# Tuesday PM, July 27

**Poster Session: 2:00 pm – 4:30 pm**
**Proteins/Enzymes**

## B-138

**Establishment of a differential biomarker signature for muscle diseases**

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A comprehensive mass spectrometry-based proteomic survey has been initiated to establish a differential biomarker signature for common skeletal muscle diseases, such as muscular dystrophy, myotonia, diabetes-related muscle weakness and age-related muscle wasting. The biochemical identification and characterization of new markers of neuromuscular disorders will be crucial for (i) furthering our understanding of the molecular mechanisms of muscle pathology, (ii) improving diagnostic approaches, (iii) developing superior methods for monitoring of disease progression and (iv) finding novel therapeutic targets. Established animal models have been employed to study global abnormalities in the dystrophic mdx mouse, myotonic adr mouse, diabetic GK rat and aged Wistar rat. Comparative proteomics was carried out with two-dimensional gel electrophoresis and various dyes including colloidal Coomassie Blue, silver and fluorescent CyDyes. We have also started to test the reliability of new signature molecules with human muscle biopsy samples. The soluble protein complement from skeletal muscle homogenates derived from normal versus pathological samples was extracted and separated by high-resolution two-dimensional gel electrophoresis (1st dimension: pH 4-7, pH 6-11 and pH 3-10; 2nd dimension: 10 kDa to 220 kDa). Depending on the dye staining method, between 600 and 2500 muscle proteins could be visualized per two-dimensional gel. For statistical purposes, routinely 4 biological repeats and 3 technical repeats were performed per analysis. Densitometric scanning was performed with a Typhoon Trio variable mode imager from Amersham Biosciences/GE Healthcare. Gel images were analysed using Progensis Samespots analysis software from Non Linear Dynamics. All analytical gels were aligned to a reference gel. Both, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and electro spray ionization mass spectrometry were used to identify muscle proteins with a differential expression pattern in dystrophic, necrotic, myotonic, diabetic or aged muscle tissues. Immunoblot analysis and immunofluorescence microscopy was carried out to verify altered concentration levels or abnormal localization of specific proteins in pathological muscles. The different groups of identified proteins were involved in metabolic pathways (glycolysis, citric acid cycle, oxidative phosphorylation, nucleotide metabolism), metabolite transportation (oxygen shuttle, fatty acid binding), ion handling (ion binding, ion fluxes, ion uptake, regulation of excitation-contraction coupling), the muscle contraction-relaxation cycle (actomyosin apparatus, troponins, tropomyosins), and the cellular stress response (detoxification, protein folding, protein aggregation). A preliminary list of potential markers, as detected by gel electrophoresis-based proteomics, contains adenylate kinase, small heat shock proteins and calsequestrin for muscular dystrophy, small heat shock proteins for hyperexcitability and relaxation disorders, monoglyceride lipase for diabetes-related muscle weakness, and mitochondrial enzymes of the citric acid cycle and contractile elements for age-related muscle degeneration. In the long-term, the biochemical verification and cell biological characterization of these newly identified biomarker candidates may lead to a better comprehension of general degenerative pathways versus more disease-specific abnormalities that trigger inherited or acquired skeletal muscle diseases.

## B-139

**Analytical validation of the Abbott Architect urine NGAL.**

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**Introduction**: The urine Neutrophile Gelatinase-Associated Lipocalin (NGAL) is a promising early new marker of acute kidney injury. Different studies have shown that it was one of the earliest protein to rise after kidney insult. However, most of these studies have been obtained with cumbersome techniques, particularly difficult to implement in an emergency laboratory. Very recently, Abbot Laboratories launched the urine NGAL on the Architect platform. The aim of this study was to perform a complete and strong analytical validation of this new test. We verified the reference range of the parameter in a healthy population. Finally, we studied the stability of urine NGAL at room temperature, +4°C, -20°C and -80°C.

**Material and methods**: We evaluated the precision with a modified protocol based on CLSI EP-5A2: six urine pools were assayed in triplicate once per day on five different days. Linearity was evaluated based on CLSI EP-6A. Recovery was determined according to CLSI EP-6P. Finally, we evaluated the measurement uncertainty, accuracy and β-expectation limits by assaying 6 urine pools in triplicate during five different days. We settled the β-expectation tolerance limits with β=0.95 and considered the method as valid if each future measurements of the same level had a probability of 95% to fall in the ±20% accepted limits of accuracy. For stability studies, 9 samples were assayed in duplicate at T0 and after 5 and 24 hours of storage at 23°C, and after 24 and 48 hours at +4°C. Stability after storage at -20°C and -80°C was studied by assaying in duplicate the 9 same pools at T0 and after 1,2,8,15,30 and 60 days. Forty-five healthy laboratory volunteers' urine samples were used to verify the 95$^{th}$ percentile proposed by the manufacturer.
**Results**: Repeatability did not exceed 4% and the intermediate precision 6% in the concentration range 22 to 1315 µg/L. The mean recovery was 101.8±6.7%. The method was found to be linear until the 1/10 dilution. Measurement uncertainty was comprised between 3.4 and 12.5%. The accuracy profile built with the predictive tolerance interval method shows that, on average, 95% of the future results that will be generated by this method will be included in the computed tolerance intervals of ±20% in the 22-1315 µg/L studied range. NGAL was shown to be stable after 24 hours of storage at +23°C, 48 hours at +4°C and up to 60 days at either -20°C or -80°C. In the healthy population, the 95$^{th}$ percentile was found at 148.5 µg/L, (median: 23 µg/L), very close to the cut-off proposed by the manufacturer (131.7 µg/L).
**Conclusions**: Abbott Architect urine NGAL is a very robust method. The accuracy profile shows that the method is completely validated between 22 and 1315 µg/L: in this range, we are sure that the values obtained are not at risk of being over ±20% of the true values. We confirmed the expected range proposed by the manufacturer. Samples can be stored up to 1 day at room temperature, 2 days in the fridge and up to two months at -20°C or -80°C.

## B-140

**Diagnostic importance of the kallikrein-kinin system enzymes activity in some pathologic conditions of an organism**

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Background: Study condition of kallikrein-kinin system (KKS) enzymes represents prognostic and diagnostic importance in diseases accompanied by inflammatory processes, also by virus pathology. Proceeding from this studying condition of KKS enzymes at patients with nasal papilloma and accessory nasal sinuses and their interrelation with immune system parameters is actual.
Objectives and Methods: 66 patients with nasal papilloma and accessory nasal sinuses at the age from 15 to 70 and 30 practically healthy (control group) were involved in observation. The patients were investigated by clinic-laboratory, immunological, biochemical, histological, instrumental (computer tomography, nuclear magnetic resonance tomography) and statistical methods.
Results: Obtained values of the KKS enzymes activity and their comparisons with the control are presented in table.
It is shown from the table that an authentic decrease of activity of such enzymes as kallikrein, BAEE-esterase, GAA (general antitryptic activity), kininase ($P<0.001$) is observed, but prekallikrein activity - increases ($P<0.001$) relatively to the control group parameters.
As our researches show studied enzymes activity gives high correlation with parameters of cellular and humoral immunities. Namely kallikrein with T-supressors - 0.975, kininase with immunoglobulin G - 0.995, BAEE-esterase with B-lymphocytes - 0.999, T-lymphocytes - 0.867, IgA - 0.99, IgG - 0.986, IgM - 0.996, prekallikrein with IgA - 0.879, IgG - 0.977, IgM - 0.785.
The established high correlation gives an opportunity to evaluate immune condition of an organism at patients with nasal papilloma and accessory nasal sinuses on the KKS enzymes activity.
We have also confirmed studied interrelation including cycloferone (immunomodulator) and contrical (inhibitor of proteolytic enzymes) in patients therapy. As a result of these measures a sharp reduction of relapse and malignization of diseases is observed.
Conclusion: Thus on the KKS enzymes activity it is possible to evaluate pathological condition of an organism at patients with nasal papilloma and accessory nasal sinuses.

| Enzyme | Measuring unit | Healthy | Patient |
|---|---|---|---|
| Kallikrein | mkmol arginin/(min•l) | 7.5±0.6 | 6.3±0.5 |
| Kininase | mkmol GA/(min•l) | 243.3±15.6 | 204.5±14.8 |
| BAEE-esterase | mEU/ml | 284.8±22.3 | 235.2±20.5 |
| GAA | g/l | 31.2±2.2 | 27.4±2.1 |
| Prekallikrein | mkmol arginin/(min•l) | 350.0±28.1 | 380.6±28.6 |

## B-141

**Development of a latex-enhanced turbidimetric immunoassay for low-grade albuminuria**

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Urine albumin is a well-established marker for diabetic nephropathy. Recent clinical interests are focused on a mortality and morbidity maker for cardiovascular and neurovascular events. Elevation of minute change of albumin may be of clinical significance for early detection and prevention of all above pathophysiologic changes.
We newly developed a novel latex-enhanced turbidimetric immunoassay for low-grade albuminuria. 4 different clones of mouse monoclonal antibody were used. All the procedure for value assignment for HSA in calibrator was performed by weighing. The assigned value was confirmed to be very close to that assigned from ERM DA-470. 0.006 ml of samples was reacted with optimal concentration of antibody-attached latex solution incubating at 20°C for 122 seconds. The detection limit was 0.16 mg/L. An actual analytical range was 0.16-25 mg/L. By setting parameter, it can be extended to approximately 100 mg/L. Three different concentrations of urines were measured for 10 consecutive times until days 14. Intra-assay CVs were 0.5, 0.5, and 0.4 %, respectively, while inter-assay CVs were 1.2, 0.3, and 0.3%, respectively. Recovery tests were satisfactory without effects of 7 different substances. Although varied between urines, the protein was stable at 4°C and -80°C. However, it was unstable at -20°C beyond at day two. More than two cycles of freeze and thawing gave a gradual decrease of the measured value. Vibrating urine vigorously for more than one hour at room temperature resulted in varied value in the range between -94% and 107% beyond 48 hours. Non-specific adsorption of urine albumin was observed on hydrophobic and hydrophilic but not super-hydrophilic plastic tube. Degree of adsorption was much varied depending on urine sample. The lowest recovery rate was 65.6%, that is, approximately 5 mg/l loss when hydrophobic tube is used. Good recovery rate was obtained with hydrophilic tube, but not hydrophobic tube by using belonging sample dilution buffer. Reference intervals were 5.61 ± 3.28 mg/g•Cr (mean ± 1SD) ranging from 1.14 to 17.28 mg/g•Cr in 155 healthy individuals.
The present established assay system was functionally reliable for accurate and precise measurement of low -grade albuminuria. Non-specific adsorption can, however, affect the measured value which was unpredictable. Use of hydrophilic tube in optimized sample buffer solution cleared the problem. The present assay may be a harbinger as an early, sensitive, diagnostic and preventive marker for renal and cardiovascular disorders.
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## B-142

**Evaluation of a Cystatin C Urine application on the Abbott Aeroset**

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OBJECTIVE: To assess the performance of urine samples with the Genzyme Diagnostics Cystatin C Reagent on the Abbott Aeroset.
INTRODUCTION: Cystatin C is a 13 kDa protein that is a member of the cystatin super-family of cysteine proteinase inhibitors. Cystatin C is freely filtered by the healthy glomerulus and is neither secreted nor taken up by cells lining the nephron. In normal kidney function, re-absorption of cystatin C is almost complete and is only detected in small quantities in urine. Given these attributes cystatin C has been proposed as a sensitive marker of glomerular filtration rate. Recently, elevated levels of urinary cystatin C (uCys C) have been associated with renal tubular dysfunction and the onset of Acute Kidney Injury (AKI).
METHODS: The evaluation was conducted using commercially available Genzyme Cystatin C Reagent & Calibrator (Genzyme Corp, Framingham, MA) on an Abbott Aeroset Clinical Chemistry Analyzer (Abbott Labortories, Abbott Park, IL). The assay was calibrated using recombinant cystatin C standards at 0, 0.50, 1.00, 2.00, 4.00, and 8.00 mg/L following the manufacturer's instructions for serum application for the Abbott Aeroset.
Total precision testing was determined by testing frozen aliquots of human urine at Low, Medium, and High levels of CysC over 5 nonconsecutive days, with a separate calibration performed in the morning of each day of testing (CLSI EP10). Linearity was determined by testing a 6 point linearity dilution series derived from a high level cystatin C urine sample (7.1 mg/L) mixed in ratios with a low level cystatin C urine sample (1.41 mg/L). Limit of detection was determined by testing two low level samples which were manufactured by diluting a suspected AKI urine sample with a normal urine sample at two different ratios (CLSI EP17). The Limit of Blank was determined by assaying 20 replicates of a normal urine sample and averaging the assay noise around the blank. The exercise was repeated with a saline blank.
A sample comparison study was conducted using 17 urines collected from normal kidney function subjects and 12 urines collected from suspected kidney injury subjects. Urine Cystatin C and Creatinine values were determined for all specimens.
RESULTS: Total Precision is under 5.0% for the mid and high levels and 15.1% for the low level. The assay was linear from 1.41 mg/L to 7.1 mg/L (R2 0.999, Slope 0.981, Int. 0.021mg/L). The Limit of Detection and the Limit of Blank of the assay were determined to be 0.044 mg/L and 0.025 mg/L, respectively.
Sample comparison testing data shows a demonstrable difference in uCys C levels between normal samples and those suspected of kidney injury.
CONCLUSION: Data generated from this evaluation demonstrates an ability to differentiate between normal urine samples and those from patients suspected of kidney injury with the Genzyme Cystatin C Reagent.

