assay repeatability and within-lab precision were ≤1.6% CV and ≤2.4% CV, respectively. Aspartate aminotransferase assay repeatability and within-lab precision were ≤1.8% CV and ≤1.8% CV, respectively. Alanine aminotransferase assay repeatability and within-lab precision were ≤1.8% CV and ≤2.7% CV, respectively. Limit of blank, limit of detection, and limit of quantitation were observed to be 1, 6, and 6 U/L for creatine kinase; 0, 1, and 7 U/L for ASTPLc; and 2, 3, and 5 U/L for ALTPLc, respectively. Minimal interference (≤10%) was observed for the creatine kinase assay with hemolysate (125 mg/dL), bilirubin (60 mg/dL), and lipemia (1000 mg/dL). Minimal interference (≤10%) was observed for both the ALTPLc and ASTPLc assays for bilirubin (20 mg/dL) and lipemia (500 mg/dL). Agreement of serum and plasma in the CK_L assay is represented by plasma = 0.99 * serum − 0.3 U/L (r = 1.00, n = 57, range: 57-1062 U/L); in the ASTPLc assay by plasma = 0.99 * serum + 2 U/L (r = 1.00, n = 56, range:10-982 U/L); and in the ALTPLc assay by plasma = 0.96 * serum + 0 U/L (r = 0.999, n = 56, range: 9-936 U/L).

Conclusion: The creatine kinase, alanine aminotransferase (with P5P), and aspartate aminotransferase (with P5P) assays tested on the Atellica CH Analyzer demonstrated acceptable performance in all tested areas. *Under development. Not available for sale.

B-351

Performance Evaluation of the ADVIA Chemistry System Creatine Kinase Liquid Assay

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Background: The ADVIA Chemistry Liquid Stable Creatine Kinase Liquid assay (CK_L) is a calibrated, IFCC traceable, chemistry assay. This assay is an improvement over the ADVIA CKNAC assay by virtue of its calibration, ready to use reagents, and longer onboard stability. Performance of the Creatine Kinase assay is described below.

Methods: Creatine Kinase reacts with creatine phosphate and adenosine diphosphate (ADP) to form adenosine triphosphate (ATP), which is coupled to the hexokinase-G6PD (glucose-6-phosphate dehydrogenase) reaction, generating NADPH (reduced nicotinamide adenine dinucleotide phosphate). The concentration of NADPH is measured by the increase in absorbance at 340/596 nm. The resulting reaction rate

signal is proportional to the concentration of analyte in the sample. The ADVIA Chemistry CK_L assay is an adaptation of the IFCC Reference Method. The ADVIA Creatine Kinase assay provides IFCC reference assay traceable results within the analytical range of 15-1300 U/L undiluted (up to 7800 U/L with dilution) on both serum and plasma. The assay accomplishes this with a 4.5 µL sample size at a time to first result of 10 minutes. The assay has an onboard stability and calibration interval of 30 days - an increase of 10 days from those of the CKNAC assay.

Results: Observed agreement in patient sample method comparison studies versus two different systems: ADVIA Chemistry CK_L = 1.05 * IFCC Reference Assay - 6.9 U/L (r = 1.00, n = 100, range=16-1245 U/L), ADVIA Chemistry CK_L = 1.01 * ADVIA Chemistry CKNAC - 1.8 U/L (r = 1.00, n = 116 range = 22-1280 U/L). Precision was evaluated across the assay range per CLSI EP05-A2 using serum/plasma pools and commercial quality control materials. Repeatability and within-lab precision were ≤ 2.0 %CV and ≤ 3.4 %CV, respectively. Limit of Blank, Limit of Detection and Limit of Quantitation were observed to be 3, 6 and 15 U/L respectively. Minimal interference (≤ 10%) was observed with Hemolysate (125 mg/dL), Bilirubin (60 mg/dL), Lipemia (1000 mg/dL), Ascorbic Acid (6mg/dL), Sulfasalazine (300 mg/L), and Sulfapyridine (300 mg/L). Agreement of serum and plasma in this assay is represented by: Plasma = 1.01 * Serum - 0.6 U/L (r = 1.00, n = 55, range=37-1282 U/L).

