# Wednesday, July 29, 2015

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

Proteins/Enzymes

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

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**Background:**

The lack of appropriately partitioned age- and gender-specific reference intervals can compromise the ability of physicians to correctly interpret laboratory test results. The Canadian Health Measures Survey (CHMS; a program of Statistics Canada) collected comprehensive nationwide health information and biological specimens from the Canadian household population. The Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) has collaborated with Statistics Canada to gain access to the CHMS data and develop a robust national database of reference intervals for 24 routine chemistry biomarkers for pediatric, adult, and geriatric populations.

**Methods:**

Between 2007-2011, the CHMS collected health information, physical measurements, urine, and serum blood samples from approximately 12,000 Canadians aged 3-79 years. The Ortho Vitros 5600 analyzer was used to measure 23 chemistry based analytes and a manual microplate analysis was used for measurement of urine iodine. Exclusion criteria were applied to remove subjects that were pregnant, had serious medical or chronic illness, or that used prescription medication. Reference intervals were calculated using SAS and R software in accordance with CLSI C28-A3 guidelines. Extreme outliers were removed by visual inspection of scatter and distribution plots, and suspected partitions were statistically verified using the Harris and Boyd method. The normality of the data was assessed and data was transformed using the Box-Cox method. The Tukey or adjusted Tukey tests were used to remove outliers from normal or skewed partitions, respectively, Reference intervals were then calculated for partitions with >120 samples using the nonparametric method, and for partitions with >40 but <120 samples using robust methods. Finally, 90% confidence intervals were calculated for the upper and lower limits of each reference interval.

**Results:**

All analytes were grouped into four categories based on the trends in concentration. (1) Sodium, chloride, potassium, bicarbonate, albumin, total calcium, total protein, and total bilirubin remained relatively constant throughout the age range. (2) Phosphate, ALKP, AST, LDH, and urine iodine all had higher concentrations in childhood that declined with age. (3) Creatinine (both serum and urine), total cholesterol, HDL, LDL, triglycerides, uric acid, and urea all had higher concentrations in adulthood compared to childhood. (4) GGT, ALT, and glucose had substantial fluctuations in concentration throughout the age range. Gender partitions were required for most biomarkers, except bicarbonate, total cholesterol, total protein, urine iodine, and potassium.

**Conclusion:**

This is the largest population study to simultaneously determine pediatric, adult and geriatric reference intervals for most commonly used biochemical markers from a large cohort of apparently healthy subjects. The data is particularly useful for clinical laboratories using Ortho chemistry assays, but should be further validated by each laboratory on their analytical platform and local population as recommended by CLSI. Clinical implementation of the robust CHMS database will also significantly improve biochemical test interpretation across pediatric, adult, and geriatric age groups and contribute to better clinical decision making and healthcare delivery.

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

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Background: Hb A1c assays are used to diagnose Type 2 Diabetes and require minimal bias and high precision. Four common commercial Hb A1c assays were evaluated using accuracy based grading and Six Sigma metrics.

Methods: Eight frozen whole blood samples from the European Reference Laboratory (ERL) for Glycohemoglobin were tested. Sample target values were assigned by the IFCC Hb A1c reference method. Assays evaluated: Abbott ARCHITECT Next Generation enzymatic; Roche Tina-quant A1c-2; Tosoh G8 HPLC; and the Bio-Rad Variant II Turbo HPLC. Reference samples were tested in two separate trials, five replicates/trial, n = 10 results per sample per assay. The mean values and % CV were calculated for each sample and assay and Sigma metrics were estimated [Sigma = (TEa − bias)/% CV], bias = absolute difference between the target values and the observed values, TEa (total error allowable) = 6%.

Results: The table lists mean observed bias and precision for all assays. Results for each assay expressed as number of samples with a Sigma value of $\geq 6$ are as follows: Abbott ARCHITECT 6/8 (range of 3.5 - 30 Sigma); Roche Tina-quant 2/8 (range of 0 -7.2 Sigma); Tosoh G8 0/8 (0 – 4.2 Sigma); and Bio-Rad Variant 5/8 (range of 0.4 – 21 Sigma).