## B-143

**Validation of the ImmunoCAP® ISAC.**

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Background : In the laboratory, the diagnosis of Type I allergy is generally performed by measuring the specific IgE antibodies (sIgE) using allergen extracts. In the recent years, a complex allergen expression pattern has been described for some sources. Recombinant allergens can be used for Component-Resolved-Diagnosis (CRD) of the patients'allergen sensitization profile, whereas allergen extracts allow us to identify allergen-containing sources. CRD permits to diagnose the genuine sensitization of patients towards a given allergenic source or cross-reactive molecules that point to cross-sensitization to several allergen sources. Recently, microarrays have been developed to run CRD. The ImmunoCAP® ISAC (VBC Genomics, Vienna, Austria / Phadia, Uppsala, Sweden) allows the determination of sIgE against 103 recombinant or purified allergen components from many different allergen sources in a single analytical step. Our objective was to establish the validation of the method in allergy diagnosis and to see whether it could be interesting for clinical practice.
Methods : We selected 19 patients with a clinical anamnesis and diagnosis of Type 1 allergy and on the basis of their sIgE tests for recombinant allergens performed with the ImmunoCAP® 250 (Phadia, Uppsala, Sweden). Secondly, we selected two patients with a high total IgE rate ($_{Tot}$IgE) (above 10.000 kU/L) to evaluate the potential unspecific binding of IgE. All the samples and controls were screened for an allergen-specific IgE determination applying the allergen microarray ImmunoCAP ISAC according to the manufacturer's recommendations. Then, we compared the results of the 157 sIgE for recombinant allergen components measured on the ImmunoCAP 250 and those obtained by the ImmunoCAP ISAC microarray platform. We evaluated the concordance between the 2 different techniques and the effects on the ImmunoCAP ISAC results of 2 patients with a high $_{Tot}$IgE rate.
Results : Seventeen of the 122 results found to be positive with ImmunoCAP 250 were found negative with ISAC (concordance 86%). Two results on 35 negative with ImmunoCAP 250 were positive with ISAC (concordance 94%). No unspecific binding was observed up to 150.000 kU/L.
Conclusion : Concerning the positive results, the discrepancies were probably due to a higher positive threshold of the ISAC method (>0.30USI) compared to the ImmunoCAP 250 (>0.10kUA/L). The 2 discrepancies in the negative results concerned two clinically important allergens of peanut (Ara h 1-3). When we looked back to the clinical anamnesis of the 2 patients, we found out that they had undergone a positive oral provocation tests for peanut. Our conclusion came to judge that these patients were sensitized to these major allergens of peanut. Moreover, we found a good specificity towards $_{Tot}$IgE as no unspecific binding was observed with the microarray technique on two sera presenting $_{Tot}$IgE above 10.000kU/L. Our study shows that the ImmunoCAP ISAC performs analytically and clinically well. As it provides many informations in a single step, the results need to be confronted to the clinical profile of the patients.

## B-144

**Development and validation of 14 human serum protein assays on the Roche cobas® c501**

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Objective: We describe the development and performance of 14 human serum protein assays (alpha-1-antitrypsin, alpha-2-macroglobulin, albumin, apolipoproteins AI and B, complement components 3 and 4, haptoglobin, immunoglobulins A, G, and M, orosomucoid, prealbumin, and transferrin) on the cobas c501.
Methods: Calibrators, controls and monospecific antisera were obtained from commercial suppliers. Each antiserum was diluted in 0.2M Tris-HCl and placed in position B of individual cobas c pack MULTI cassettes. Reaction buffer (0.01M PBS containing 48 g/L polyethylene glycol) was placed in position A. Calibrators, controls and patient sera were

diluted off-line in PBS or PBS containing detergent. Assay performance was characterized using CLSI-derived protocols.
Results: We obtained excellent precision at low, normal, and high physiologic concentrations for each protein (within-run imprecision CVs < 2.5%, total imprecision CVs < 3.6%). Calibration for the 14 protein assays was stable for at least two weeks. Linearity for all protein assays was within 5% of the expected value across the calibration range. We observed no significant interference from bilirubin (up to 70 mg/L), hemoglobin (up to 8.9 g/L), triglyceride (up to 28 g/L), or rheumatoid factor (up to 3,930 IU/mL). Samples outside the assay's calibration range were detected and reassayed using the c 501's automatic rerun feature. Antigen excess detection capabilities exceeded 4,900 mg/dL, 5,200 mg/dL, and 20,500 mg/dL for IgA, IgM, and IgG, respectively. Method comparison studies to either the Roche turbidimetric or Siemens' nephelometric assays (BN II) were in good agreement (r > 0.975).
Conclusions: The newly developed assays on the c501 offer precise and accurate results with high throughput and equivalent performance to existing commercial immunoassays. The c501 requires only 60 uL of sample to measure all 14 proteins making it ideal for pediatric samples, research samples of limited volume, or samples that must be split for processing.

Method Comparison Studies (Deming regression)

| Protein | Comparative method (Instrument, reagents) | Range (mg/dL) | n | Slope | Intercept | Sy/x | r |
|---|---|---|---|---|---|---|---|
| A1AT | c501, Roche | 58 - 377 | 67 | 1.12 | -9.3 | 5.2 | 0.998 |
| A2M | BN II, Siemens | 116 - 535 | 97 | 0.90 | 6.0 | 17.6 | 0.985 |
| Alb | BN II, Siemens | 972 - 5,750 | 100 | 0.99 | -71 | 167 | 0.988 |
| Apo AI | c501, Roche | 73 - 261 | 72 | 1.01 | 1.0 | 3.7 | 0.998 |
| Apo B | c501, Roche | 52 - 212 | 66 | 0.99 | 0.9 | 3.4 | 0.997 |
| C3 | c501, Roche | 44 - 300 | 64 | 1.00 | -3.3 | 6.4 | 0.995 |
| C4 | c501, Roche | 10 - 58 | 72 | 1.20 | 1.9 | 2.3 | 0.991 |
| Hpt | c501, Roche | 7 - 455 | 72 | 0.88 | 1.9 | 8.6 | 0.997 |
| IgA | c501, Roche | 25 - 688 | 100 | 1.02 | 0.4 | 10.5 | 0.998 |
| IgG | c501, Roche | 444 - 2,454 | 100 | 1.11 | -16.6 | 40.8 | 0.996 |
| IgM | c501, Roche | 26 - 560 | 99 | 1.04 | -4.7 | 10.4 | 0.996 |
| Oro | c501, Roche | 43 - 246 | 65 | 1.01 | 1.9 | 3.3 | 0.999 |
| Pal | c501, Roche | 10 - 42 | 72 | 1.18 | -2.7 | 1.8 | 0.986 |
| Tf | c501, Roche | 120 - 403 | 72 | 0.94 | 7.3 | 5.2 | 0.997 |

## B-145

### Plasma and tissue fibronectin and serum procollagen III peptide in chronic liver disease patients as reliable biomarkers for hepatic fibrogenesis.

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**Objective:** This work was designed to assess the role and distribution of hepatic tissue fibronectin (FN) and both plasma FN and serum procollagen III peptide (PIIINP) in fibrogenesis in chronic liver disease (CLD) of different etiologies and to investigate the correlation of plasma FN and serum PIIINP with the grades of inflammatory activity, stages of hepatic fibrosis and the intensity of expression of hepatic tissue FN (intracellular or extracellular) in the different studied groups of CLD.
**Materials and methods:** Eighty five patients with chronic liver disease (CLD group) were enrolled in the study, further subdivided according to the etiological pathogenesis into 6 subgroups: chronic hepatitis C group (HCV) (n= 28), HCV and hepatic schistosomiasis group (HCV+Sch) (n=21), HCV and chronic hepatitis B group (HCV+HBV) (n= 6), chronic HBV group (HBV) (n= 12), HBV and Sch group (HBV+Sch) (n = 9) and Sch group (n=9). Fifteen chronic calcular cholecytitis patients were also included as a control group after exclusion of concomitant liver disease. Plasma fibronectin was measured by a sandwich ELISA, while serum PIIINP was performed using a competitive radioimmunoassay technique. FN expression, localization and intensity were assessed in hepatic unstained tissue sections of all subjects by the indirect immunohistochemistry technique using the polyclonal rabbit anti-human-FN-antibody.
**Results:** Plasma fibronectin levels showed a significant increase in both HCV ($p<0.05$) and HCV+HBV ($p<0.05$) groups as compared to controls. Serum PIIINP showed significant elevation in HCV ($p<0.01$), HCV+Sch ($p<0.01$), HCV+HBV ($p<0.01$), HBV ($p<0.05$), HBV+Sch ($p<0.01$), Sch ($p<0.01$) groups as compared to controls. In CLD group, plasma fibronectin correlated positively with serum PIIINP ($r=0.48$, $p<0.01$), the grades of activity ($r=0.8$, $p<0.01$) and hepatic extracellular fibronectin ($r=0.29$, $p<0.01$) but not with hepatic intracellular FN. Serum PIIINP in CLD group correlated with the grades of activity ($r=0.54$, $p<0.01$), and hepatic fibrosis ($r=0.72$, $p<0.01$). On the other hand, the increased expression of extracellular FN in CLD group was found directly correlated with the grades of inflammatory activity ($r=0.35$, $p<0.01$) and stages of fibrosis ($r= 0.29$, $p<0.01$).
**Conclusion:** We concluded that hepatic tissue FN, plasma FN and serum PIIINP could be considered reliable biomarkers for the hepatic fibrogenesis in chronic liver diseases.

## B-146

### Characterization of a mutant R11H αB-crystallin protein that is associated with human inherited cataract

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**Background:** Recently, we identified a missense mutation corresponding to a change that replaced the argnine residue at codon 11 with a histidine residue (R11H) in the αB-crystallin in a large family with autosomal dominant congenital nuclear cataract.
**Objective:** This study aimed to investigate the molecular mechanism of cataract that may develop due to the R11H mutation in αB-crystallin.
**Methods:** Recombinant human wild-type and R11H mutant αB-crystallin were expressed in *Escherichia coli* and purified to homogeneity. The recombinant wild-type and mutant αB-crystallin were characterized by UV circular dichroism, Bis-ANS fluorescence and thermal stability. The chaperone activities of wild-type and mutant αB were compared by using insulin as substrate. The expression constructs for wild-type or mutant αB-crystallin were transfected into HLE and HeLa cells, respectively. The subcellular distributions of mutant R11H versus wild-type αB-crystallin in HLE and HeLa transfectants were compared using confocal microscopy. The levels of cell death in HLE transfectants were determined by FACS.
**Results:** Circular dichroism spectra indicated that the mutant protein exhibited altered secondary, tertiary structures. In addition, bis-ANS fluorescence spectra showed that the mutation had resulted in a decreased surface hydrophobicity. However, the mutant αB-crystallin showed an enhanced chaperone-like activity when insulin was used as a target protein. Furthermore, the mutant αB-crystallin was remarkably similar to the wild-type protein in its subcellular distribution and the thermal stability. The results showed that R11H increased the percentage of HLE cells in apoptotic state in R11H-expressing cells compared with control cells.
**Conclusions:** The R11H mutation in αB-crystallin resulted in altered structure and unusual ability to trigger cell death which could contribute to turbidity and loss of transparency of the lens.

## B-147

### Quantitative electrophoretic profiling for diagnostic prediction of major serum constituents.

### - Comparison of Protein6(Sebia) and CZE 2000(Beckman Coulter) -

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Background: We recently reported that electrophoretic profile by CZE2000 (CZE), Beckman Coulter, could be used to diagnose the severity of DM and metabolic syndrome through standardization of mobility and precise calibration of the wave height. However, CZE was cut out of supply last year and is being replaced by Protein6 (P6), Sebia, in Japan. The two systems differ in assay conditions such as detecting wavelength and inlet voltage, resulting in somewhat different wave patterns.
Aim: In order to elucidate the cause of difference in wave patterns, we matched test results for major serum constituents with the heights of specific segments of the wave profile.
Methods: Duplicate run using the two analyzers was carried out for 3,000 serum samples from healthy volunteers, which were originally obtained to derive reference intervals for 90 major laboratory tests. Raw curve data were retrieved directly from P6 and CZE. Each wave profile was standardized based on locations of peaks for albumin and N,N-dimethylformamide (DMF). The area under the curve was adjusted in reference to total protein concentration. The wave pattern was divided into 300 segments and a segment of the highest correlation with serum concentration of each of 90 analytes was determined.
Results: Serum concentrations of iron, UIBC, LDL, ApoB, and TTR showed close correlations to the heights of specific wave segments in P6, while those of HDL-C, ApoA1, C3, and C4 were closely associated with distinct wave segments in CZE. The most prominent difference between the two systems was the segment matched to serum HDL-C: its location was between albumin and alpha-1 in CZE while it was between prealbumin and albumin in P6. The wave based diagnosis of latent DM and metabolic syndrome was less accurate by P6 than by CZE.
Conclusion: Although the two analyzers give seemingly similar basic peaks in the electrophoretic pattern, detailed analysis of wave segments revealed a grossly different

composition of the curvature. Some adjustment of electrophoretic conditions may be required for P6 to have diagnostically more relevant wave profile.

