Wednesday, July 30, 2014

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

Proteins/Enzymes

B-364

Determination of reference value for ALT in our laboratory population

N. Z. Maluf, C. F. d. Pereira, M. L. Garcia, N. Silva, C. C. V. Ricardo, F. S. Fukuoka. *DASA, Barueri, Brazil*

Background: As the reference values informed in the instructions of use (IFU) are just a guidance, we know the ranges can vary from lab to lab and so each lab may have a different range for what´s normal. Thereby, the ranges for ALT (alanine aminotransferase) numbers may differ slightly depending on the technique and protocols used by different laboratories worldwide. However, normal reference ranges are routinely provided by each laboratory in their printed individual patient's reports. This study aims to establish the reference value for ALT in our laboratory population in order to ensure the best follow-up for each patient. The objetive is to evaluate and standardize the reference values of the UF1000 measured parameters.

Methods: 100 serum samples from healthy individuals (based on current IFU Siemens Dimension® ALTI method reference values: 12 - 78 U/L) were tested for ALT in two different instruments: Siemens ADVIA 2400 and Siemens Dimension RxL Max. The Siemens Dimension® ALTI and the Synermed ALT (measured on Siemens ADVIA 2400) are an L-alanine pyridoxal-5-phosphate (P5P) methodology. The reference value for Synermed ALT is from 7 to 35 U/L. We used D'Agostino-Pearson test to establish the reference values for ALT to our hospital´s patients and it represents the central 95% of results determined from a healthy population.

Results: The results are summarized in the table below.

Conclusion: The obtained ALT reference value (5.5 - 45 U/L) is in agreement with the values from the literature. Finding our own reference values is crucial to ensure reliable results and, consequently, improve the patient´s quality of life.

	N	Mean	Median	SD	Lowest value	Highest value	Lower limit	Upper limit
ALT, U/L Dimension ALTI	100	25.24	24.20	9.67	12.2	71.6	5.5	45
ALT, U/L Synermed ALT	100	22	21	7.7	8	54	7	35

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Evaluation of a novel enzymatic HbA1c test on the fully automated system respons®910

A. Lein, H. Mueller, J. Rink, S. Rosenthal, K. Hahne, D. Vendt, A. Grzesista, H. Baethies, T. Maerker, E. Metzmann, M. Grimmler, G. Gorka. *DiaSys Diagnostic Systems, Holzheim, Germany*

Background: Glycated Hemoglobin A1c (HbA1c) is a well established parameter for long-term monitoring and diagnosis of diabetes. Here, we present a novel enzymatic HbA1c test (HbA1c net FS) for highly specific detection of HbA1c, excluding putative interferences by common hemoglobin (Hb) variants. HbA1c net demonstrates excellent precision based on the application type (twin-test). This test links 2 calibrations and 2 detections for Hb and HbA1c in only **one** determination. The test principle is defined by Hb determination after sample hemolysis at 570 nm and H_2O_2 release after oxidative cleavage of fructosylated dipeptides in the same cuvette. H_2O_2 concentration is determined colorimetrically at 660 nm, whereas delta absorbance is proportional to the HbA1c concentration. The aim of this study was to establish a novel enzymatic HbA1c test, with superior performance, convenient handling and optimized workflow compared to other common methods in the market (as immunoassays or HPLC).

Methods: Assay adaption and performance verification have been carried out on respons®910. All reagents,calibrators and controls were from DiaSys Diagnostic Systems GmbH. Method comparisons were performed against HPLC as reference system. 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). Determination of hemoglobin subtypes was carried out by recovery measurements of IFCC Hb-subtype evaluation samples.