| Target Value (% A1c) | Architect Bias (%) | Architect Precision (%CV) | Roche Bias (%) | Roche Precision (%CV) | Tosoh Bias (%) | Tosoh Precision (%CV) | Bio-Rad Bias (%) | Bio-Rad Precision (%CV) |
|---|---|---|---|---|---|---|---|---|
| 4.99 | 1.4 | 1.0 | 5.0 | 4.6 | 1.8 | 3.4 | 3.8 | 3.3 |
| 5.70 | 0.9 | 0.9 | 6.8 | 1.8 | 1.6 | 2.9 | 1.6 | 0.5 |
| 6.72 | 0.3 | 0.8 | 3.7 | 2.9 | 4.0 | 3.4 | 2.4 | 0.9 |
| 7.55 | 1.1 | 0.6 | 0.8 | 1.9 | 6.5 | 1.2 | 2.0 | 0.0 |
| 8.44 | 1.7 | 0.5 | 0.8 | 1.1 | 5.6 | 1.3 | 2.4 | 0.6 |
| 9.33 | 2.9 | 0.0 | 0.9 | 1.5 | 6.5 | 1.2 | 2.9 | 0.0 |
| 10.36 | 1.4 | 0.3 | 2.0 | 1.2 | 3.9 | 4.9 | 0.5 | 0.5 |
| 11.26 | 1.9 | 0.4 | 2.5 | 1.5 | 4.4 | 1.6 | 1.4 | 2.4 |

Conclusion: Hb A1c is a critical assay because of the world-wide diabetes epidemic and analytical quality is imperative for early detection of patients at risk. The IFCC reference method for Hb A1c is internationally accepted and commutable whole blood samples with reference method target values are available so "true bias" of assays, instead of "relative bias," can be measured. A TEa of 6% based on clinical needs for diagnosis is established. Sigma metrics allows assay quality to be objectively assessed. Comparison of analytical quality of common Hb A1c field methods demonstrated some marked differences.

### Metrological Traceability of ARCHITECT Amylase and Alkaline Phosphatase Assays to IFCC Reference Methods

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Background: ISO 18153, In vitro diagnostic medical devices — Measurement of quantities in biological samples — Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials, describes traceability of enzyme assays. The IFCC has established reference measurement procedures (RMPs) to standardize the measurement of enzyme catalytic concentrations. This study validated the metrological traceability of optimized enzyme calibration factors for the ARCHITECT alkaline phosphatase (ALP) and amylase (AMY) assays to ensure agreement with the RMPs. It's necessary for the three ARCHITECT systems (c4000, c8000, and c16000) to not only to provide comparable test results but accurate results as determined by comparison to results from the "gold standard" reference procedures.

Methods: Human serum samples were assigned enzyme activity target values for ALP and AMY using the primary RMPs maintained at the IFCC reference laboratory in Hannover, Germany. Sample aliquots were stored at –75 C before testing using the field assays on two ARCHITECT c8000 systems using different reagent lots.

Results: The mean results from both ARCHITECT systems using different reagent lots were compared to the results from the RMPs for ALP and AMY. The mean % bias was 1% (95% limits of agreement; range -8.8 to +10.8%) for ALP and 0.1% (95% limits of agreement; range -8.5 to +8.2%). The Passing-Bablok regression data is presented in the table below.

| Assay | N | Correlation Coefficient (r) | Slope (95% CI) | Intercept (95% CI) |
|---|---|---|---|---|
| Alkaline Phosphatase | 57 | 0.9965 | 1.02 (1.01, 1.04) | -1.09 (-6.33, 2.25) |
| Amylase | 51 | 0.9973 | 0.99 (0.96, 1.01) | 2.35 (-0.65, 4.24) |

Conclusions: The metrological traceability of the ARCHITECT enzyme assays for ALP and AMY to the internationally recognized reference procedures of the highest metrological order, RMPs listed in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database, was established. The optimized enzyme calibration factors determined allow ARCHITECT results to be in excellent agreement with the IFCC reference methods. Metrological traceability is necessary for the global standardization of clinical laboratory practice and direct comparability of patient test results.

### B-286

**Development of a New ELISA Kit for the Specific Detection of Monoamine Oxidase B (MAO-B) in Gel Filtered Platelet Preparations**

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**Background:**Monoamine oxidase B (MAO-B) is a dimeric, integral outer mitochondrial membrane flavoenzyme that catalyzes the oxidative deamination of arylalkylamine neurotransmitters such as dopamine and serotonin. MAO-B activity increases with age in humans. It has been reported that Alzheimer's plaque-associated astrocytes presented elevated MAO-B levels when compared with age-matched controls. The changes in