## B-148

**Measurement of ceruloplasmin and haptoglobin with assay kits for application to RX series analysers.**

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**Background:** Ceruloplasmin and haptoglobin are glycoproteins produced by the liver. Ceruloplasmin is measured for the diagnosis of Wilson's disease and other diseases related to the liver as well as in the detection of estrogen administration. Measurement of haptoglobin is indicated in haemolytic anemia. They are, among other proteins, acute-phase reactants associated with inflammation. The availability of convenient, specific and precise methods for the assessment of these molecules in biological samples is advantageous for research and clinical applications.
**Relevance:** We report the performance evaluation of two developed latex enhanced immunoassays for the quantification of ceruloplasmin in serum or plasma and haptoglobin in serum for application to RX series analysers.
**Methodology:** Immunoturbidimetric assays are employed for the determination of these analytes. The concentration of analyte in the sample is proportional to the increase in absorbance at 340nm. The assays are applicable to the fully automated RX series analysers (RX Daytona and RX Imola) that include dedicated software for data management. Assay traceability was assessed by testing CRM470 reference material. Within-run and total precision were assessed by testing samples at defined medical decision levels, 2 replicates twice a day for 20 days. Correlation studies were conducted with 45 serum samples for ceruloplasmin and haptoglobin.
**Results:** Evaluation of the performance parameters shows an assay range of 3.27mg/dl up to 80 mg/dl in serum/plasma for the ceruloplasmin assay. The assay range of the haptoglobin assay is 0.13g/l up to 4.18g/l in serum. The within-run precision and total precision for three different concentration levels (n=80) and expressed as %C.V. was typically ≤6 for both assays. Correlation with other commercially available assay generated the following linear regression equations: Y = 0.90x + 10.57; r = 0.96 for ceruloplasmin and Y = 0.94x - 0.01; r =0.98 for haptoglobin.
**Conclusion:** Data shows optimal analytical performance of the assays for determination of ceruloplasmin and haptoglobin in biological samples on the RX series analysers. This is of value as analytical tool for application to research and clinical settings.

## B-149

**Preliminary commutability study on a candidate reference material for cystatin C**

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*Background:* Plasma (or serum) cystatin C has been proposed as a marker for the glomerular filtration rate, a measure of the capacity of the kidneys to filter plasma (GFR) [1]. Several studies, as well as one meta-analysis [2], have suggested that it is superior to serum creatinine for estimation of GFR. Particularly, cystatin C could be used for tests for children, elderly, patients with low muscle mass, and the early stages of kidney problems, where creatinine measurements do not perform well. Additionally cystatin C is used as a marker for cardiovascular risk and pre-eclampsia.
The IFCC working group for the standardisation of cystatin C has, in collaboration with the IRMM, prepared a candidate reference material (RM) for cystatin C. Homogeneity and stability studies have been completed successfully. The characterisation of the RM was performed using immunoassays, calibrated with a pure protein preparation to which values had been assigned by dry mass determination. Here the results from a preliminary commutability study, performed in order to optimise the design of a planned large-scale commutability study, are presented.
*Methods:* Commutability studies have been performed using four assays, all employing antibodies from different sources. Thirty serum samples were measured together with dilutions of the candidate RM (mass fractions of 0.1, 0.2, 0.25, 0,3, 0.6 in assay diluent) as well as the undiluted material, in one analytical run, in triplicate. The methods used were the Siemens N Latex Cystatin C Test Kit run on a BN ProSpec®, the Sentinel CH assay run on an Architect c16000, the Roche tina quant cystatin C assay run on a Cobas c501 and the Gentian cystatin C immunoassay run on an Architect.
In order to assess the commutability of the material it was investigated whether the measurement results of the dilutions and the candidate reference material were within the 95 % prediction interval of the linear regressions.
*Results:* The data were first analysed by performing linear and polynomial regressions on the means of the measurement results of the patient samples for each combination of assays. For comparisons of measurements performed with the Siemens, Sentinel and Gentian assays the results showed a good linear correlation. For comparisons of the results of these three methods with the Roche assay a better $R^2$ was obtained with a polynomial regression as there was a deviation from linearity at high concentrations. The candidate RM and its dilutions are commutable for combinations of the Siemens, Gentian and Sentinel assays. Dilutions with a mass fraction equal to or below 0.6 are commutable for combinations of these three assays and the Roche tina quant assay.
*Conclusions:* The candidate reference material is commutable for combinations of methods tested, although sometimes only for dilutions. The planned large scale commutability study should include appropriate dilutions of the RM, and test plasma as well as serum.
[1] A. Grubb, O. Simonsen, G. Sturfelt, L. Truedsson, H. Thysell Acta Med Scand 218 (1985) 499-503. [2] V. R. Dharnidharka, C. Kwon, G. Stevens Am J Kidney Dis 40 (2002) 221-6

## B-150

**Evaluation of Immunoturbidimetric Specific Protein Assays on the Abbott ARCHITECT® *c*4000 Clinical Chemistry System**

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*Objective*: Evaluate the performance of immunoturbidimetric Specific Protein assays on the Abbott ARCHITECT® *c*4000 Clinical Chemistry System.
*Methodology*: The ARCHITECT *c*4000 Clinical Chemistry System uses the reagent configuration, calibration processes, reaction modes, photometric technology and Integrated Chip Technology® (ICT) that are available on the Abbott ARCHITECT® *c*8000 and *c*16000 Systems and the Abbott AEROSET® System. The ARCHITECT *c*4000 is a fully automated analyzer that can be run as a stand-alone instrument or integrated with the ARCHITECT® $i1000_{SR}$ (the immunoassay module in the ARCHITECT family of instruments).
*Results*: Assay performance (Precision, Sensitivity (Limit of Quantitation), Linearity and Method Comparison) was characterized using CLSI-derived protocols. Precision was determined using three levels of controls. LoQ was performed on three ARCHITECT cSystems. The analytical range represents the low and high linearity claim. Method Comparison was evaluated by assaying serum patient samples across the entire range of the assay comparing the *c*4000 to the *c*16000. Bias was determined at the medical decision level(s). Sigma Metrics were calculated and ranged from 5.0 (Immunoglobulin G) to 13.7 (Immunoglobulin A).

| Assay | Units | Control Imprecision (Total %CV) Level 1 | Level 2 | Level 3 | Limit of Quantitation (Sensitivity) | Table of Contents Analytical Range Analytical Range | Method Comparison (vs c16000) Slope | R | % Bias |
|---|---|---|---|---|---|---|---|---|---|
| Apolipoprotein A, | mg/dL | 1.1 | 0.8 | 1.8 | 2 | 16 - 310 | 1.03 | 0.999 | 2 |
| Apolipoprotein B | mg/dL | 1.6 | 1.0 | 1.0 | 2 | 11 - 240 | 0.99 | 0.998 | 0 |
| Complement C3 | mg/dL | 1.4 | 1.4 | 1.7 | 3 | 11 - 315 | 0.98 | 0.998 | 1 |
| Complement C4 | mg/dL | 1.4 | 1.3 | 1.4 | 0.8 | 2.0 - 59.8 | 1.00 | 0.998 | 3 |
| Haptoglobin | mg/dL | 1.7 | 1.5 | 1.5 | 2 | 12 - 230 | 1.01 | 0.999 | 2 |
| Immunoglobulin A | mg/dL | 1.4 | 1.2 | 1.5 | 2 | 5 - 3400 | 1.01 | 0.999 | 1 |
| Immunoglobulin G | mg/dL | 1.5 | 1.6 | 2.4 | 40 | 94 - 3839 | 0.97 | 0.998 | 1 |
| Immunoglobulin M | mg/dL | 3.8 | 1.7 | 1.5 | 2 | 14 - 1537 | 1.01 | 1.000 | 2 |
| Prealbumin | mg/dL | 1.1 | 1.2 | 1.6 | 0.3 | 3 - 54 | 1.00 | 0.998 | 1 |
| Rheumatoid Factor | IU/mL | 2 sd | 2 | 1 | 12 | 10 - 200 | 1.01 | 0.999 | N/A |
| Transferrin | mg/dL | 2.1 | 1.3 | 1.4 | 7 | 19 - 477 | 1.00 | 1.000 | 1 |

*Conclusion*: The ARCHITECT *c*4000 Clinical Chemistry System demonstrated acceptable performance characteristics that achieved or exceeded all pre-established analytical goals. The method correlation data support the equivalence of the ARCHITECT *c*4000, *c*8000, *c*16000 and AEROSET Systems. The common reagent and commodity requirements across the ARCHITECT system family allows the laboratory the flexibility to use any of these instruments depending on the user needs and still maintain commutable results. Further, the ability to integrate the *c*4000 with an $i1000_{SR}$ immunoassay module provides the capacity to analyze routine clinical requests on a single platform.

## B-151

**Measurement of urinary cystatin C by particle enhanced turbidimetric immunoassay (PETIA) on automated biochemistry analyzer**

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**Background:** The measurement of specific proteins in urine is important in differentiating glomerular from tubular proteinuria. Cystatin C (CysC), is produced all nucleated cells of human body, is freely filtered by the kidney glomerulus and reabsorbed by the tubules,

where it is almost totally catabolized, with the remainder then eliminated in urine. Tubular re-absorption occurs by endocytosis, through a receptor (megalin) which is common to many proteins. It is widely accepted that no tubular secretion of CysC occurs. Raised urinary levels are believed to indicate tubular damage. We report here for the first time the development of a quantitative assay to measure urinary cystatin C (uCysC) using a commercial CysC kit (Sentinel) based on a latex particle enhanced turbidimetric immunoassay (PETIA), on Architect-ci16200 analyzer (Abbott).

**Methods**: The standard spline data reduction method of calibration protocol was used, using 6 calibrators and a reagent blank. A calibrator with an initial value of 2780 ug/L was used in order to prepare 5 dilutions reaching a minimum concentration of 60 ug/L. Dilutions were prepared automatically by the analyzer and measured in duplicates. The clinical relevance of the above assay was tested on several kidney disease patients (20 patients with kidney tubular disease [KTD], 20 patients with stable chronic kidney disease [SCKD], 10 patients with prerenal azotemia [PRA], 15 patients with normal kidney function [NKF]) and in 24 healthy controls. Spot-urine collections were used from the above patients. All patient samples were tested in triplicates. Statistical analysis was performed with SPSSv16 software.

**Results.** The limit of detection of this assay was determined at 20 ug/dL. Precision (within-run and total CV) was tested with four control serum preparation at the following concentrations 79, 203, 401 and 1242 ug/L, for 20 consecutive days with the use of two different reagent lots and it was acceptable in all cases (<5%). Calibration was stable for at least 20 days. Urinary-CysC was stable, at urine pH≥5, at -20°C for at least 14 days, at 4°C for at least 7 days, and at 20°C for 48 hours. Repeated freezing and thawing did not influence uCysC concentration.

Values in healthy controls ranged from non-detectable (<20 ug/L) to 86.67 ug/L (median 41.7, and interquartile range 22.5-86.7 ug/L). Median uCysC concentration in KTD patients (1473 ug/L, interquartile range 505.8-9030.0 ug/L) was significantly higher (Kruskall-Wallis test) than in NKF patients (43.3, 36.7-70.0;p<0.0001), SCKD patients (66.7, 45.0-626.7;p<0.0001) and PRA patients (110.0, 95.8-206.7;p<0.0001).

**Conclusion**: Our Data indicate that uCysC can be processed on automated clinical chemistry analyzers. Preliminary data show that increased uCysC levels may indicate tubular dysfunction and allow its accurate detection of among various types of kidney disease. Therefore its measurement could easily be added to the standard panel used to screen kidney pathologies even in emergency situations.

## B-152

### Estimating glomerular filtration rate: Comparison of Cystatin C-based formulas with Chrome-EDTA clearance

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Correctly estimating glomerular filtration rate (GFR) is fundamental when making a clinical assessment of the kidney function. GFR can be determined by measuring the clearance of exogenous or endogenous substances. It can also be estimated by means of formulas based on variables such as the serum level of a substance and an individual's personal characteristics, such as weight, height, sex, age and ethnic origin. Chrome-EDTA clearance has a correlation of more than 97% with inulin clearance and is accepted as being an accurate method for determining the renal function. Cystatin C, a 13 kD protein produced at a constant rate and present in all nucleated cells, has been proposed to estimate GFR, In the last few years several Cystatin C-based formulas have been developed to estimate the GFR.

The aim of this study was to compare the performance of eight different Cystatin C-based formulas in estimating the glomerular filtration rate in patients with renal diseases or with risk factors for renal diseases. Blood samples from 30 adult patients were collected to measure Cystatin C levels (BN II - Siemens), after which GFR was calculated by several formulas that do not consider gender in the equation. These formulas included the simple Cystatin-C formula and those described by Grubb et al, Hoek et al, Hojs et al, Larsson et al, Le Bricon et al, MacIsaac et al and Rule et al. Chrome-EDTA clearance was also measured. Simple linear regression (least square method) and variance analysis (one way ANOVA) were used to the statistical analysis. The performance of Cystatin C-based formulas in assessing GFR was determined in terms of its sensitivity, specificity, positive and negative predictive values, and accuracy.

The statistical analysis (one way ANOVA) revealed no difference between the GFR estimated by all the studied formulas (p>0.05). There was an extremely high correlation between the GFR calculated from serum Cystatin C-equations and the Chrome-EDTA clearance (r = 0.95 in all cases, sensitivity = 100%, specificity > 89%, accuracy > 93%). Despite these data, we observed that the simple Cystatin C-formula (100/Cystatin C) tends to overestimate the GFR when compared to the Chrome-EDTA clearance and to the other formulas. This difference is more evident in the lower GFR levels.Our data showed that all the proposed Cystatin C-based formulas have a similar performance compared to the Chrome-EDTA clearance and can be safely used in the clinical practice to estimate GFR. Special care should be taken if the simple Cystatin C-formula is used.