Conclusion: The ADVIA Clinical Chemistry Creatine Kinase assay exhibits equivalent performance characteristics and shows improvement to the current Siemens Healthineers ADVIA CKNAC assay by virtue of its ready-to-use reagents, calibration and improved onboard stability.

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B-352

A mutation at factor VII protease domain *N*-glycosylation site may contribute to the coagulation disorder

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Background:

Factor VII is an important protein in coagulation. Plasma factor VII interacts with the tissue factor released from the injured blood vessel and initiates coagulation. Factor VII deficiency may cause the coagulation disorder and bleeding. In clinical laboratories, it is diagnosed by factor VII assay.

Factor VII is secreted from hepatocytes into the circulatory system. Impaired secretion of factor VII is a cause of factor VII deficiency. Factor VII mutations with poor secretion have been identified in patients with factor VII deficiency. Genetic testing for factor VII deficiency is offered clinically.

Factor VII has one *N*-glycosylation site in protease domain. Previous studies showed that lack of protease domain *N*-glycans impaired factor VII secretion. However, the underlying mechanism is unknown. Meanwhile, a mutation at factor VII protease domain *N*-glycosylation site has been reported in human, but it has not been characterized yet.

Objectives:

The aims of our study were to investigate the underlying mechanism of protease domain *N*-glycans promoted factor VII secretion and to characterize the reported mutation at factor VII protease domain *N*-glycosylation site.

Methods:

cDNA of factor VII was inserted into the expression vector containing C-terminal V5 tag to generate wild type (WT) factor VII plasmid. Plasmids expressing factor VII mutants, N360Q and N360D, were made by PCR-based mutagenesis. HEK293 cells were transfected with plasmids for WT factor VII and mutants, respectively. After one-day culture, proteins binding to factor VII in the cells were co-immunoprecipitated with factor VII and factor VII in the conditioned medium was immunoprecipitated. The accumulated proteins were analyzed by SDS-PAGE and Western blotting.

Results:

Factor VII levels in the cell lysate were similar for WT factor VII and mutants. Compared with WT, N360Q had reduced level of factor VII in the conditioned medium (45 ± 9% *vs*. WT). In the meantime, the level of calnexin binding to N360Q was higher than that in WT (124 ± 6% *vs*. WT). Moreover, the level of BiP binding to N360Q increased significantly (232 ± 45% *vs*. WT). N360D, which is corresponding to the reported human mutation, had a reduced level of factor VII in the conditioned medium (32 ± 9% *vs*. WT). N360Q and N360D in the cell lysate and conditioned medium migrated faster than WT, supporting that the two mutants lacked *N*-glycans.

Conclusion:

The mutations of asparagine at *N*-glycosylation site disrupted the attachment of *N*-glycans to protease domain of N360Q and N360D, hence, proteins migrated faster in SDS-PAGE. Without protease domain *N*-glycans, secretion of N360Q was impaired, showing protease domain *N*-glycans are important in factor VII secretion. At the same time, more factor VII was retained with calnexin and BiP, indicating lower efficiency of utilizing calnexin-mediated protein folding and more misfolded protein removed by BiP. These data reveal that factor VII protease domain *N*-glycans are critical for protein folding and impaired protein folding leads to poor protein secretion. The reported mutation in human also impaired factor VII secretion as shown in N360D, suggesting the mutation disrupting factor VII protease domain *N*-glycosylation exists in human and it may contribute to coagulation disorder.

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Evaluation of serum zinc concentration using protein fraction waveform data

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Background: Zinc (Zn), one of the essential trace elements of human body, has a wide role including metabolism of the skin and hair, cell division, cell metabolism, taste and immunity. In a case of elderly, fall in serum Zn concentration has a risk for appetite loss and malnutrition, and generation of pressure ulcer. On the other hand increase of serum Zn concentration is observed in hyperthyroidism, polycythemia, essential hypertension and hemolytic anemia. Therefore, measurement of serum Zn concentration is important, but as the cost of measurement is expensive frequent measurement of Zn is unreasonable. The aim of this study was to examine serum Zn concentration using the protein fraction waveform with a relatively low cost, and to determine the clinical utility of this method for predicting serum Zn concentration.