Results: A direct method comparison of HbA1c net values obtained on r910 against HPLC (BioRad Variant II) with 90 native samples demonstrated excellent correlation [r=0.9977; Passing/Bablok: y=1.015 x - 0.23%(DCCT)]. DiaSys HbA1c net FS test is highly precise with an intra-assay precision of CV<0.7% (for HbA1c values from 5.7 to 13.0%) and an inter-assay precision of CV<2.1% (for HbA1c values from 4.4 to 9.9%). High accuracy of HbA1c net FS was demonstrated by recovery IFCC controls (with varying Hb and HbA1c levels) within ± 3% of the target value. Various Hb variants as HbAA, HbAC, HbAD, HbAE, HbAJ, HbAS, HbCC, HbEE, HbSC, HbSS, elevated HbF and b-Thalassemia showed no significant interference with HbA1c net FS.

Conclusion: DiaSys new enzymatic HbA1c assay reveals outstanding specificity and precision. This test highly correlates to HPLC (NGSP/DCCT) but also to IFCC reference material and is unaffected by interferences from common Hb variants. By application of HbA1c net to the fully automated DiaSys system respons910, HbA1c workflow is optimized, due to the implemented on-board hemolysis eliminating error-prone and time-consuming manual preparation.

B-366

Platelet Hyaluronidase-2 as a Potential Novel Biomarker for Both Inflammatory Bowel Disease and Platelet Function.

S. Albeiroti, K. Ayasoufi, C. de la Motte. *Cleveland Clinic, Cleveland, OH*

Background: Inflammatory Bowel Disease (IBD), a group of chronic inflammatory conditions of the intestine, consists of two main types: Crohn's Disease (CD) and Ulcerative Colitis (UC). Different platelet abnormalities are associated with IBD including reactive thrombocytosis, decreased mean platelet volume (MVP) and increased state of activation in the circulation. Our lab has recently reported that platelets contain the enzyme hyaluronidse-2 (HYAL2), which is a GPI-anchored protein that digests hyaluronan (HA). HA is a major polysaccharide component of the extracellular matrix and its deposition is increased during intestinal inflammation. Importantly, HA fragments resulting from HYAL2 digestion can promote inflammatory cytokine production. We therefore hypothesize that platelet HYAL2 plays a role in IBD.

Objective: To compare platelet HYAL2 levels between IBD patients and non-IBD controls and to define normal platelet expression parameters in resting and activated platelets.

Methods: Platelets from IBD patients and non-IBD controls were isolated, washed and lysed (n=12). Protein contents of platelet lysates were measured by Bradford assay and HYAL2 levels were compared using immunoblot assay standardized to protein content (25 µg of total protein). Flow cytometry was used to analyze surface HYAL2 levels of paraformaldehyde fixed platelets from normal donors (n=9). Platelets were activated using thrombin receptor activating peptide (TRAP) and surface P-selectin was used to assess platelet activation state.

Results: 1) Immunoblot analysis of platelets from IBD patients showed an average reduction of 41% in total HYAL2 levels compared to their non-IBD counterparts (p-value < 0.05). 2) Flow cytometric analysis showed that only 6.8 ± 1.3% (±SE) of isolated, normal, non-activated (P-selectin negative) platelets express surface HYAL2. However, after activation the percentage of platelets expressing surface HYAL2 increased to 37.6 ± 6.4% (±SE) (p-value < 0.001).

Conclusion: Platelet HYAL2 levels are significantly lower in patients with IBD compared to non-IBD controls, which demonstrates its potential usefulness as a marker in screening patients with IBD. In addition, we showed that platelet HYAL2 surface expression is significantly higher in activated platelets compared to resting platelets. This reveals that platelet HYAL2, similar to P-selectin, is a potential marker for platelet activation, indicating usefulness in platelet function tests.