## B-153

### New Pregnancy-Associated Peptide Found and Characterized by a Novel Serum Proteomics Method

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Objective: To determine whether a recently identified peptide may be pregnancy specific.

Background: A recently developed serum proteomics approach involving capillary liquid chromatography (cLC) coupled to electrospray ionization mass spectrometry (ESI-QqTOFMS) has been applied to serum from both non-pregnant human control specimens and women in the second half of their pregnancy. This proteomic approach probes both the low molecular weight and low abundance serum 'proteome'.

Methods: Sera were submitted to a published protein depletion step prior to introduction onto the cLC system. A 250 µm C8 equivalent column was put through a 50 min gradient elution and eluate was passed through an electrospray needle and into a tandem QqTOFMS instrument (Applied Biosystems) for the initial data survey to locate species of interest. Additionally, those species selected were then reanalyzed using an in-line collision cell and collision fragments analyzed in the second MS allowing for amino acid sequence identification. Thereafter a synthetic analogue was synthesized using stable heavy isotopes to allow for more accurate quantitation. To conserve specimen, improve reproducibility and increase throughput, subsequent research used an Agilent 6510 QTOF with 1200 series HPLC and Chip Cube nanospray source. One species, found in serum of pregnant women between 20-30 wks gestation with uncomplicated pregnancies, appeared to be absent in control specimens. This led to further characterization of this peptide. Quantitation was achieved by spiking serum with 66.0 pg/mL of the calibrating analogue prior to processing and MS analysis.

Results: A peptide having m/z of 677 in its predominant +3 charge state was observed only in the sera of pregnant women studied here compared with serum from men and non-pregnant women (controls, n=140, 0.0+0.0 vs pregnant women (n=41), 3.0+0.5 pg/mL, p=1.1x10-20). Concerns over the origin of the peptide led to stability studies. Stable isotope spiked into non-pregnant serum in the absence of protease inhibitors showed the following 24 hr reductions versus water control (frozen: 9%; 4oC: 7% loss: room temp: 17% loss). However, the 24 hr stability profile appeared to be different when the spike was introduced into pregnant serum in the absence of protease inhibitors: (4oC: 2.1% loss; room temp: 66.3% loss). Storage of serum from pregnant women for 24 wks at -20oC did not appreciably change levels of the endogenous peptide. The month-to-month interassay CV over this period was on average 8.2%. Finally, sera from non-pregnant individuals stored for 4 yr (n=4) and 10 yr (n=4) did not demonstrate the peptide.

Conclusions: A previously undescribed peptide was located and identified by means of a serum proteomic approach. Studies to date suggest that this peptide may be specific to pregnancy and does not appear to arise from long term storage and is relatively stable in the absence of protease inhibitors in frozen specimens for at least 24 wks and at 4oC for up to 24 hr. The effect of gestational age has not been studied. Likewise, the function of this peptide, if any, and its potential diagnostic utility are currently unknown but under investigation.

## B-154

### Clinical Utility of Cystatin C in screening of Kidney Dysfunction

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**Objective:** We evaluated the clinical utility of Cystatin C in screening and monitoring of kidney dysfunction in comparison to β2-microglobulin and Creatinine using samples from patients at various stages of Chronic Kidney Disease (CKD).

**Methods:** Latex-enhanced TIA for Cystatin C (Denka Seiken, Japan) was validated on a Hitachi 7600-310s P-Module (Hitachi High-Technologies Corporation, Japan). Within-run precision, between-day precison, linearity, sensitivity, prozone and interference from various endogenous substances were asseseed prior to the study with clinical samples. Clinical utility was assessed using samples from hospitalized patients (99 males and 47 females) at various stages of CKD. Cystatin C and β2-microglobulin were tested in serum samples. Creatinine was tested both in serum and urine samples to obtain Creatinine Clearance and eGFR values.

**Results:** The Cystatin C assay was confirmed to have CVs below 2%, the assay range up to 10 mg/L, and prozone tolerance up to 70 mg/L. There was no significant level of interference with 4.83 g/L of hemoglobin, 19 mg/dL of conjugated- and unconjugated-bilirubin and 725 U/L of rheumatoid factor. Cystatin C showed good correlation with β2-microglobulin, Creatinine Clearance and eGFR. The correlation between serum Cystatin C and serum Creatinine was also good when Creatinine was relatively low. When Creatinine level was over 4 mg/dL, however, the slope of the correlation curve tended

to be compromised. Clinical sensitivity was compared at each stage of CKD among Cystatin C, β2-microglobulin and Creatinine. There was no difference at Stage 3, 4 and 5 among 3 parameters. At Stage 2, however, only Cystatin C showed a positive rate over 50% (58%). At Stage 1, while Creatinine showed no single positive result, Cystatin C gave a positive rate of 27%. Through detailed investigation with individual samples, it was revealed as a false negative of Creatinine due to loss of muscles in such hospitalized patients. **Conclusions:** Cystatin C was revealed as the more sensitive and more specific biochemical marker in screening of kidney dysfunction better representing GFR than Creatinine and β2-microglobulin.

## B-155

### Analytical and clinical evaluation of an enzyme-linked immunosorbant assay for measurement of afamin in human plasma

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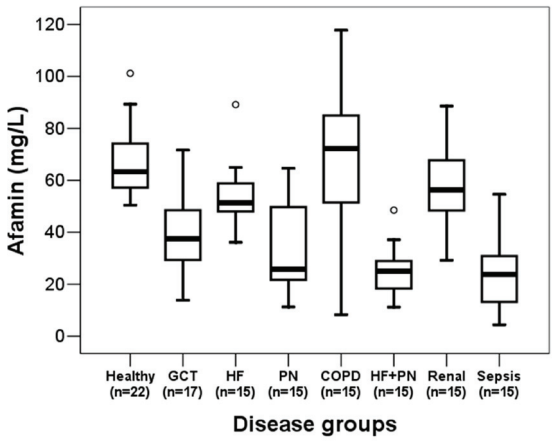
**Background:** Comparative proteomics has recently identified afamin, the newest member of the albumin gene family, as a potential biomarker for ovarian cancer. The aim of this study was the analytical and clinical evaluation of a sandwich ELISA assay for the determination of afamin in human plasma.
**Methods:** We evaluated precision, linearity, and detection limit of the assay, analyte stability and biological variability, determined reference values and afamin concentrations in fasting vs. non fasting status. For the clinical evaluation we compared afamin plasma concentrations in a healthy control group with patients with various diseases [i.e., primary testicular germ cell tumor (GCT), heart failure (HF), pneumonia (PN), chronic obstructive disease (COPD), HF and co-morbidity of PN, renal disease, and sepsis].
**Results:** Within-run and total coefficients of variation were 3.3% and 6.2% at a mean concentration of 73 mg/L. The method was linear across the whole measurement range of the assay. Detection limit was 7 mg/L for the assay. The analyte was stable for 24 hours at room temperature, for 48 hours at 4°C, and for at least 1 year at -20°C and -80°C. The reference change value for healthy individuals was 24%. Age- and sex-independent reference values were 45-99 mg/L (median 68 mg/L). There was no significant difference in afamin plasma concentrations in fasting vs. non-fasting status. In the clinical assay evaluation (see Figure 1) median afamin concentrations were modestly decreased in patients with HF. Patients with GCT, PN, HF and co-morbidity of PN, and sepsis exhibited markedly decreased afamin concentrations. However, in patients with chronic renal disease or COPD there was no difference compared to healthy individuals.

**Fig. 1. Afamin plasma concentrations in various diseases**




**Conclusion:** The afamin assay meets the needs of quality specifications of laboratory medicine. The results of the clinical assay evaluation are novel with respect to afamin in various diseases and should initiate further studies.

## B-156

### Performances of two cystatin-c reagents suitable for Beckman Coulter Dx-C analysers

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**Background:** Cystatin-C is a low-molecular weight protein freely filtered by the kidney glomerular cells, then completely reabsorbed and catabolised in the proximal tubular cells. These physiological proprieties make cystatin-c as a relevant biomarker to monitor kidney function and to estimate the glomerular filtration rate (GFR). The aim of our study was to evaluate the analytical performances and reliability of two turbidimetric methods for cystatin-c determination on Beckman Coulter Dx-C system.
**Method:** Cystatin-C levels were measured on Dx-C analyser (Beckman Coulter, Brea, USA) with the Gentian® reagent (Gentian®, Moss, Norway) and with the Dako® reagent (Dako®, Glostrup, Denmark). Method imprecision was evaluated with pools of plasma and linearity was confirmed through serial dilutions of patient samples. Method comparison was performed with BN-II nephelometric method (Siemens®, Erlangen, Germany) through 128 patient samples. Evaluation of reference ranges was done with 44 healthy volunteers. GFR estimations were calculated for each cystatin-C methods on the basis of standardised equations. Statistical analyses were performed with Medcalc® software and CBstat®.
**Results:** Intra-run coefficients of variation (CV) for Cystatin-C concentrations about 1 and 2 mg/L were: 5,20% and 3,23% for the Gentian® method and 5,33% and 6,13% for Dako® method. Inter-run CV were < 7% for Gentian® and < 13% for Dako®. The functional sensitivities determined for a CV of 10% were 0,56mg/l and 0,96mg/l for Gentian® and Dako®, respectively. Both methods were linear and mean recoveries were 98,42% and 122% for Gentian® and Dako®, respectively. The reference values were between 0,65mg/l and 1,14mg/l for Gentian® and between 0,65mg/l and 1,24mg/l for Dako®. Both, Gentian® and Dako® methods, were significantly correlated to the Siemens® reference method ($r = 0,9820$, $p < 0,0001$ and $r = 0,9569$, $p < 0,0001$ for Gentian® and Dako®, respectively). Method comparison by Passing and Bablok regression analysis between Gentian® and Siemens® methods displayed a slope of 1,005 (95% Confident Interval: 0,971 to 1,047) and an intercept of 0,078 (95% CI: 0,076 to 0,082). Passing and Bablok analysis between Dako® and Siemens® methods gave a slope of 0,871 (95% CI: 0,830 to 0,920) and an intercept of 0,107 (95% CI: 0,105 to 0,113). Bland and Altman plots only showed limited bias. The eGFR equations, adapted with the Passing and Bablok regression data from a published formula of the Siemens method, were 76,7*{10^[(log(Cystatin-c concentration)-0,07781)/1,0054]}^-1,19 for Gentian® and 76,7*{10^[(log(Cystatin-c concentration)-0,1074)/0,8711]}^-1,19 for Dako®. The mean eGFR values were 77,6ml/min/m² (95% CI: 71,7to 83,5) for Siemens®, 78,1ml/min/m² (95% CI: 71,9 to 84,3) for Gentian® and 80,4ml/min/m² (95% CI: 73,5 to 87,3) for Dako®.
**Conclusions:** Our results demonstrate that analytical performances obtained on Beckman Coulter Dx-C analyser were excellent for Gentian® and fair for Dako®, allowing their use for routine measurement of cystatin-C. Furthermore, using standardized equations, both methods appear as similar for the estimation of eGFR.

## B-157

### The Activity Of Lipoperoxidation Processes And Tubular Enzymes In Progressing Diabetic Nephropathy

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INTRODUCTION The lysosomal enzymes (N-acetyl-ß-D-glucosaminidase (EC 3.2.1.30, NAG), β-galactosidase (EC 3.2.1.23,β-GAL) and mitochondrial enzyme L-canavanine: ornithine amidinetransferase (EC 2.1.4.1, COAT) is well studied as markers of the "cellular membrane pathology" showing the changes in proximal nephrothelium in renal diseases.
AIMS The work presents the results of studying in urine the activity of tubular enzymes having the organ specific peculiarities in relation to kidneys -NAG, its thermostable isoenzyme NAG B and β-GAL having lysosomal localization, and COAT having mitochondrial localization and also the activity of process of lipoperoxidation judged about by the level malondialdehyde (MDA) in blood serum, erythrocytes and in urine in 82 patients with diabetes mellitus in progressing the late state of diabetic nephropathy (DN): group I - 42 patients (20 patients with type 1 and 22 - type 2 diabetes) with the preserved function of kidney (GFR 65,5-114,3 ml/min,), group II -40 patients (20 patients with type 1 and 20 - type 2 diabetes) with the broken function of kidney (GFR 23-49 ml/min) when chronic renal failure of various degree developed. Patients have been included in research with subcompensation of a carbohydrate exchange. Also 30 healthy patients of the same age (controls) were surveyed.
RESULTS It was noted, that for DN patients, an increase in NAG, especially its thermostable isoenzyme NAG B and also β-GAL activity in urine was typical, and the occurrence of COAT activity, usually absent in urine of healthy persons, was also

characteristic. The changes of COAT activity in the certain degree depend on type of diabetes: the maximal activity are ascertained in patients with diabetes types 2.
In parallel, at all DN patients had large contents of MDA in serum, erythrocytes and urine against normal data. The DN progression in patients lead to significant increase of MDA level in blood serum: maximal level of MDA was registered in group II (in particular in the patients with type 1diabetes mellitus).
CONCLUSIONS The precise dependence of levels of NAG, NAG B, β-GAL, COAT activity and MDA level on functional state of renal parenchyma was found, in particular on nephrocytes of tubular nephron, that allows to regard the given parameters as markers of progressing of diabetic process in kidneys in patients with diabetes. It was marked, that the type of diabetes mellitus in the certain degree influences the changes of researched parameters, in particular it concerns MDA and COAT.