Methods: Serum Zn concentration was measured (colorimetric method) using 277 specimens submitted to routine laboratory test. Protein electrophoresis was normalized using raw electrophoresis waveforms obtained by Sebia CAPILLARYS2 PROTEIN(E)6. Raw waveform data were normalized without eliminating the information between the albumin (ALB) and α1 fractions. Mobility data was corrected by positioning the ALB peak at 75, and N,N-dimethylformamide (DMF : used as internal standard in pre-examination) at 300 and β1 peak position at 203. ROC analysis (≦65µg/dL : 1,≧66µg/dL : 0) was conducted at each 300 point of the normalized mobility. Logistic regression analysis was performed using these data as explanatory variables and a prediction formula was obtained to detect serum Zn concentration.

Results: Decrease in serum Zn concentration was associated with drop (AUC = 0.737) in the tail position of preALB (mobility : M33), drop (AUC = 0.750) in the tail position of ALB (mobility : M77) and drop (AUC = 0.705) in the peak position of β1 (mobility : M203) by the ROC analysis. The logistic regression formula : 3.607+(-0.065) × M33+(-0.012) × M82+0.015 × M83 (AUC =0.791) was obtained.

Conclusion: A high diagnostic characteristic associated with decrease in serum Zn concentration existed at the mobility 33, 77 and 203 of the protein fraction pattern. In addition to evaluation of 5 to 6 fractions of the protein electrophoresis, detecting distortion of the wave form peak of the wave form mobility by applying the normalization technology was also important to predict serum Zn concentration.

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Evaluation of Immunoturbidimetric Assays Using the SPA_{PLUS}: Comparison with Immunonephelometry

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Background: Specific proteins have been traditionally analyzed with immunonephelometry. Improvements led immunoturbidimetry to be considered as a validated alternative to immunonephelometry. Before switching to turbidimetric assays, a laboratory should ensure that performance is acceptable for clinical needs, or "fit for purpose". We aimed to verify the performance claims for precision and trueness of IgA, IgG_4 and kappa (κ) / lambda (λ) free light chains (FLC) and total light chains (TLC) assays on SPA_{PLUS}, that were previously validated by the manufacturer. We also evaluated low values of IgA for patients diagnosed with selective IgA deficiency based on serum IgA concentration. According to the European Society for Immunodeficiency, the definitive diagnosis of (selective) IgA deficiency can be

made in patients older than four years of age with a serum IgA of less than 0.07g/L. **Methods:** Assay imprecision was evaluated following the Clinical Laboratory Standards Institute (CLSI) protocol EP15-A2, using three replicates per day for two levels of serum pool over 5 days (n=15 per level.). In order to demonstrate trueness, we used materials of minimum of two analyte concentrations sent for external quality control program. These materials were measured by a number of laboratories, and their peer group mean value (assigned value) was used to assess agreement. In addition, results obtained with SPA_{PLUS} were compared to those obtained with the nephelometer Immage 800. The comparison also included Ig A results less than 0.0667 g/L, which is the limit of quantitation (LOQ) of Immage 800. **Results:** The total precision (CV %) ranged from a low of 0.93% (Ig G_4) to a high of 11.86% (κ FLC). The precision was less than 2.0% for 4 of the 12 serum pool samples. As the estimated within-run and total imprecision for all parameters except Ig A, kappa and lambda TLC were less than or equal to the verification value, we concluded that the data were consistent with the manufacturer's claim. Total imprecision for Ig A, kappa and lambda TLC were checked to see whether they are within the acceptable desirable specifications on Westgard's website updated in 2014 and none were outside desirable limits of imprecision (2.4%, 2.7%, 2.7%; respectively). In addition, the verification of manufacturer's claimed bias was achieved as the verification interval included the assigned value for all parameters. The correlation coefficients of linear regressions were between 0.84 and 0.98 for the analytes measured. Among the 37 patients with Ig A values <0.0667 g/L on Immage 800, 21 had results below the LOQ of SPA plus (<0.02 g/L), 2 had results greater than 0.07 g/L and the 14 remaining patients' results were between 0.02 g/L - 0.0667 g/L. **Conclusion:** In conclusion, SPA_{PLUS} immunoturbidimetric assay is suitable for routine use, and correlates well with representative immunonephelometric assays on the Beckman Immage 800 analyzer. It might also be an increasingly accepted alternative to nephelometry especially for some selected parameters like IgA.