MAO-B activity in the prefrontal cortex seem to occur very early in Alzheimer's disease (AD) patients and remain relatively constant as the disease progresses. Furthermore, it was shown that MAO-B activity in platelets is increased in Alzheimer's, but not in Parkinson's disease patients. The availability of convenient immunoassays for the detection of this enzyme is relevant for research applications. This study reports the development of a new ELISA kit incorporating lyophilised calibrators containing recombinant MAO-B for the specific detection of this protein in platelet preparations. Platelets express MAO-B and are a model system for neuronal cells. This kit will facilitate research into the potential

use of patient stratification for treatment strategies in Alzheimer's disease. Methods:Sheep were immunized with recombinant MAO-B expressed in *E. coli*. Lymphocytes were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of specific MAO-B antibody using ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by direct binding ELISA to determine their specificity MAO-B. Initial screening results indicated optimal antibody combinations that were likely to meet assay requirements. These antibody combinations were then assessed and an antibody pair was selected for the sandwich ELISA. The ELISA experimental procedure involved an initial incubation of 60 minutes at +25°C upon addition of assay diluent and sample, followed by a wash step. Conjugate was then added and after a second incubation of 60 minutes at +25°C and wash step, signal reagent was added. After 20 minutes at room temperature +19°C - +25°C the reaction was stopped. The absorbance was measured at 450 nm. 50 μl of sample were required. A total protein determination of the sample was also required to allow for normalization of samples following lysis.

**Results:**The assay was specific for MAO-B (cross-reactivity <0.8% for MAO-A). The assay sensitivity was 3.1 ng/mL (calibration range: 0-75 ng/mL). The spiked recovery of recombinant protein from platelet lysate ranged from 100.4 to 122.8%. The intra-assay precision expressed as %CV (n=12) was <10% for different concentration levels.

**Conclusion:**The results show applicability of this new ELISA kit to the specificdetection of MAO-B from gel filtered platelet lysates. Platelets express MAO-B and are a model system for neuronal cells. Limited alternatives using other matrices are currently available. Furthermore, this ELISA kit incorporates calibrators containing recombinant MAO-B, hence facilitating the assay procedure. This represents a useful analytical tool in research studies into stratification of patients for

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

### B-287

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

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**Background:** Cystic Fibrosis (CF) is a debilitating hereditary disease in which the lungs produce thick sputum difficult to clear and is classically

associated with chronic bacterial infection resulting in an increased,

detrimental inflammatory response. One such effect of this chronic infection is disruption of the body's natural defence to proteases; the antiprotease screen. One antiprotease that is affected is human Secretory Leucocyte Protease Inhibitor (SLPI), which in general, protects the body's tissues from the detrimental effects of different proteases such as human neutrophil elastase (HNE). Previous research has shown that, when CF patients are chronically infected with *Pseudomonas aeruginosa*, SLPI is cleaved due to the excess level of HNE resulting from the recruitment of activated neutrophils to the sites of infection. HNE cleavage of SLPI results in the generation of a C-terminal polypeptide fragment, cleaved SLPI (C-SLPI). The current methodology of testing for bacterial infection during an exacerbation in CF patients involves classical culture-driven microbiological techniques. The availability of rapid, less laborious tests will facilitate a more efficient diagnosis leading to quicker treatment strategies to reduce the pathological burden of infection within CF patients. The aim of this study was to generate monoclonal antibodies for the development of efficient immunoassays based on the detection of C-SLPI as a potential biomarker for bacterial infection.

**Methods:** An amino acid sequence within C-SLPI was used as an immunogen to produce hybridoma clones expressing monoclonal antibodies to C-SLPI. Basic Local Alignment Search Tool (BLAST) analysis of the immunogen against the Pfam protein families database was performed as was *in silico* modelling of the predicted C-SLPI epitope. Monoclonal antibodies purified from the supernatants of these hybridoma clones via affinity chromatography were assessed by ELISA for activity against both C-SLPI and full-length SLPI and antibodies recognising specifically C-SLPI were selected. Additionally, 26 CF patient sputum samples were assayed.

**Results:** Respectively, BLAST analysis and *in silico* modelling showed the immunogen to be specific for C-SLPI and that the epitope is exposed on the C-SLPI peptide but not on full length SLPI. ELISAs using recombinant C-SLPI demonstrated that monoclonal antibodies purified from supernatants of the hybridomas clone lines were able to detect C-SLPI ranging in concentration from 2.70 ng/mL to 2000 ng/mL. Using a C-SLPI monoclonal antibody paired with a conjugated detector antibody the cross-reactivity with full-length SLPI was <0.5% indicating high specificity for C-SLPI. Initial analysis of sputum samples from CF patients experiencing an exacerbation showed higher levels of C-SLPI antigen when compared to those patients who were not experiencing an exacerbation.

**Conclusion:** The results indicate the successful development of antibodies applicable to the development of efficient immunoassays for the specific detection of C-SLPI. This is relevant in clinical settings to facilitate a more rapid testing of bacterial infection status. These novel antibodies could be employed on various diagnostic platforms to detect bacterial infection or exacerbation in patients with chronic infective lung disease.

### B-289

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

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Background: Mildly increased urinary albumin excretion (3-30 mg/dL) is considered a clinically important indicator of progressive renal disease, atherosclerotic disease and cardiovascular mortality. It is used to predict the development of diabetic nephropathy as this protein tends to appear ahead of other serum proteins in urine during the course

of renal glomerular damage. Screening for urinary albumin is therefore recommended by the American Diabetes Association and other guidelines for all diabetic patients.