## B-158

**Polyethylene Glycol Precipitation of Eight Enzymes for Macroenzyme Detection**

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Serum macroenzymes may cause elevations in total enzyme activity leading to diagnostic confusion. Polyethylene glycol (PEG) precipitation is a useful technique which can help detect macroenzymes in serum. However, reference intervals need to be established for PEG precipitation. We analyzed alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMYL), aspartate aminotransferase (AST), creatine kinase (CK), γ-glutamyltranspeptidase (GGT), lactate dehydrogenase (LD) and lipase (LIP) before and after PEG precipitation on the Roche Modular E170. We evaluated 120 samples from healthy subjects to establish reference intervals. For comparison an additional 79-237 samples with enzyme activities greater than the upper reference limit (URL) were tested for each analyte. An additional five confirmed macro-CK samples were analyzed for macro-CK by PEG. Reference intervals (RI) and mean recovery were determined for all analytes (Table). Using the reference intervals for total enzyme activity established from the healthy subjects, the mean recoveries were determined for all samples with activities > URL. A URL of 200 U/L was used for CK. Mean recoveries ranged from 29-96% for healthy subjects and 29-80% for samples above the URL. Using a -2SD cutoff from the mean recovery for CK samples above the URL we were able to identify 3 possible macro-CK samples of which 2 were confirmed to contain macro-CK by electrophoresis. Of the 5 confirmed macro-CK samples, 4 were below the -2SD. In the current study, 4 of the 8 analytes had mean recoveries for samples with activities above the URL that compared well to those established with healthy subjects. In conclusion, ALP, GGT, LDH and LIP had recoveries after PEG precipitation that differed between the reference interval and greater than URL sample sets. Further analysis including definitive macroenzyme detection needs to be performed on these analytes before PEG precipitation can be implemented for clinical use.

| Analytes | Reference intervals, central 95% (N=120) | | | Samples with total enzyme activities > URL | | |
|---|---|---|---|---|---|---|
| | Neat samples (U/L) | PEG supernatant (U/L) | Mean recovery, % (± 2SD) | N | Mean recovery, % (± 2SD) | N with mean % recovery, < -2SD |
| ALP | 33-112 | 28-124 | 96 (68-124) | 117 | 80 (51-110) | 5 |
| ALT | 9-49 | 2-14 | 29 (5-54) | 143 | 29 (8-51) | 1 |
| AMYL | 23-112 | 12-64 | 57 (37-78) | 100 | 58 (30-87) | 4 |
| AST | 13-35 | 4-20 | 52 (24-81) | 231 | 47 (20-73) | 4 |
| CK | 30-471 | 20-307 | 60 (40-81) | 79 | 61(33-89) | 7 |
| GGT | 8-68 | 6-48 | 86 (50-123) | 177 | 51 (18-85) | 3 |
| LD | 110-211 | 28-88 | 33 (17-50) | 237 | 32 (2-61) | 1 |
| LIP | 18-68 | 8-30 | 49 (29-70) | 133 | 40 (18-63) | 1 |

## B-159

**Cystatin C - A newly developed assay for early detection of kidney disease**

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Cystatin C is a non-glycosylated, basic protein (120 amino acids) with a low molecular weight of 13.4 kDa. It acts as a cysteine protease inhibitor and can be found in all organs and nucleated cells investigated. Cystatin C is said to be a more reliable and sensitive measure of the glomerular filtration rate (GFR) than creatinine clearance or serum creatinine. It is endogenously produced at a constant rate by all nucleated cells investigated, freely filtered in the renal glomeruli, and almost completely reabsorbed and degraded in the renal tubuli in healthy people. Almost no secretion into urine takes place. Beside this, the cystatin C level is independent of age, sex and muscle mass. Especially children under 4 years of age are going to benefit from this analyte as cystatin C enables an early and reliable detection of a moderate GFR decrease which is not possible with creatinine clearance. Moreover, cystatin C determination is recommended in patients with potential glomerular impairment like in diabetes, liver cirrhosis and after renal transplantation.
We developed a liquid-stable, particle enhanced, turbidimetric test, using a polyclonal goat antibody. Controls (normal and pathological range) and calibrators (5 levels) have been also part of the development and are based on recombinant cystatin C. Because an internationally accepted IFCC standard is still not available a traceability chain to another commercial assay was established and an uncertainty of value assignment of 3 % has been calculated.
In an assay protocol for a Hitachi 917 analyzer a sample volume of 2 µL is added to 180 µL of reagent 1. After incubation for 5 minutes at 37 °C the addition of 60 µL of reagent 2 (first absorbance reading) is followed by a further incubation for 5 min at 37 °C (second absorbance reading). The absorbance difference of the two readings is proportional to the amount of cystatin C concentration. The calibration curve is fitted by a cubic spline algorithm. Intra- and interassay precision of the assay are less than 3.0 % and 5.0 %. Total precision (CLSI guideline EP5-A2) is 3.92 % for 1.05 mg/L cystatin C and 1.88 % for 2.67 mg/L respectively. Detection limit is 0.02 mg/L. Linearity is given up to 8 mg/L cystatin C. No interference is observed at bilirubin concentrations up to 60 mg/L, triglycerides up to 1000 mg/dL, rheumatic factor up to 600 IU/L and hemoglobin up to 1000 mg/dL. No prozone effect was observed up to 30 mg/L. Our test shows a good correlation for sera and plasma in comparison studies against a commercial nephelometric assay (slope m=0.972, intercept b=0.049, correlation coefficient r=0.999, n=100). Calibration stability is given for 6 weeks and onboard stability for 12 weeks.
In summary the newly developed cystatin C assay demonstrated good reagent performance and can be easily adapted to most clinical chemistry analyzers on the market.

## B-160

**Serological markers of gastric mucosa**

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**Purpose**. The aim of this study was to evaluate results of serological tests for Pepsinogen I [PGI], Pepsinogen II [PGII], Gastrin 17 [G17] and anti Helicobacter pylori IgG antibodies [HP-IgG] (Gastropanel), to assess which of these markers provide more useful information on the status of the gastric mucosa.
**Materials and methods**: We analyzed test results performed by ELISA techniques (Biohit, Helsinki, Finland and Euroclone Milan, Italy) from March 2009 to February 2010 (Cut-offs: PGI: 40-100 µg/L; PGII: 2.5-10; ratio PI/PII >3; G17: 2.0-7.0; HP-IgG:<32 IU). Data were retrieved for 522 gastropanels performed on 199 men (mean age ± SD: 50.5±16.6) and 323 women (age: 49.7±16.3).
**Results**: The population was clustered into three classes. Class 1 included 331 patients with *normal gastric mucosa* (age: 48.1±15.8; females/males:199/132; PGI [median, 5-95° percentiles]: 70.7, 45.6-146 µg/L; PGII: 5.8, 2.9-9.5 µg/L; G17: 1.3, 0.2-24.2 pmol/L; HP-IgG: 2.4, 0-86 U/L) including 188 patients with probable gastroesophageal reflux (age: 47.6±16; females/males: 107/81; PGI: 66.8 µg/L, 45.3-105 µg/L; PGII: 5.2, 2.7-9.1 µg/L, G17: 0.4, 0-1.3 pmol/L; HP-IgG: 1.7, 0-55 U/L). Class 2 included 141 patients with *non-atrophic gastritis* (PGII>10µg/L), 66 of which were HP positive (females/males: 45/21; PGI: 124, 71.4-286.2 µg/L; PGII: 16.5, 10.9-33 µg/L, G17: 6.8, 1.2-58.4 pmol/L; HP-IgG: 81.6, 34-337 U/L) and 75 HP negative (females/males: 41/34; PGI: 159, 47.6-298 µg/L, PGII: 13.6, 10.2-51.4 µg/L, G17: 5.5, 0.2-40 pmol/L, HP-IgG: 3.6, 0-24 U/L). Class 3 included 50 patients with possible *atrophic gastritis* (PGI <40µg/L), including 35 with *probable widespread atrophic gastritis* (females/males: 28/7; PGI: 33.2, 3.9-39.3 µg/L, PGII: 2.8, 1.4-8.0 µg/L, G17: 0.9, 0-5.0 pmol/L; HP-IgG: 2.7; 0.1-84 U/L) and 15 with *probable atrophic gastritis of the gastric body* (females/males: 10/5; PGI: 18.2, 5.4-38.3 µg/L, PGII: 5.9, 1.7-14.6 µg/L; G17: 36.6, 8.7-104.4 pmol/L, HP-IgG: 13.7, 2.3-94 U/L).
**Conclusions**: We observed a substantial prevalence of women in the number of tests requested, as well as of patients with signs of gastroesophageal reflux. The frequent occurrence of PGI levels <40 µg/L lead us to suggest that further investigations (i.e., gastroscopy) might be advisable for detecting possible atrophic changes of gastric mucosa, which are often linked to pre-existing or chronic HP infection or autoimmune polyendocrinopathies. Although esophagogastroduodenoscopy with multiple biopsies remains the gold standard for diagnosing gastric disorders, its clinical usefulness might be improved by additional information from results of Gastropanel.

## B-161

### Evaluation of the Performance Characteristics of a new Immunoturbidimetric Assay for HbA1c on the Beckman Coulter AU® Clinical Chemistry Systems.*

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**Objective:** Hemoglobin A1c (HbA1c) is an important tool in the management of diabetes. Formed from the non-enzymatic covalent glycation of free amino groups at the N-terminus of the β-chain of hemoglobin $A_0$, HbA1c provides an indication of the mean daily blood glucose concentration over the preceding three months. The objective of this study was to evaluate the performance of a new immunoturbidimetric HbA1c assay on the Beckman Coulter AU Clinical Chemistry systems. .

**Methods:** The new HbA1c assay for use on AU Clinical Chemistry Systems is an immunoturbidimetric inhibition assay, suitable for use with human whole blood. Samples are pre-treated off-line with hemolyzing reagent prior to analysis. HbA1c is measured in an immunoinhibition assay whereby HbA1c in the hemolyzed sample reacts with anti-HbA1c antibody in the R1 reagent to form soluble antigen-antibody complexes. Polyhaptens in the R2 reagent react with excess antibodies to form an insoluble complex which is measured immunoturbidimetrically. Total hemoglobin in the hemolyzed sample is measured in a separate reaction.

**Results:** The new Beckman Coulter HbA1c assay was evaluated on a series of AU Clinical Chemistry Systems and demonstrated acceptable within-run imprecision. Observed imprecision on the AU680 analyzer was less than 1.5 %CV. Method comparison versus the current assay for AU Clinical Chemistry Systems shows substantial correlation with the existing method; r = 0.992, slope = 1.030 and intercept of -0.2065 %HbA1c, when tested with a panel of National Glycohemoglobin Standardization Program (NGSP) samples (n=40; Range 5.1 - 11.3 %HbA1c).

The assay showed no significant interference from bilirubin, Intralipid** (Kabivitrum Inc.), and ascorbate up to concentrations of 30 mg/dL, 500 mg/dL, and 50 mg/dL respectively. The reagent had a calibration stability of 14 days and on-board reagent stability of greater than 14 days. A study of 40 NGSP samples showed that the assay was capable of fulfilling the new criteria for NGSP certification (95% of the differences between the test and SRL method to be ±0.75%HbA1c), and all samples recovered within ±6% of NGSP target.

**Conclusion:** The new HbA1c immunoturbidimetric inhibition assay provides a rapid, accurate and convenient means of measuring HbA1c in human whole blood on the Beckman Coulter AU Clinical Chemistry Systems.

* Assay currently under development and not available for clinical use.

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## B-162

### IFCC Traceability of MULTIGENT HbA1c Assay on Abbott ARCHITECT and AEROSET Clinical Chemistry Systems

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***Objective*:** Establish IFCC Traceability of MULTIGENT HbA1c assay on Abbott ARCHITECT and AEROSET Clinical Chemistry Systems.

***Methodology*:** Abbott MULTIGENT HbA1c measures percent HbA1c in human whole blood and is traceable to National Glycohemoglobin Standardization Program (NGSP). Traceability was extended to the International Federation of Clinical Chemistry (IFCC) Hb A1c reference system. IFCC value assignment was performed at an IFCC Hb A1c reference system Network Lab in Zwolle, The Netherlands, through certified value assignment using TinaQuant Reagent on Roche Integra.

***Results*:** Assay performance (Sensitivity/LoD, Linearity, Method Comparison and Interference) with IFCC traceable calibrators was corroborated using CLSI-derived protocols. Limit of detection was demonstrated to meet or exceed values pre-determined for the ARCHITECT *c*Systems. The analytical range represents the observed linear low and high levels verifying the Linearity claim. Method Comparison was evaluated by assaying serum patient samples spanning the range of the assay, comparing ARCHITECT *c*System to TinaQuant assay on Integra. Interference data (% Hb A1c) generated with the NGSP certified MULTIGENT HbA1c assay was converted to mmol/mol using the Master Equation: **NGSP = [0.09148 * IFCC] + 2.152.** The data met the IFCC manufacturer's accuracy specification of ± 6.9 mmol/mol.

| Study | HbA1c Assay Results | |
|---|---|---|
| | **NGSP Traceable HbA1c Calibrators** | **IFCC Traceable HbA1c Calibrators** |
| Limit of Detection of HbA1c | 0.1 g/dL | 0.062 mmol/L |
| Analytical Range<br>(a) THb Linearity<br>(b) HbA1c Linearity | 7 to 23 g/dL<br>0.400 to highest calibrator g/dL | 4.34 to 14.27 mmol/L<br>0.248 to highest calibrator mmol/L |
| Method Comparison vs. Comparative Method | Slope: 0.998<br>Intercept: 1.41<br>R: 0.994 | Slope: 0.991<br>Intercept: -1.708<br>R: 0.992 |
| Interference<br>RF<br>Bilirubin<br>Sodium Cyanate<br>Gamma Globulin<br>Ascorbic Acid<br>Acetylsalicylic Acid<br>Lipemia (Triglyceride) | Up to<br>3100 IU/mL<br>50 mg/dL<br>50 mg/dL<br>5 g/dL<br>50 mg/dL<br>50.8 mg/dL<br>1000 mg/dL<br>All recoveries were within ± 10% of target<br>All biases within ± 0.75 % NGSP HbA1c and within ± 6.9 mmol/mol IFCC HbA1c | |

***Conclusion*:** The MULTIGENT HbA1c assay on the Abbott ARCHITECT *c*Systems demonstrated acceptable sensitivity, linearity, interference and method correlation using IFCC traceable calibrators. The assay is now traceable to both the NGSP and IFCC reference systems and results may be reported both as % Hb A1c and mmol/mol. The MULTIGENT HbA1c assay is supported across Abbott's Clinical Chemistry family of instruments - Abbott ARCHITECT *c*4000, c8000, *c*16000 systems and Abbott AEROSET System.