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Reference values capillary protein electrophoresis of a carefully selected healthy population adhering to IFCC recommendations

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Objective: We established percentual and absolute reference values for electrophoresis fractions on the Sebia Capillarys system.

Methods: The study group consisted of healthy individuals from a geriatric study supplemented with working individuals (age range 19-89, n= 478). Malnutrition (transthyretin), immunological status (IgG, IgA, IgM), fatty acid metabolism (cholesterol, triglycerides), iron metabolism (Iron, ferritin, transferrin, soluble transferrin receptor), kidney (creatinine), liver function (ASAT; ALAT, GGT) and blood differential were used to rigidly biochemically classify the study participants.

Results: 42.7% (n=204) had all values in the reference range, at least one value was elevated in 48.5% (n=232) and 8.8% (n=42) showed a monoclonal gammopathy. Subjects above 60 presented with statistically significantly lower albumin. We found no sex related differences, but women using oral contraceptives (n=41) had a statistically higher alpha-1 (median increase: 0.5 g/L) and beta-1 fraction (median increase: 0.85 g/L). These were excluded, yielding 69 men and 106 women (n=175) with a median age of 63 years (range 19- 92)..

Fractions	**g/L**		**%**	
	P 2.5	P 97.5	P 2.5	P 97.5
Albumin < 60 years	41	49.9	56.2	69.5
> 60 years	36.4	45.9	55.5	67.4
Alpha-1	2	3.5	2.7	4.7
Alpha-2	5.2	9.2	7.4	13
Beta	5.6	9.8	8.1	13.4
Beta-1	3.5	5.3	5	7.2
Beta-2	1.8	5.1	2.6	7
Gamma	6.2	15.3	9.6	19.6

Conclusion: To our knowledge this is the first study that establishes percentual and absolute electrophoresis reference ranges according to IFCC criteria. Our rigidly selected reference range group markedly surpasses the recommended IFCC sample limit of 120 and showed a normal Gaussian distribution in all fractions. Albumin age related differences or the effect of oral contraceptives, however, still need further verification with additional larger, rigidly classified subgroups.

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Validation of Alkaline Phosphatase Isoenzyme Assay by Electrophoresis

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Background: Alkaline Phosphatase (ALP) is the enzyme that catalyzes hydrolysis of phosphate esters at an alkaline pH. The liver, bone, intestine, and placenta are of the most ALP-abundant, producing organ specific isoforms. Differentiating these ALP isoforms in circulation may provide valuable diagnostic information. Here we report performance characterization of alkaline phosphatase isoenzyme by electrophoresis.

Method: Validation testing was performed on the SPIFE 3000 with SPIFE alkaline phosphatase isoenzyme gels/reagent (Helena Laboratories, Beaumont, TX, USA) using leftover patient serum samples in our laboratory. Performance validation included precision, method comparison, stability, and reference intervals. Data was analyzed using EP Evaluator Version 10 (Data Innovations LLC, Burlington, VT, USA).