Beckman Coulter has developed a new sensitive albumin assay for the quantitative measurement of albumin in urine and CSF. The performance of this assay was evaluated on the Beckman Coulter AU5800® Clinical Chemistry System.

Methods: This method was compared with existing Beckman Coulter Urine and CSF albumin assays and the Siemens BN ProSpec following CLSI guideline EP09-A3. Precision was assessed following CLSI guideline EP05-A2. The linear range was assessed following CLSI EP06-A and the high dose hook effect was evaluated. Interferences for endogenous analytes and common drugs were assessed following CLSI guideline EP07-A2.

Results: The new assay demonstrated a good correlation with other Beckman Coulter and competitor assays with Deming regression results vs.: Beckman Coulter AU Microalbumin of y = 1.12x + 0.28 mg/dL; Beckman Coulter DxC of Microalbumin y = 1.09x + 0.03 mg/dL; Beckman Coulter Immage CSF Albumin of y = 1.05x -0.77 mg/dL; Siemens BN ProSpec urine of y = 0.96x - 0.16 mg/dL; Siemens BN ProSpec CSF of y = 0.97x - 0.03 mg/dL.

Estimates of precision were obtained using three urine pools with repeatability of 0.9% CV 0.6% CV & 1.4% CV, and within laboratory precision of 4.3% CV 2.5% CV, 2.1% CV at albumin concentrations of 1.68, 3.28 & 20.0 mg/dL respectively.

The assay was shown to be linear up to 450 mg/L and there was no high dose hook effect up to 20,000 mg/L.

No significant interference was observed up to: 300 mg/dL Creatinine; 3000 mg/dL Glucose; 5000 mg/dL Urea; 500 mg/dL Ascorbate; 50 mg/dL Citrate; 400 mg/dL Magnesium; 30 mg/dL Oxalate; 40 mg/dL Conjugated Bilirubin; 500 mg/dL Hemoglobin; 350 mg/dL Acetone; 10 mg/dL Uric Acid; 2.25 mg/dL Urobilinogen; 300 mg/dL Acetaminophen; 400 mg/dL Ibuprofen; 600 mg/dL Metronidazole; 150 mg/dL 5-Aminosalicylate; 78 mg/dL Calcium.

Conclusion: The results of our study demonstrate excellent performance for the new Beckman Coulter AU Urine/CSF assay demonstrating that the assay is accurate, precise and reliable. Method comparison results show good agreement with existing assays.

### B-290

**Determination of storage conditions for candidate SRM2924 C-reactive protein in solution.**

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**Background:** The objective of this study was to determine the effects of a freeze/thaw cycle on the concentration and structure of candidate reference material SRM2924 C-reactive protein (CRP) in solution, currently stored at 4 °C. Stability of this material in long-term storage would be enhanced if the material were kept in a frozen state (-80 °C). This material is intended to provide a "pure substance" calibrant material for the concentration assignment of future CRP reference material in biological matrices relevant to clinical chemistry such as plasma, serum, urine, etc. SRM2924 was generated in a lot size projected to be sufficient for 3-5 years of demand, therefore, determination of optimum storage conditions over this period is critical to providing a stable reference material.

**Methods:** Vials of SRM2924 (n=24) were randomly selected and assigned to one of two groups (n=12) which were either frozen (-80 °C) or kept at 4 °C. Six vials were removed from each group after one week for analysis. Concentration was determined by amino acid analysis (AAA) and pentameric structure by size exclusion chromatography (SEC). The remaining vials were kept in storage for an additional twenty-one weeks prior to SEC analysis. AAA was conducted by isotope dilution mass spectrometry using leucine, isoleucine, proline, valine and phenylalanine amino acids liberated by vapor phase acid hydrolysis (6 M HCl, 120 °C, 48 hr) of the sample in two separate reactions. Concentration was determined using an external calibration curve generated using ratios of pure substance amino acids in both natural and isotopically enriched forms using triple quadrupole mass spectrometry (Agilent 6490). SEC was conducted by HPLC (Thermo Scientific 3000 UltiMate) using isocratic flow through column (TSKgel) and detected by fluorescence (excitation- 295 nm, emission- 350 nm). AAA and SEC were validated by using reference material NMIJ CRM6201b C-reactive protein in solution. This material is certified for both concentration and structure and was included in all runs of AAA and SEC. The slopes (range = 0.93 to 1.12) and y-intercepts (range = 0.002 to 0.020) of the individual amino acid linear regressions were acceptable and all had r2 values greater than 0.999.