## B-163

### Evaluation of the Hitachi 911/Roche total alpha amylase assay for the measurement of human salivary amylase activity

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The objective of this study was to determine if α-amylase activity could be reliably measured in human saliva (sAA) with the Hitachi 911/Roche total α-amylase reagent system. The measurement of sAA is rapidly gaining popularity in clinical research as a non-invasive marker of the sympathetic nervous system's response to stress. At our institution, sAA has been successfully measured in microtiter plates with a reagent kit available from Salimetrics, LLC. This kit method is widely used in stress research; however, it requires two manual dilutions of each saliva specimen, and manual pipetting of diluted saliva and substrate reagent into the wells of the microtiter plate. Preliminary experiments in our lab revealed that sAA can be measured in a single 1/100 dilution of saliva, with the Hitachi 911/Roche total α-amylase reagent system. The Roche reagent system is FDA approved for serum and urine, but has not been evaluated for use with saliva.

We assessed the within-run and total imprecision (CLSI EP 5-A2), analytical measurement range (CLSI EP 6), and functional sensitivity of the Hitachi 911/Roche and the Salimetrics methods, using pools of human saliva either neat or adjusted with physiological saline to achieve low levels, or adjusted with purified human salivary α-amylase (Lee Biosolutions) to achieve high levels. We assessed recovery (CLSI EP 9-A2) and agreement between methods (CLSI EP 15-A2) with individual human saliva specimens. We collected saliva from apparently healthy volunteers using Salivette® collection devices. Saliva recovered from the cotton roll by centrifugation was frozen, thawed, vortexed, and recentrifuged to obtain clear saliva for pooling or analysis. Pooled saliva was adjusted if necessary, aliquoted, and frozen at -70°C. Individual saliva specimens were also stored at -70°C. Specimens were thawed and analyzed by both methods on the same day.

The analytical measurement range for the Hitachi 911/Roche method was verified between 4 and 846 U/mL, with functional sensitivity to 0.4 U/mL. The analytical measurement range for the Salimetrics method was verified between 3 and 595 U/mL, with functional sensitivity to 1.5 U/mL. Within-run C.V.s ranged from 0.4-1.8% for Hitachi 911/Roche, and from 2.2-7.3% for Salimetrics. Total C.V.s were 2.6% (mean = 15.0 U/mL) and 5.4% (mean = 277 U/mL) for Hitachi 911/Roche. Total C.V.s were 5.5% (mean = 9.5 U/mL) and 8.9% (mean = 169 U/mL) for Salimetrics. Recoveries ranged from 83-119% for Hitachi 911/Roche and from 78-121% for Salimetrics. Excellent correlation between the two methods was observed for 39 individual saliva specimens, ranging from 1.3-150 U/mL by the Salimetrics method ( r = 0.99). However, a significant proportional bias was observed with Deming regression: (Roche) = 1.79(Salimetrics) - 0.82

Salivary α-amylase activity may be reliably measured with the Hitachi 911/Roche method for total α-amylase activity, although numerical results are not interchangeable with those obtained by the Salimetrics assay. The proportional bias between the two methods is likely due to the utilization of different substrates, and different calibration strategies.

## B-164

**European Multicenter Evaluation of the NR2 Peptide and Antibody Biomarkers for Hyper-Acute Stroke within 4 Hours of Onset**

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Introduction: Rapid management of acute ischemic stroke in the emergency setting, especially if the CT scan is normal or MRI is contraindicated or not available, is essential to ensure that patients receive thrombolysis within the therapeutic window. To speed up ruling in or ruling out of ischemic stroke prior to CT imaging using a blood test in the ambulance, for example, would also be extremely useful in this critical diagnostic interval. Abilities of the NR2 peptide to signal acute and NR2 antibodies detect prior and multiple cerebrovascular events have been studied.

Objective: European multicenter trials were conducted to evaluate diagnostic accuracy of NR2 peptide and antibody biomarkers for rapid differentiation of cerebral ischemia *vs* intracerebral hemorrhage.

Methods: Plasma specimens collected at 13 clinical sites (NCT00916864) were tested using an NR2 peptide assay based on a magnetic particle (MP) ELISA and an NR2 antibody ELISA assay (CIS Biotech, Inc., Atlanta, GA). Of 186 patients recruited between June 2009 and January 2010, 94 had acute and 43 had prior and/or multiple ischemic stroke. To define tissue-based evidence of injury, all patients had DWI/MRI. Blood samples were drawn within 4 hours of acute hemispheric stroke onset. Plasma samples were also drawn from patients with intracerebral hemorrhage (n=47).

Results: In adult patients >18 years old NR2 peptide measurements > 0.3 ng/mL indicates acute hemispheric ischemic stroke within 4 h of onset. Detection of NR2 antibody test (cut off of 2 .0 ng/mL) determines prior and/or multiple ischemic events. In this 186-patient cohort, prevalence of intra-cerebral hemorrhage (by MRI) was 25%. For those with intra-cerebral hemorrhage, mean NR2 peptide was 0.21 ± 0.05 ng/mL, with a 95% reference interval of 0.12 to 0.29 ng/mL. Mean NR2 antibodies were 1.19 ± 0.27 ng/mL, with a 95% reference interval of 0.65 to 1.70 ng/mL. In patients with acute ischemic stroke, NR2 peptide level was detected with a 95% reference interval of 0.32 to 10.87 ng/mL and NR2 antibodies were measured with a 95% reference interval of 0.68 to 5.76 ng/mL.

Conclusion: The NR2 peptide and antibody brain biomarkers provide reliable measurements of stroke across a broad, clinically important range and should prove useful in urgent situations. Simultaneous detection of NR2 peptide and antibodies improves diagnostic certainty of cerebral ischemia and helps to rule-out hemorrhagic stroke within 4 hours of onset.

## B-165

**AMPAR Peptide Assay for Assessment of Mild Traumatic Brain Injury (TBI)**

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Introduction: Mild TBI affects electrical and chemical circuits, leading to edema-activating necrosis. AMPA receptors are key components that control these processes. Excessive amounts of glutamate activate AMPA receptors, triggering an excessive influx of calcium, leading to overexpression of glutamate receptors in the extra-synaptic region. Degradation of receptors by thrombin-activated serine proteases results in peptide fragments entering the bloodstream via the compromised blood-brain barrier. AMPAR peptide is tissue-based evidence of neuronal dysfunction due to mTBI.[1] A magnetic particle (MP) ELISA was used to measure AMPAR peptide and assess its concentrations in healthy persons.

Methodology: A sensitive, 1-step bridging-type enzymatic MP ELISA has been developed* that measures AMPAR peptide in 20 µl of EDTA plasma sample against recombinant AMPAR peptide calibrators (0-12 µg/L). The antibody pair used in the assay measures free AMPAR peptide in equimolar concentration and does not cross-react with other neuroreceptors.

Results: The within-run and total precision for AMPAR peptide in quality control material was 6.3% and 9.8% for the low concentration (1.25 µg/L); 5.7% and 7.2% for mid-level (5.0 µg/L); and 5.3% and 5.9% for high concentration (10.0 µg/L). The average analytical sensitivity calculated by the interpolation of the mean plus 2 standard deviations of 20 replicates of the zero calibrator on 2 independent lots is 0.26 µg/L. Functional sensitivity of the assay at 20% CV was 0.29 µg/L. Dilution studies showed an average recovery of 94%. Regression analysis yielded a good assay linearity with R2=0.999. When potential interferents (triglycerides, bicarbonate, creatinine, cholesterol, bilirubin, hemoglobin, and human serum albumin) were added at 2X physiological concentration, AMPAR peptide concentrations were within ±10% of control. Significant interference was detected with hemoglobin. The mean AMPAR peptide value for a healthy male and female (n=30) was 0.337±0.036 µg/L.

Conclusions: A sensitive and reliable MP-ELISA AMPAR peptide assay has been developed to measure low levels of AMPAR peptide in EDTA plasma. Using this assay, approximate median AMPAR peptide levels in a healthy ambulatory population can be measured with <11% CV. These results suggest MP-ELISA AMPAR peptide assay performance is acceptable for further investigation of the AMPAR peptide as a biomarker of mild TBI.

1. Dambinova, SA. (2007). IVD Technology. 3:41-48. *For in vitro diagnostic use in CE-marking countries. For research use only in USA.

## B-166

**Urine neutrophil gelatinase-associated lipocalin (NGAL) predicts acute renal injury in critically ill patients**

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**Background:** Plasma and urine concentrations of neutrophil-gelatinase-associated lipocalin (NGAL) have been proposed as earlier biomarkers of acute kidney injury (AKI). However, most analysis have been performed in patients after cardiac surgery, and data are lacking for patients in the intensive care unit (ICU).

**Aim:** To analyze whether urine NGAL could predict AKI in critically ill patients admitted to a general intensive care unit for different pathologies, particularly severe sepsis and septic shock.

**Methods:** NGAL was measured by a chemiluminiscent microparticle immunoassay (Abbott Diagnostics) in an Architect i 1000SR analyzer in urine samples collected at admission (6h urine collection) and 24 and 48h later in 24h-urine collection. Kidney function was evaluated by the RIFLE score [RIFLE 0=Normal function, R=Injury risk, I=Existing injury, F=Kidney failure (ARF)] at the same times as urine collection. According to the clinical picture, patients were classified as having systemic inflammatory response syndrome (SIRS), severe sepsis or septic shock.

**Results:** The study included 100 consecutively-admitted patients (40 female) with mean age 59.1±17.8 years, and length of ICU stay of 10.3±9.6 days. Seventy-one patients developed SIRS, 9 severe sepsis and 20 septic shock. Mortality was 22%. Eighteen patients developed ARF, 14 at ICU admission, and extracorporeal renal therapies were required in 9 cases. When patients were classified according to RIFLE score at 24h after admission, NGAL values at admission (as median (IQR) in ng/mL, throughout the text) were 26.3 (<10-80.8) in 48 patients with RIFLE 0, 163 (49.1-997) in 5 with RIFLE R, 356 (151-597) in 8 with RIFLE I and 384 (31.2-539) in 10 with RIFLE F ($p<0.0001$ for trend among groups). The area under ROC curve for AKI prediction with NGAL concentration at ICU admission was 0.83 (95% CI: 0.74-0.92, $p<0.0001$); a cutoff value of 114 ng/mL showed 69% sensitivity and 84% specificity. In 54 patients with SIRS, NGAL at admission was 30.1 (<10-132), whereas in 6 patients with severe sepsis and 16 patients with septic shock it was 35.9 (<10-235) and 400 (126-592), respectively ($p=0.001$ for trend). Finally, NGAL at admission differed in accordance with changes in the RIFLE score during the first 48h of ICU stay; median NGAL was 23.2 (<10-67.5) in patients with sustained normal RIFLE score, 119 (31.9-341) in those whose scores improved, 263 (27.3-517) in those whose scores remained abnormal during evolution and 370 (328-611) in those whose scores worsened ($p<0.0001$).

**Conclusions:** Urine NGAL concentrations at admission appeared to be predictive of AKI in ICU patients. Highest NGAL median values were observed in patients with kidney failure or worsening renal function during ICU stay and lowest values in those with normal renal function scores. Interestingly, urine NGAL showed an increasing concentration pattern according to severity of disease from SIRS to severe sepsis and septic shock.

## B-167

**Evaluation of a fully automated NGAL particle-enhanced turbidimetric assay**

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**Background** NGAL (neutrophil gelatinase-associated lipocalin) is a new, promising marker of acute kidney injury (AKI) in both urine and plasma. In AKI, NGAL levels in urine may increase from normal levels of up to about 20 ng/mL to levels above 25,000 ng/mL, while the range of expected levels in EDTA plasma is from about 50 to 5000 ng/mL. A fully automated, ready-to-use assay applicable to general clinical chemistry analyzers is needed in order to exploit NGAL's diagnostic potential fully. The purpose of this study was to evaluate an automated particle-enhanced turbidimetric assay for NGAL in urine and plasma samples.