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Determination of Fecal Pancreatic Elastase using an Automated Immunoassay Procedure

C. Cruzan, P. Krause, K. Urek, J. A. Maggiore. *Doctor's Data, Inc., Saint Charles, IL*

Background: The clinical laboratory's role in the assessment of chronic digestive disorders and bowel diseases continues to expand. Exocrine pancreatic insufficiency as a contributing source of bowel-related diseases is commonly assessed by measuring

fecal levels of pancreatic Elastase, a serine protease that hydrolyzes dietary proteins. The absence or insufficiency of pancreatic Elastase contributes to incomplete protein digestion leading to varying degrees of malnutrition, and to pathologies ranging from abdominal discomfort to maladies of the large intestine. Manual ELISA techniques to measure fecal pancreatic Elastase are labor-intensive and prone to high variability related to sample handling. An automated sample processing and analytical platform was sought to enhance operating efficiency, reduce variability, and retain analytical accuracy.

Methods: The BIOSERV Diagnostics solid-phase enzyme linked immunosorbent assay (ELISA) is based on a double sandwich technique employing two polyclonal antibodies engineered to recognize several epitopes on human pancreatic Elastase peptide sequences; the first is coated to the assay wells of the microplate, and the second is labeled to biotin which binds to the immobilized Elastase molecule. A streptavidin-labeled horseradish peroxidase binds to the biotin-Elastase complex, which oxidizes the tetramethylbenzidine (TMB) chromogenic substrate to a blue color in the well-characterized reaction. The required sample dilution steps and the procedural sequence of substrate addition, conjugation, washing, incubation, and reading were programmed into a Dynex DSX® analyzer. Analytical precision, linearity, recovery, and accuracy of this automated process were assessed. Further, time studies of technologist direct labor and analytical throughput were undertaken to assess the benefits of automation.

Results: Using the automated procedure on the Dynex DSX, intra-assay precision coefficients of variation (CV) (n=20) at pancreatic Elastase levels of 107 and 459 µg/g were 8.4% and 4.3%, respectively. Inter-assay CV (n=20) at the same pancreatic Elastase levels were 12.0% and 6.4%, respectively. The inter-assay CV and intra-assay CV using the manual ELISA method at a level of 484 µg/g were 4.8% and 8.9%, respectively. Assay linearity was demonstrated between 28 and 500 µg/g, with recovery of expected pancreatic Elastase concentrations ranging from 101.5% to 112.7%. Least-squares regression analysis comparing pancreatic Elastase values from diluted fecal extracts as determined by manual ELISA and automated DSX ELISA processing (n=35, range 35 - 485 µg/g) yielded a correlation coefficient (R^2) of 0.93, y = 1.10x + 58.6; standard error of estimate = 48.5. Time studies (n = 5) indicate that the automated procedure provides an average reduction of 147 minutes of direct, hands-on labor of a technologist processing a 96-well plate of samples, quality controls, and calibrators. Overall, start-to-finish analytical throughput averages 244 minutes using manual processing, compared to 303 minutes using the Dynex DSX program for pancreatic Elastase.

Conclusion: The automated ELISA procedure for fecal pancreatic Elastase using the Dynex DSX has been shown to maintain accuracy and improve analytical precision of the BIOSERV Diagnostics ELISA method. Despite increased overall analytical throughput, the automated procedure provides operating efficiency through the reduction of direct hands-on time.

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Clinical application of urinary protein profiling using cellulose acetate membrane electrophoresis for patients with IgA nephropathy

A. Nakayama[1], M. Sakatsume[2], A. Katayama[3], H. Suzuki[3], K. Shiba[1], S. Iijima[1]. [1]Bunkyo Gakuin University, Tokyo, Japan, [2]Niigata University, Niigata, Japan, [3]Nippon Medical University, Tokyo, Japan
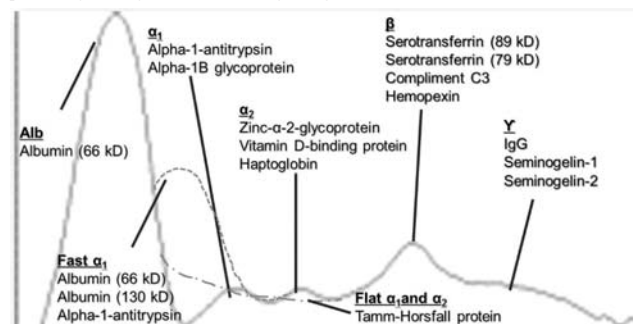
Background: Urinary protein patterns obtained from cellulose acetate membrane (CAM) electrophoresis are useful for renal damage prediction in patients with glomerulonephritis such as IgA nephropathy (IgAN). To identify the disease-causing proteins in each CAM fraction, we established a protein extraction method for proteomic analysis and developed protein profiles in the patients with IgAN.