**Results:** The concentration of CRP in the first hydrolysis were 20.7 (%cv=2.7) µmole/kg and 20.4 (%cv=0.6) µmole/kg for the -80 °C and 4 °C group, respectively, while

the values were 20.4 (%cv=1.1) µmole/kg and 20.4 (%cv=2.6) µmole/kg, respectively, in the second. ANOVA (t-test) did not indicate a difference between the either the hydrolysis or storage temperature groups. The concentration for CRM6201b was 39.5 µmole/kg and 39.3 µmole/kg for each of the respective hydrolysis groups which compares well with the certified concentration value of 40.0 (+/- 1.6) µmole/kg. SEC results were consistent through all groups, conditions, time points and validation material in that only a single peak was present in the fluorescent detection of proteins indicating retention of pentameric structure.

**Conclusion:** This study indicates that there are no adverse effects of storage of SRM2924 at -80 °C for the time studied. Periodic checks should be performed to monitor stability throughout the lifecycle of the current batch.

### B-291

**Performance of random albumin creatinine ratio for detection of micro and macro-albuminuria in patients with type 2 diabetes mellitus.**

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**Abstract**

**Background:** Twenty four hour urine albumin excretion (24 hour UAE) considered as gold standard is traditionally used to assess renal damage. This study was performed to evaluate the performance of a less cumbersome spot or random albumin creatinine ratio (RACR) for screening of renal damage in patients with type 2 diabetes mellitus (T2DM).

**Methods:** This was a cross-sectional study of 122 patients with T2DM (mean age 54±15) comprising of 104 female and 18 male patients performed at King Khalid University Hospital, Riyadh between March 2011 and June 2012. Urine samples for both 24 hour UAE and RACR were collected for assessment of albuminuria. Urine albumin levels of <30, from 30-300 and over 300 mg/g were considered as normo-albuminuria, micro-albuminuria and macro-ablbuminuria respectively.

**Results:** Concordance between the two assays was observed in 114 (93.4%) samples including 36 (29.5%) with microalbuminuria and 46 (37.7%) with macro-ablbuminuria. Whereas the mean urinary albumin among normo-albuminuria samples assessed in 24 hour UAE (7±5.4 mg/g) was lower (p=0.03) than that of RACR (9.6±7.1 mg/g), samples with micro-albuminuria (80±41vs115±73) and macro-albuminuria (1615±10vs1416±85) were no different. The sensitivity of RACR against 24 hour UAE assay was 100% and specificity was 91.3% with a positive predictive value (PPV) of 95% and a negative predictive value (NPV) of 100% in micro-albuminura range. For macroalbuminuria RACR had a sensitivity of 100%, specificity of 76% with PPV of 94.1% and NPV 100%.

**Conclusion:** RACR was comparable to 24 hour UAE assay particularly in excluding renal impairment among patients with T2DM.

### B-292

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

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**Background:**Glial fibrillary acidic protein (GFAP) is the major intermediate filament and cytoskeletal protein expressed predominantly in astrocytes. As a cytoskeletal protein, it is thought to regulate astrocyte structural stability and mobility. GFAP is primarily known for its requirement in the central nervous system (CNS), where its deregulation or loss has been linked to degenerative conditions. In healthy individuals, GFAP is maintained within astrocytes and as such is generally not detectable in the plasma or serum. It has been reported however, that GFAP can be used to distinguish ischemic stroke (IS) from acute intracerebral haemorrhage (ICH), based on the speed of its release from astrocytes upon stroke. In cases of IS a delayed release of GFAP is observed with levels below the detectable range for the first 24 hours. Conversely, GFAP is released rapidly into the blood within 2- 6 hours of an ICH event. The availability of rapid tests enabling the detection of this protein represents an advantage in clinical settings. This study aimed to develop a new rapid enzyme-linked immunosorbent assay (ELISA) for the specific and rapid detection of GFAP in human serum.

**Methods:** A colorimetric 2-step sandwich immunoassay was employed. The capture antibody is immobilised and stabilised on a 96-well microtitre plate surface. The analyte, if present in the sample, is bound to the capture antibody and then a second antibody labelled with horseradish peroxidase is bound to the analyte. Absorbances are read at 450nm. The signal is proportional to the concentration of the analyte in the sample. All assay kit reagents are ready to use and serum samples do not require dilution. Recognition of native GFAP was confirmed with analysis of serum samples from ICH patients (n=3) compared with controls (n=3). Difference was assessed with Kruskal Wallis Test (Medcalc version 12.7.8.0).