**Methods** The NGAL assay uses polystyrene particles coated with mouse monoclonal antibodies. The calibrator material is full-length recombinant human NGAL delivered in

ready-to-use solutions at concentrations of 0, 150, 750, 1500, 3000 and 5000 ng/mL. The assay was run on a Hitachi 917 clinical chemical analyzer utilizing 3 µL of sample, 150 µL of buffer and 50 µL of antibody-coated particle suspension, with a total assay time of 10 min. The within-run imprecision was estimated from 20 daily measurements of calibrator material at two concentrations. The linearity of the assay and the upper limit of the security range were determined by measurement of serial dilutions of calibrator material in steps of 10 ng/mL from 0 to 100, steps of 500 ng/mL from 0 to 5000 and steps of 8000 ng/mL up to 40,000 ng/mL. The measuring range was defined as the concentration range over which sample recovery deviated <15% from the expected value. Interference of hemoglobin (500 g/L), bilirubin (30 g/L) and emulsified lipid (5.0%) on the measurement of control material was determined. NGAL was measured with the turbidimetric assay and a commercially available ELISA kit (BioPorto) in urine samples from 130 ICU patients and EDTA plasma samples from 40 ICU patients.

**Results** The measuring range of the turbidimetric assay was specified to be 25 to 5000 ng/mL. Within-run imprecision was 2.0% and 1.2% at 200 and 500 ng/mL, respectively. The assay was linear in range from 20 to 5000 ng/mL with deviations below 5% of expected values. No effect of antigen excess was seen up to a 40,000 ng/mL. Potentially interfering substances had only marginal effects (<4%) on measurements. There was excellent agreement between the ELISA and turbidimetric measurements of both urine and EDTA plasma samples. The Deming regression fits (95% confidence intervals in brackets) were: urine, slope 0.97 (0.85 to 1.09), x-axis intercept -57 (-187 to 74), plasma, slope 1.00 (0.87 to 1.13), x-axis intercept -55(-124 to 15). These data were not significantly different from identity between the results of the two assays.

**Conclusions** The NGAL turbidimetric assay is a fast, precise and convenient test that works well with both urine and EDTA plasma samples over the clinically relevant concentration range. With this assay the promise of NGAL measurement for the diagnosis of AKI can be translated into widespread clinical use.

## B-168

### Serum and Urine Trypsinogen Activation Peptide in Assessing Post-Endoscopic Retrograde Cholangiopancreatography Pancreatitis

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**Background**. Trypsinogen activation peptide (TAP) reflects the amount of activation trypsinogen, not taking into account how much trypsin is active or linked to specific inhibitors. TAP is released into the peritoneal cavity and circulates after which the peptide, thanks to its small size, is rapidly metabolized in the kidney and excreted in the urine. It seems logical that the greater the quantity of trypsinogen activated, the more the pancreas is damaged. The aim of the study was to evaluate the serum TAP concentration elevation after ERCP and to establish its role in the early diagnosis of post-procedural acute pancreatitis. The second aim was to explore whether the administration of gabexate mesylate could prevent the activation of tripsinogen by blocking trypsin activation.

**Methods**. Sixty-five patients were enrolled in the study. Patients who were under 18 years of age, patients with a recent onset of acute pancreatitis, those who were pregnant, and patients with a known allergy to gabexate mesylate were excluded. In all patients, a 5 mL blood sample was taken immediately before the endoscopic examination and 1, 2, 3, 4 and 6 hours post-ERCP. A two mL sample of urine was also collected before ERCP and 2, 4 and 6 hours after the completion of the ERCP. Serum and urine TAPs were assayed using a technique previously described [Pancreas 2004;29:298-305]. The detection limit was 1.0 ng/mL and healthy subjects had no detectable values of TAP in serum and in urine. Serum trypsinogen concentrations were determined using commercially available RIA-kits (Sorin Biomedica). The detection limit was 2.5 ng/mL and the reference values of our laboratory were 10-57 ng/mL. The use of gabexate mesylate was decided by the endoscopist. Post-ERCP acute pancreatitis was defined as the appearance of typical abdominal pain associated with an increase in serum amylase activity greater than 3 times the upper reference limit. The severity of the pancreatitis was assessed by clinically-based Atlanta criteria.

**Results**. In the 65 patients who completed the study, 2-hour post-ERCP serum TAP concentrations were elevated (P=0.034 vs. pre-ERCP) whereas these concentrations significantly declined at 4 hours (P=0.006). Urine TAP showed a similar behavior. Mean serum trypsinogen concentrations were slightly below the upper reference limit before ERCP and then significantly increased thereafter. Serum and urine TAP levels, as well as serum trypsinogen concentration, showed no significant differences between patients who developed acute pancreatitis and those who did not. Within the group of the patients who received gabexate mesylate, serum TAP concentrations were significantly lower at 1 and 2 hours after ERCP in the patients who developed acute pancreatitis (P=0.033 and P=0.041, respectively).

**Conclusions**. Serum and urine concentrations of TAP are detectable very early in patients who undergo ERCP, i.e. within the first 6 hours. As for the other pancreatic enzymes, serum and urine TAP determination is of limited value in diagnosing post-ERCP acute pancreatic damage if used alone. Finally, more studies are necessary to precisely establish the role of TAP determination in patients treated prophylactically with drugs capable of blocking the activation of trypsin.

## B-169

### Comparison of the analytical performance of four automated assays for measurement of cystatin C

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*Background:* The analytical performance of four cystatin C assays (Siemens N Latex on BNII, Roche Gentian on Cobas c501, Genzyme on Cobas c501 and Tosoh ST AIA-PACK on Tosoh AIA-600II) were evaluated according to guidelines published by the Clinical and Laboratory Standards Institute (CLSI).

*Methods:* Total precision, limit of detection, and limit of quantitation for each assay was evaluated using patient serum pools with varying cystatin C concentrations. Conformance to peer groups was evaluated using CAP proficiency specimens. Linearity/recovery was evaluated using serial 10% dilutions of a high patient serum pool (cystatin C free serum was used as diluent). Patient comparison (n=102) was performed using the Siemens assay as a comparison method.

*Results:* All assays demonstrated sufficient limits of detection and quantitation with respect to values observed in clinical specimens. Compared with the manufacturer's specifications, the total CVs we observed were generally higher for all assays; the largest discrepancy was observed with the Genzyme assay (8.9% versus 2.3% on the respective low cystatin C pools, 3.7% versus 1.3 % on the respective medium cystatin C pools, 9% versus 2.4% on the respective high cystatin C pools). All assays demonstrated acceptable performance based on CAP criteria (SDI <3), but linearity/recovery revealed that the Roche assay tended to over recover, particularly at low concentrations of cystatin C (mean recovery 119%; 142% at 0.5869 mg/L). Deming Regression equations from the patient comparison studies were y=1.184x+0.089, r = 0.987 for Genzyme; y=0.937x+0.231, r = 0.995 for Roche; y=1.010x+0.216, r = 0.997 for Tosoh. The Genzyme assay appears to give higher result than the Siemens assay, which is consistent with a higher reference range specified by the manufacturer (0.61-1.17 mg/L for Genzyme, 0.53-0.95 mg/L for Siemens).

*Conclusions:* Although all assays tested are acceptable for clinical use according to commonly accepted criteria, their diagnostic performances are not optimal. Concerns for different assays include imprecision observed on the Genzyme assay, over recovery of the Roche assay on low cystatin C samples, as well as assay specific bias in the patient comparison studies. This means e.g., that assay-specific cystatin C-based GFR-prediction equations are required. This problem might be solved if the assays are further optimized using an international cystatin C reference standard now being developed.

## B-170

### Turbidimetric immunoassays for IgAκ and IgAλ quantification for the assessment of patients with multiple myeloma

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Detection and quantification of IgA monoclonal proteins by serum protein electrophoresis (SPE) can be difficult in patients with low levels of protein or where the band is obscured. Immunofixation electrophoresis (IFE) improves sensitivity of detection but is non-quantitative. Specific polyclonal antibodies have been produced which recognise conformational epitopes spanning the junction of the heavy and light chains of IgA. Here we describe automated, turbidimetric immunoassays for the quantification of IgAκ and IgAλ in serum on the SPA PLUS™, a small, bench-top turbidimeter available from The Binding Site. Determination of the IgAκ/IgAλ ratios can be used as an aid in the diagnosis of multiple myeloma (MM). The main assay characteristics are summarised in the table below:

| Assay | IgAκ | IgAλ |
|---|---|---|
| Measuring range (g/L) | 0.18-11.2 | 0.16-10.4 |
| Sample dilution | 1/10 | 1/10 |
| Min sample dilution | 1/1 | 1/1 |
| Sensitivity (g/L) | 0.018 | 0.016 |
| Assay time (mins) | 10 | 10 |
| Linearity | y = 1.015x -0.0187 $R^2$ = 0.996 | y = 0.993x + 0.0707 $R^2$ = 0.998 |
| Intra-assay precision %CV<br>(mean)<br>(n=10) | 1.2% (8.68g/L)<br>2.1% (2.21g/L)<br>2.3% (0.32g/L) | 1.3% (7.76g/L)<br>3.0% (2.07g/L)<br>3.9% (0.25g/L) |
| Inter-assay precision %CV<br>(mean)<br>(n=10) | 7.9% (8.92g/L)<br>5.1% (2.14g/L)<br>5.9% (0.32g/L) | 8.7% (8.48g/L)<br>6.1% (2.00g/L)<br>5.9% (0.27g/L) |

Interference was within ±2.5% when either bilirubin (20mg/dL), hemoglobin (500mg/dL) or Chyle (1530 formazine turbidity units) were added to serum samples with known IgAκ and IgAλ concentrations. IgAκ and IgAλ concentrations were measured in 120 normal (blood donor) sera; median IgAκ 1.37gL (range 0.57 - 2.08g/L), median IgAλ 1.25g/L (range 0.44 - 2.04g/L), median IgAκ /IgAλ ratio of 1.18 (range 0.78 - 1.94). IgAκ + IgAλ summation correlated well with total IgA (Binding Site SPA PLUS):- y=1.06x + 0.03 (Passing and Bablok). IgAκ and IgAλ concentrations were measured in 55 IgA (29 IgAκ /26 IgAλ) archived MM patient sera. The results correlated well with total IgA:- y=1.06x + 0.16 (Passing and Bablok). In all cases the IgAκ /IgAλ ratio correctly identified the monoclonal IgA type. We conclude that serum IgAκ /IgAλ assays provide a rapid, precise method for quantifying IgAκ and IgAλ in serum, and the presence of an abnormal ratio may be useful in identifying patients with IgA myeloma.

## B-171

### Evaluation Of Sentinel Cholinesterase Assay On Synchron®Lx20 And Unicel®Dxc Clinical Chemistry Systems

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**OBJECTIVE:** analytical performance of Sentinel Cholinesterase Liquid assay was evaluated on Beckman Coulter Synchron®LX20 and UniCel®DxC Clinical Chemistry Systems. Goals of the study were to verify the analytical performance and establish this assay's agreement with the desirable analytical specification based on biological variability as given by Ricos.

**MATERIALS/INSTRUMENTS:** Cholinesterase Liquid reagent, manufactured by SENTINEL CH., is based on the catalytic reaction of endogenous seric Cholinesterase (CHE) with butyrylthiocholine substrate, which forms thiocholine and reduces hexacyanoferrate (III) to hexacyanoferrate (II). The decrease in absorbance is directly proportional to CHE activity in the sample. The assay is optimized in according to the DGKC recommendations.

The SynchronLX20 is a fully automated, high-throughput clinical chemistry system, able to run colorimetric, enzymatic, as well as turbidimetric assays, which allows for consolidation of Lab workflow on a unique platform.

**STUDY DESIGN:** Study protocols were based on CLSI guidelines. Acceptance criteria were defined to meet desirable analytical specifications as per biological variation (TE< 7.4%) at clinical decision level (4 kU/L). Total Imprecision (EP05A); acceptable CV% </= 3%. Linearity (EP06A); acceptable % bias +/-5%. Limit of Quantitation (LOQ_EP17) was defined as the analyte concentration at which the Total Error is less than 10%. Method comparison was evaluated by comparing the results of paired patient serum samples on SynchronLX20, on UnicelDxC and on Abbott Aeroset analyzers, according EP09A. Endogenous substances interferences were investigated following EP07A; allowed bias was 0.30 Ku/L. In Use Stability was tested in terms of On Board reagents and calibration stability.

**RESULTS:** Total Imprecision as CV% were on SynchronLX20 2.2% at 5.10 kU/L and on UniCelDxC 2.1% at 5.28 kU/L. Assay was found to meet Linearity specifications from 0.3 up to 22 kU/L. LOQ was 0.30 kU/L. Comparison A) SynchronLX20 vs. Aeroset® analyzer: n 99, slope 0.98, intercept 0.13, r 0.99; B) SynchronLX20 vs UniCelDxC: n 50, slope 1.02, intercept 0.20, r 1.00. Assay was free of interferences up to Hemoglobin (1000 mg/dL), Lipids (1000 mg/dL), conjugated and unconjugated Bilirubin (60 mg/dL), Ascorbate (24 mg/dL). In use stability study proved 28 days Reagent stability and 14 days Calibration On Board stability. Plasma suitability: Li-heparin vs. Serum = n 25, slope 0.98, intercept 0.02, r 0.99; K-EDTA vs. serum = n 25, slope 0.97, intercept 0.08, r 0.99.

**CONCLUSION:** performance of Sentinel CH Cholinesterase Liquid assay on Beckman Coulter SynchronLX20 and UniCelDxC Clinical Chemistry Systems did meet the Acceptance criteria based on Ricos Biological Variation database specification. The study supported the use of the assay on clinical laboratory routine, thus allowing the consolidation of the lab workflow on a unique instrument platform.