Results: Within-day coefficient of variation (CV) was assessed by analyzing two patient pools 10 times within a single batch, and was found to be 0.4-1.7% for the liver isoenzyme, 1.1-3.0% for the bone isoenzyme, and 17.2% for the intestine isoenzyme. Between-day CV was evaluated by assaying the same pools twice a day for 10 days, and was found to be 2.8%-3.5% for the liver isoenzyme, 3.0-3.6% for the bone isoenzyme, and 207.0-330.4% for the intestine isoenzyme. The large CV for intestine fraction was due to the small quantity in the specimens. This method was compared with a similar agarose electrophoresis method offered by a reference laboratory using 38 leftover patient samples. The comparison offered a slope of 0.988, an intercept of 2.11, and an R value of 0.9662 for the liver fraction, a slope of 1.059, an intercept of 1.96, and an R value of 0.9568 for the bone fraction, and a slope of 0.849, an intercept of 0.36, and an R value of 0.9877 for the intestine fraction. All isoenzyme fractions were found stable in serum for 24 hours at room temperature, 7 days under refrigeration, and 14 days at -20 °C. Specimens (n=209) used for reference range studies had normal total ALP and no clinical indication for abnormal liver, bone, or intestine conditions. The reference intervals indicated in the package inserts were verified using the reference specimens: liver isoenzyme was 26.0-86.2% and the bone isoenzyme was 10.7-68.3% for both genders. We established reference intervals for the intestine isoenzyme fraction (0.0-24.2%) and calculated isoenzyme activities: 14.9-66.8 U/L (liver), 12.2-51.3 U/L (bone), and 0.0-15.3 U/L (intestinal).

Conclusion: The SPIFE 3000 alkaline phosphatase isoenzyme assay by electrophoresis was validated for patient testing. Reference intervals were either verified to the package inserts or established using the reference specimens collected locally.

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Evaluation of free light chains and various protein assays on the Optilite® turbidimetric analyser

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Background: The measurements of serum free light chains (FLC), immunoglobulins (Ig), complement factors 3 (C3) and 4 (C4), and acute-phase proteins are used routinely for the management of various diseases. Serum levels of intact Igs and FLC can indicate plasma cell dyscrasias or immunodeficiencies while levels of complement and acute-phase proteins can reflect the presence of autoimmune disorders, hematologic syndromes or rare diseases such as Wilson's disease.

Objectives: To evaluate the performance of the IgG, IgA, IgM, kappa FLC (κ-FLC), lambda FLC (λ-FLC), C3, C4, haptoglobin (HG), and ceruloplasmin (CP) assays on the Optilite® turbidimetric analyser.

Methods: Precision was assessed using three levels of third-party quality control material tested over 10 working days in duplicate once per day. Correlation studies were performed with a minimum of 40 patient samples compared against the Siemens BN™II Nephelometer or the Binding Site SPAPLUS® Analyser (FLCs only). Furthermore, the FLC assays were evaluated for antigen excess detection and carryover.

Results: All assays gave imprecision of <8% CV at every level. Deming regression showed good agreement with slopes of 1.00 ± 0.2 with the exception of κ-FLC (0.72), HG (0.89), and CP (1.38). All analytes displayed R>0.9. For the FLC assays, antigen excess detection was confirmed using n = 7 (κ-FLC) and n = 3 (λ-FLC) samples and carryover was not observed.

Conclusions: The Optilite® analyser provides robust measurements for Igs, FLCs and acute-phase proteins.

Table 1 - Precision and correlation statistics

Study	Metric		IgG	IgA	IgM	κ-FLC	λ-FLC	C3	C4	HG	CP
Precision	Total CV (%)	Level 1	3.5	3.5	2.4	2.9	0	5.1	3.6	7.2	7.5
		Level 2	3.2	3.0	2.1	1.4	3.7	4.0	4.4	3.9	6.1
		Level 3	2.4	2.9	1.4	2.2	4.7	2.9	4.4	3.1	4.1
Correlation	Deming slope		1.08	1.05	1.08	0.72	1.21	1.13	1.06	0.89	1.38
	Deming y-intercept		-1.02	-0.12	0	11.02	-21.94	0.13	0	0.06	-0.04
	R		0.992	0.997	0.999	0.976	0.988	0.975	0.990	0.987	0.914
	n		57	57	59	54	55	76	72	52	59