**Results: The ELISA was specific for GFAP, with cross-reactivity of <0.1% for vimentin, desmin and peripherin. The assay exhibited a functional sensitivity of 0.29 ng/mL and a measuring range of 0-100 ng/mL. Median concentration of GFAP from ICH serum samples (Median 8.796 ng/mL) was significantly higher than controls (Median<0.29 ng/mL), p<0.01. Inter- assay and intra-assay precision values (n=10) were expressed as CV and the values were 7.2% and 3.8% respectively.**

**Conclusion:** The results show applicability of the developed ELISA for the specific and sensitive detection of GFAP in serum. Median concentration values were significantly higher in ICH serum samples when compared with controls. The assay presents all kit reagents ready to use, the total assay time is less than 3 hours and serum samples do not require dilution. This assay is a useful analytical tool for clinical research studies.

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## B-293

### Molecular Defects in Proexosites of Prothrombin Molecule Results in Abnormal Thrombin Generation

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Uncontrollable bleeding at the site of vascular injury is the inception of diseases like stroke, cardiac arrest and other cardiovascular diseases. Upon vascular injury, proteolytic conversion of prothrombin (Pro) to thrombin occurs in the presence of the prothrombinase complex. Prothrombinase is an enzymatic complex between factor Va (fVa) and factor Xa (fXa) assembled on a membrane surface in the presence of divalent metal ions. Although fXa is capable of activating Pro through initial cleavage at Arg271 followed by the cleavage at Arg320 (pre2-pathway), it would take approximately six months to form a clot, which is not physiologically compatible with life. However, the incorporation of fVa into prothrombinase results in a 300,000-fold increase in the catalytic efficiency of fXa for thrombin generation and the order of cleavages reversed (initial cleavage at Arg320 followed by Arg271 (meizo-pathway), which is physiologically compatible with life. Recently, we have shown that the concentration of fVa locally at the place of vascular injury dictates the pathway of Pro activation and that fXa has a fVa-dependent interactive site on Pro within amino acid region 478-482. In addition, several specific basic amino acid residues within Pro have been shown to interact with fXa in a fVa-dependent manner (proexosite I). Thus, in order to elucidate the contribution of amino acid residues from both proexosite I and the region 478-482 in Pro activation by fXa-alone or prothrombinase we constructed several recombinant Pro (rPro) molecules. The first rPro was mutated with two point alanine mutations at Arg382 (Arg382→Ala) and Lys385 (Lys385→Ala) known herein as rProW2. Next, a rPro molecule containing the two previous mutations and the deletion of amino acid residues 478-482 (rProΔ478-482/W2) was also constructed. The two mutant rPro molecules and wild type Pro (rProWT) were stably transfected in BHK-21 cells, and all rPro molecules were purified to homogeneity according to a well established protocol. The last step of the procedure utilized a Fast Performance Liquid Chromatography instrument equipped with a strong anionic exchanger that employed the use of a step-wise calcium gradient to isolate fully carboxylated rPro. The rPro molecules were analyzed for their ability to be activated by both fXa-alone or the prothrombinase complex by SDS-PAGE. Gel electrophoresis revealed fXa-alone exhibited slightly impaired catalytic activity toward rProW2 and rProΔ478-482/W2 when comparing to rProWT activation, whereas prothrombinase activity towards both rPro molecules was severely impaired. Subsequently, we further analyzed prothrombin times (PTs) for plasma-derived Pro, rProWT and both rPro mutants. While rProWT and plasma-derived Pro had standard PTs, the clotting assay employed revealed that rProΔ478-482/W2 and rProW2 were devoid of functional activity. In conclusion, our data suggest that amino acids Arg382 and Lys385 together with a previously described deletion of amino acids 478-482 of Pro hold combined significance for the fVa-dependent binding of fXa on prothrombin within prothrombinase. Our results also provide further explanation for a natural mutation in proexosite I (Arg382→Cys) that was reported in the literature. Patients harboring this natural mutation have dysfunctional abilities to form a fibrin clot and, thus, are prone to be severe bleeders.

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## B-294

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

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

Background: Expansion of instrument analyte menus is often needed to accommodate country specific needs. To address specific needs in China, seven clinical chemistry assays have been modified for evaluation on the ARCHITECT c system. These analytes are used in the investigation of several diseases including disseminated intravascular coagulation, liver damage, lung cancer, diabetes, and myocardial damage.

Methods: The following analytes were evaluated: Fibrin/fibrinogen degradation products (FDP), fibronectin (FN), glutamate dehydrogenase (GLDH), glycated albumin (GA), heart fatty acid binding protein (H-FABP), myeloperoxidase (MPO), and sialic acid (SA). Reagents from Beijing Strong Biotechnologies Inc. (BSBE) were evaluated on the ABBOTT ARCHITECT c system. Precision, accuracy, LoQ, and linearity range were evaluated with guidance from CLSI documents EP15-A2, EP6-A and EP17-A2. Correlation to other commercial kits was performed using human serum/plasma samples across the measuring range of each analyte.