## B-172

### The role of Brain-Fatty Acid-Binding Proteins in silent cerebral ischemia monitoring undergoing carotid trhomboendoarterectomy

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**BACKGROUND** Nowadays the true incidence of silent cerebral ischemia undergoing carotid thromboendoarterectomy (CEA) is unknown: against an apparent obscure clinic, it has been pointed out a possible decline of neuropsychological functions. The B-FABPs (Brain-Fatty Acid-Binding Proteins) have shown high sensitivity and specificity for the neurological damage in stroke patients (pz), while H-FABPs (Heart -Fatty Acid-Binding Proteins) are issued by myocardium in the course of ischemic damage. We have considered the possible role of B-FABPs and H-FABPs as potential indicators of silent cerebral ischemia during CEA. Availability of an early marker of damage may offer a timely neuroprotective rescue especially in the period between the neuronal injury and the cells death.

**MATERIALS AND METHODS** This observational study was approved by Ethics Committee of IRCCS Humanitas Hospital (Rozzano, Italy). We enrolled: 71 patients (age 72.8 ± 7.8; 51M and 20 F) showed carotid stenosis undergoing CEA; 5 healthy academic volunteers such as negative controls; 4 patients with sequelae of neurosurgical approach for brain hemorrhage as positive controls. Timed blood samples were collected for 71 patients: one preoperative (pre time) and two postoperative (post time) respectively after 24 hours and 48 hours. On serum samples were determined two proteins: H-FABPs using a commercially available ELISA kit (CTHK402,2*96; HyCult Biotechnology, Uden, NL) and B-FABPs using a Home Made kit. It was developed by polyclonal antibodies against the Human B-FABPs (HP9029, HyCult Biotechnology, Uden, NL).

**RESULTS** The analysis of protein H showed at pre time the value 221 pg/mL ±167.7; at post time at 24 hours the value 497 pg/mL ±138.4 and at 48 hours the value 375 pg/mL ±487.7. The values of pre time are significantly less than of post time (Wilcoxon test $p<0.001$). The analysis of protein B, obtained without an internal standard, is performed on Arbitrary Units (UA/mL) on the basis of binding between antigen and antibodies. The healthy volunteers showed an average value equal to 0.04 UA/mL; the positive controls gave an average value equal to 2.1 UA/mL (Wilcoxon test $p<0.001$). At pre time the group of 71 patients showed an average value to 1.71 ± 0.25 UA/mL, at post time an average value equal to 1.75 ± 0.25 UA/mL at 24 hours and equal to 1.78 ± 0.28 UA/mL at 48 hours. In patients that showed cerebrovascular lesions confirmed by imaging magnetic resonance (MRI) we recorded significant different values in post time, values equal to positive controls.

**CONCLUSIONS** In case of ischemic cardiomiopaty, patients showed an increase of protein H values and this evidence overlapped with the literature datas. The light of results obtained we can say that the preoperative samples presented positive values of B-FABPs and they showed invariant marker's values if the surgery was without complications. The protein B could be a clinical marker of ischemic damage of central nervous system and its value seemed to correlate with the dimension of damage. A good diagnostic sensitivity was observed in one case of asymptomatic silent ischemia documented by MRI.

## B-173

### Quantitative Analysis of Intact IGF-1 in Human Serum Using High Resolution Mass Spectrometry

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**Introduction:** Measurement of IGF-1 levels is useful in the diagnosis and treatment of growth hormone deficiency. Traditionally, this analysis has been achieved by immunoassay with RIA or sandwich approaches. Methodologies suitable for the clinical lab have been extensively developed over the last 30 years but criticisms remain regarding precision, accuracy, and inter-platform variability. We have developed an LC-MS method for the quantitative analysis of IGF-1 which provides excellent performance, excellent agreement to RIA, and freedom from interferences by IGF binding proteins without the use of a blocking step. The methodology takes advantage of high resolution, high mass accuracy MS to measure intact IGF-1. In contrast to bottom-up or signature peptide-based approaches, our method is technically simple and suitable for use in a high-throughput clinical lab.

**Method:** Intact IGF-1 was quantified by high resolution SIM using an Agilent 6530 time-of-flight MS interfaced with an Aria TX-4 operating in focus mode. Patient samples were extracted with acid ethanol prior to online solid phase extraction followed by analytical chromatography. All data were collected at a resolution of >23,000 FWHM with an observed accuracy of <5 ppm using an internal reference mass. Oxidized rat IGF-1 was used as an internal standard. Extracted ion chromatograms were generated using a 10 ppm

symmetric window and integrated peak areas were used for all quantitative calculations. Isotope pattern matching was used to insure the identity and integrity of all quantitative measurements.

**Results:** An assay suitable for use in the clinical lab has been validated with the following characteristics: LOD 4 ng/mL, LOQ 15 ng/mL, ULOQ 2000 ng/mL, intra-assay precision <3% CV, inter-assay precision <6%. Accuracy was determined by spike recovery (average recovery: 104% at 100ng/mL, 103% at 400ng/mL , 102% at 700ng/mL). Method comparison analyzed using Deming regression between the LC/MS approach and 3 immunoassays revealed good agreement: RIA vs LC/MS, n=100, m= 0.9625 +/- 0.0145, b= 11.12 +/- 5.330, Sy.x=34.7; Siemens vs LC/MS, n=63, m= 1.202 +/- 0.0341, b= -52.62 +/- 14.95, Sy.x=75.8; Meso Scale Discovery vs LC/MS, n=100, m= 1.203 +/- 0.04596, -55.50 +/- 16.82, Sy.x=104.2 . Bland-Altman analysis revealed that the LC-MS method has negligible bias when compared to the RIA. No interferences were observed.

**Conclusion:** A LC/MS method for IGF-I was successfully validated and is suitable for use in a high-throughput laboratory.

## B-174

### Relative utility of urinary neutrophil gelatinase-associated lipocalin (NGAL) and urinalysis for detecting acute kidney injury

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Background: There is great interest in developing sensitive and specific markers of acute kidney injury (AKI) in order to facilitate rapid diagnosis and initiation of therapy. Therefore, we analytically and clinically validated urinary neutrophil gelatinase-associated lipocalin (NGAL) and compared performance to the traditional urinalysis.

Methods: Trained technicians in the Mayo Clinic Renal Laboratory quantitated NGAL by ELISA (BioPorto Diagnostics) and performed microscopic urinalysis blinded to the study. Twenty four hour urine samples were prospectively obtained from 115 normal volunteers (mean age 45.7; range 22-77 yrs), and waste urine samples obtained from 363 consecutive patients admitted from the St. Marys Hospital Emergency Department. Medical records were abstracted to stage patients for AKI occurrence over the next 2 days after admission (AKIN criteria stages 0-3).

Results: NGAL was stable in urine up to 7 days when ambient, 4ºC, or frozen (-20 or -70ºC). The assay was linear between 0.24 -10,000 ng/ml with a lower limit of quantification of 0.24 ng/ml. Intra- and inter-assay precision were excellent (CV<5%). High levels of hemoglobin (>4g/L) and albumin (>4g/L) interfered with NGAL measurement in neat urine samples, but not when diluted >1:40 as per the ELISA protocol. WBCs (>3 per HPF) increased NGAL measurement in samples that were frozen without prior centrifugation; however WBCs did not influence urinary NGAL levels in fresh never-frozen urine specimens. In the reference value study mean urinary NGAL excretion was greater in females (n=67) than males (n=58) whether normalized for creatinine (F: 41.5 ng/g (31.7, 51.4); M: 12.8 ng/g (10.2, 15.3)) or not (F: 33.4 ng/ml (26.0, 40.8); M: 14.0 ng/ml (11.6, 16.5); P<0.001 for each.

In the clinical validation study urinary NGAL levels (ng/ml) increased significantly with AKIN stage from a median of 23.0 ng/ml in patients without AKI to 153.2 ng/ml in those with AKIN stage 3. This trend was also evident for NGAL:creatinine ratios, however unlike NGAL concentration the ratio decreased between AKIN stages 2-3. By microscopy the presence of at least one of renal epithelial cells (REC), epithelial cell casts (ECC), and granular casts (GC) had a 22.4% sensitivity and 91% specificity for AKI (PPV=39.5% and NPV 91.7%). Using 42.71 ng/ml as a cutoff, urinary NGAL had only fair sensitivity (65.5 %) and specificity (64.7 %) to differentiate AKIN stage 0 versus stages 1, 2, or 3, (AUC 0.70). By comparison, in the ROC curve analysis for the urinary sediment the AUC was 0.57.

Conclusions: NGAL is stable and can be reliably measured in clinical urine samples. However, careful attention must be paid to sample handling prior to long term storage to avoid WBC lysis. Baseline urinary NGAL levels appear to be higher in women than men and must be accounted for in studies where a subtle increase in NGAL must be detected. In our cohort of emergency department patients an increasing level of urinary NGAL was associated with degree of AKI, however sensitivity and specificity were only fair. Conversely, the presence of RECs, ECCs, and GCs by traditional urinalysis is very specific, but lacks optimal sensitivity.

## B-175

### New Multi-Analyte Human-Based Calibration Verification / Linearity Test Kit for Enzymes on the Ortho Vitros System.

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Objective: To produce a liquid, multi-analyte calibration verification/linearity test kit in a human serum albumin matrix optimized for the Vitros Fusion 5.1 analyzer (VALIDATE® GC3vt). Relevance: Many commercial calibration verification materials are manufactured in animal protein matrices and give rise to response deviations in methods optimized for analysis in human serum. The new material is built in a human serum albumin matrix and should better reflect performance of native samples. This product also has better coverage of the claimed range for many analytes. Methodology: VALIDATE® GC3vt linearity set was run and compared with the bovine serum albumin-based VALIDATE® Chem 10 (Maine Standards Company). Samples were analyzed in triplicate on a Vitros Fusion 5.1 using Ortho-Clinical reagents. Results were processed using MSDRx® Calibration Verification software (Maine Standards Company). Validation: All products were manufactured in a manner to produce an equal distance between consecutive levels. Data regression statistics, shown in Table 1, represent observed values regressed against software-calculated target values. Conclusions:

Regression parameters for the new human-based product show excellent agreement with the original product and demonstrate improved linearity performance for the majority of analytes compared to that of the bovine-based product. Additionally, the human material covers a greater percentage of the linear range for most of the enzymes.

| Table 1 | GC3vt | | | Chem 10 | | |
|---|---|---|---|---|---|---|
| **Analyte** | **slope** | **int** | **% of High** | **slope** | **int** | **% of High** |
| ALP | 0.95 | 21.92 | 90.8 | 0.86 | 40.82 | 72.7 |
| ALT | 1.05 | -9.33 | 86.7 | 1.04 | -7.08 | 85.8 |
| AMY | 0.99 | 2.74 | 94.3 | 0.94 | 13.61 | 82.1 |
| AST | 1.04 | -3.59 | 94.2 | 1.07 | -6.66 | 94.2 |
| CK | 0.90 | 43.38 | 96.4 | 1.00 | 0.21 | 94.7 |
| GGT | 0.94 | 19.03 | 83.3 | 1.01 | 0.92 | 86.6 |
| LD | 1.07 | -33.62 | 92.8 | 1.13 | -53.88 | 84.9 |
| LIP | 0.98 | 8.52 | 92.2 | 1.07 | -30.01 | 100.3 |

## B-176

### Variations in Alanine Aminotransferase Levels Within the Normal Range Predict Metabolic and Androgenic Phenotypes in Women of Reproductive Age.

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Obesity plays pathogenetic roles in nonalcoholic fatty liver disease (NAFLD) and hyperandrogenic states like polycystic ovary syndrome (PCOS). As PCOS and NAFLD have insidious onset and share common pathophysiology, we hypothesise that alanine aminotransferase (ALT), a marker of NAFLD and liver dysfunction, will show significant associations with spectrum of endocrine and metabolic abnormalities in women with normal ALT. Fasting glucose, insulin, total testosterone, DHEAS, 17-hydroxyprogesterone, prolactin, leptin, soluble leptin receptor (sOb-R), free leptin index (FLI), lipid profile, ALT, gonadotropins, and sex hormone binding globulin (SHBG) were measured in the early follicular phase in 200 women aged 18 - 48 years. Beta cell function (%B), insulin sensitivity (%S) and insulin resistance (IR) were calculated using the homeostasis model assessment (HOMA). Subjects were classified a-posteriori, after full clinical and laboratory evaluation. The upper 95th percentile for ALT (ALT = 18 IU/l) in metabolic syndrome negative apparently healthy female subjects was used to categorise the subjects into subgroups (ALT < 18 IU/l or ALT ≥18 IU/l). Results showed that ninety-two women had PCOS (Rotterdam criteria); 64 had idiopathic hyperandrogenism; 44 were normal controls. ALT showed significant positive correlations with waist circumference (WC), systolic blood pressure, glucose, leptin, FLI, triglycerides, HOMA-IR and androgens and significant inverse correlations with sOb-R, HDL-cholesterol, %S and SHBG. Partial correlation, correcting for WC showed that the associations between ALT and glucose, HOMA-IR and androgens are independent of obesity. Binary logistic regression analysis showed significant association of ALT with PCOS and hyperandrogenemia. ALT ≥18 IU/l also showed significant association with PCOS with OR = 2.28, p = 0.043. We conclude that in women of reproductive age, ALT is a useful tool for identification of metabolic and androgenic phenotypes. We suggest routine estimation of ALT and extension of its routine use beyond the diagnosis of liver disease. Studies in different populations are necessary to ascertain the significance of routine ALT estimation as an adjunct for the early detection of insulin resistance.