Results: In the studies, all assays demonstrated good performances. Key performance characteristics are summarized in the following table:

| Analytes | Precision: Within-run CV | Accuracy: Bias from Target Value | Linearity (correlation factor r) | LoQ | Correlation (correlation factor r) |
|---|---|---|---|---|---|
| FDP | 1.8% | -0.42% | 0.9987 | 0.83 µg/mL | 0.9921 |
| FN | 0.8% | -2.99% | 0.9993 | 40 mg/L | N/A |
| GLDH | 2.0% | 4.7% | 0.9991 | 10.66 U/L | 0.9995 |
| GA | 0.8% | -4.69% | 0.9998 | 7.13 mg/dL | 0.9917 |
| H-FABP | 6.9% | 5.69% | 0.9999 | 2.5 ng/mL | 0.9838 |
| MPO | 3.2% | 0.75% | 0.9975 | 13.97 ng/mL | 0.9937 |
| SA | 1.3% | 5.4% | 0.9999 | 2.89 mg/dL | 0.9918 |

Conclusions: These initial results are very promising. All the assays tested exhibit good precision, accuracy, linearity, anti-interference, and LoQ performances on ARCHITECT c system. In addition, they also have excellent correlation with commercial assay kits on market e.g. GLDH kit from Roche, MPO kit from Diazyme, FDP kit from Leadmanbio, GA kit from Asahi Kasei, SA kit from Dongou, and H-FABP kit from Randox.

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## B-295

### Evaluation of an advanced Cystatin C assay on DiaSys automated analyzer respons®920

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**Background:**

Cystatin C is an endogenously expressed, non-glycosylated protein that represents an excellent biomarker for moderate impairment of kidney function. Increased Cystatin C levels indicate an even slightly reduced glomerular filtration rate (GFR) compared to conventional parameters like e.g. Creatinine. Since kidney diseases develop slowly and at least in the beginning painless, the majority of individuals with early stages of chronic kidney disease remain undiagnosed. Therefore, early detection of renal insufficiency by a sensitive marker as Cystatin C is of increasing importance to avoid the irreversible condition of renal failure.

The aim of this study was to establish an advanced particle-enhanced Cystatin C assay for DiaSys respons®920, a bench top random access clinical chemistry analyzer. The requirements for this test were superior performance and traceability to IFCC reference material for reliable detection of impaired GFR.

## Methods:

Assay adaption as well as performance verification have been carried out on DiaSys respons®920. All reagents, calibrators and controls were provided by DiaSys Diagnostic Systems GmbH. Calibration stability was optimized by the use of an aqueous 5-level calibrator set containing recombinant Cystatin C, reflecting various conformations of native Cystatin C in different sample material. Method comparisons were performed against nephelometric and immunoturbidimetric competitor assays. Data have been evaluated by using regression analysis according to Passing and Bablok. Inter- and intra-assay imprecision were performed according to the CLSI protocol (EP5-A2). Traceability was investigated by using IFCC reference material ERM-DA417/IFCC.

## Results:

Comparative studies of Cystatin C FS on respons®920 were carried out with 104 native serum and heparin-plasma samples against Hitachi as a common laboratory analyzer [r=0.999; Passing/Bablok: y=0.977 x + 0.006 mg/L] confirming equivalent performance. Good correlation of Cystatin C FS against latest immunoturbidimetric [r=0.9975; Passing/Bablok: y=0.984 X + 0.032 mg/L] as well as a current nephelometric competitor assays [r=0.9970; Passing/Bablok: y=0.974 X + 0.017 mg/L] was demonstrated. Moreover, DiaSys Cystatin C FS is highly precise with an intra-assay precision of a CV≤ 2.53% and an inter-assay precision of CV ≤ 3.71% on respons®920. Based on an advanced calibration approach high calibration stabilities of up to 6 weeks were achieved. Due to good correlation of DiaSys calibrator to IFCC reference material traceability was demonstrated [r=0.999; Passing/Bablok: y=1.0 X + 0.02 mg/L].

## Conclusion:

Here we present a Cystatin C assay with outstanding performance especially for specificity and precision.

This test performs very well on common analyzers as Hitachi systems, but was also proven for equivalent performance on DiaSys respons®920 systems. The advantages of combining Cystatin C FS with this flexible and convenient system are reliable results, optimized workflow and high efficiency (achieved by the perfect match of analyzer, system reagents and applications). Moreover, Cystatin C FS highly correlates to nephelometric and immunoturbidimetric tests and is traceable to ERM-DA471/IFCC reference material. In summary, DiaSys Cystatin C assay represents an excellent tool for early and reliable detection of even slightly impaired kidney function.

### B-296

**Development of a latex-enhanced immunoturbidimetric assay for the measurement of L-FABP levels on automated clinical chemistry analyzers**

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Liver-type fatty acid binding protein(L-FABP) is a 14kDa protein found in the cytoplasm of human renal proximal tubules. It has been demonstrated that measuring urinary L-FABP levels is useful for early diagnosis of renal disease accompanying tubular dysfunction.

We have developed a new method for measurement of urinary L-FABP levels for use in clinical chemistry analyzers. This method is based on latex-enhanced immunoturbidimetry, using anti-human L-FABP mouse monoclonal antibodies. The concentration is determined by measuring the change in absorbance that results from agglutination of latex particles.

The reagents are supplied ready-to-use, and the assay can be completed within 10 min. Using a Roche/Hitachi 917 auto analyzer, 3.0 μL of human urine was mixed with 150 μL of the first buffer solution and incubated for 5 min at 37ºC. Subsequently, 50 μL of the second reagent, which contains the monoclonal antibody-coated latex particles, was added and the absorbance was monitored at 570 nm/800 nm (main/sub wavelengths) for 5 min.

The lower detection limit for L-FABP was 1.0 ng/mL, and the upper quantitation limit was 200 ng/mL. No prozone effect was observed in L-FABP samples of concentrations from 200 to 2000 ng/mL. The within run C.V. (n=20) at 10 ng/mL, 50 ng/mL, and 100 ng/mL was 2.3 %, 1.1 %, and 1.0 %, respectively. The between run C.V. (n=10) at 10 ng/mL, 50 ng/mL, and 100 ng/mL was 1.9 %, 2.3 %, and 2.2%, respectively. Interference studies showed no effect from bilirubin, hemoglobin, glucose, ascorbate,

rheumatoid factor, or chyle at concentrations of 50 mg/dL, 500 mg/dL, 4000mg/dL, 100mg/dL, 500 IU/dL, and 2000 formazin turbidity units, respectively.

Comparison of our assay kit with a commercially available kit , the principle of which is enzyme immunoassay(EIA), yielded a correlation coefficient of 0.984 and an equation of Y (present method) = 0.98X (the EIA kit) - 0.16 (n = 321 urine samples).

We concluded that this assay reagent provides an accurate, precise, and simple method for routine measurement of L-FABP levels in urine samples.

### B-297

**Extending Capillary Zone Electrophoresis (CZE) of Serum Proteins**

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The objective of this study is to extend the clinical utility of CZE. The CZE system used is CapillaryS(tm). The system's server software can provide data downloads to a PC client. Our lab uses data downloads and our middleware to transform and analyze the serum protein electrophoresis (SPE). The SPE zones are: albumin, alpha-1, alpha-2, beta-1, beta-2 and gamma. Reference ranges for the protein in these zones assist the SPE interpretation. The peaks of these zones consist mainly of albumin, alpha-1-antitrypsin, alpha-2 globulins, transferrin, C3+C4 complement and immunoglobulins, respectively, and the zone protein is often used as a surrogate measure of the main zone protein. The higher resolution of CZE allows better estimates of a peak's area, shape, center and height, and the UV absorbance of the eluate provides a better estimate of SPE protein than the scanning of stained gels. These features, for example, allow our middleware to find a quantifiable prealbumin peak in more than 90% of specimens, but this peak is usually not seen in routine gel electrophoresis. This study emphasizes the beta-1 and beta-2 peaks. Several algorithms for quantifying the area of these peaks, which translates directly to protein mass, were tested. The areas of the better algorithms were found to agree well with routine clinical assays of transferrin and C3+C4 complement. Subtraction of these peaks from the ELP curve also allowed an estimate of other beta zone proteins, particularly immunoglobulins (IGs), which can be added to the gamma zone IGs to form an estimate of the total IGs. In specimens with hypergammaglobulinemia, the estimates of total IG agreed well with the sum of IgG, IgA, and IgM IGs. For a systematic comparison, SPE specimens that also had a clinical result for transferrin or C3+C4 complement or quantitative IgA, IgM and IgG, were chosen at random until at least 20 specimens for each analyte or analyte group were found. The values of the clinical analytes were then paired with the corresponding beta peak protein and with the beta zone protein. The quantitative clinical immunoglobulins were paired with the total IG as decribed above, and with the gamma zone protein. Bland-Altman plots of the pairs were made, and the variances of the peak values versus zone values were calculated and compared. In each case the peak protein pairs showed a significantly smaller $F$-test (variance ratio), p<0.01, than the zone protein pairs.

This study shows the measurement of peak areas helps in the detection of abnormal results. Further benefits of our middleware are the assessment of monoclonal gammopathy peaks and assistance in the interpretation of results. A similar system for immunotyping analysis and interpretation has been developed.