Methods: Urine samples from 30 patients with IgAN were each applied onto 10 lanes of a CAM. After electrophoresis, the first and last lanes were cut, silver-stained, and used as a guide for fractions in the unstained region. These sections were fragmented, and proteins were extracted with Laemmle's sample buffer, separated by non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis, silver-stained, and identified by liquid chromatography-tandem mass spectrometry.

Results: Urinary protein profiles for 30 patients with IgAN showed 3 main patterns (types A - C): in type A (13 patients), 5 fractions were similar to the normal serum pattern; in type B (14 patients), mobility of the α1-globulin fraction was relatively fast, similar to that of albumin; and type C (3 patients) showed a lack of α1-, α2-, and γ-globulin fractions. The fast α1-globulin fraction of type B mainly comprised an albumin dimer, which is formed in response to oxidative stress, and α1-antitrypsin. Although there were no bands corresponding to α1- and α2-globulin in type C, a significant amount of Tamm-Horsfall protein, reflecting normal tubular function, was

detected. A schematic diagram of the overall urinary protein pattern in IgAN was established (Figure 1) based on proteins identified in more than 50% of subjects of each profile pattern.

Conclusion: The type B urinary protein profile pattern indicates increased oxidative stress compared to type A, whereas type C indicates mild renal impairment compared to types A and B. Our detailed analysis provides a valuable non-invasive tool for predicting the degree of renal damage in IgAN.

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Performance Evaluation of ERM-DA471/IFCC standardized Tina-quant® Cystatin C Generation 2 Assay on Roche Clinical Chemistry Analyzers

C. Schmidt[1], C. M. Cobbaert[2], J. A. Bakker[2], H. P. C. Schippers[2], W. Endlich[2], M. Flodin[3], P. Eliasson[3], D. Hermsen[1]. [1]University Hospital of the Heinrich-Heine University, Central Institute of Clinical Chemistry and Laboratory Diagnostics, Düsseldorf, Germany, [2]Leiden University Medical Center (LUMC), Department of Clinical Chemistry and Laboratory Medicine, Leiden, Netherlands, [3]University Hospital, Clinical Chemistry and Pharmacology, Uppsala, Sweden

OBJECTIVE Cystatin C has been proposed as a more sensitive endogenous biomarker regarding the estimation of glomerular filtration rate when compared to serum creatinine. But Cystatin C assays of different manufacturers showed a lack of comparability before the ERM-DA471/IFCC reference material was available. In our study we evaluated the analytical performance of the new ERM-DA471/IFCC standardized Tina-quant® Cystatin C Gen. 2 immunoassay (Roche Diagnostics, Mannheim, Germany).

METHODS AND RESULTS The analytical performance of the new assay was evaluated in three laboratories using Roche/Hitachi **MODULAR ANALYTICS**, COBAS INTEGRA® 800, **cobas c** 701 and **cobas c** 501 analyzers. Cystatin C concentration was measured turbidimetrically by a particle enhanced immunoassay standardized against the ERM-DA471/IFCC. The analytical performance of the new assay was investigated under routine laboratory conditions using samples covering the entire measuring range of cystatin C (0.4 - 6.8 mg/L).

Within-run imprecision data were collected using three control levels (Roche Diagnostics) and three self prepared pools of human sera (single run, n = 21 replicates per sample) covering a concentration range from 0.75 to 5.02 mg/L. Coefficients of variation (CVs) were determined to be less than 3.3 % for control materials and less than 2.8 % for pooled samples. Between-day imprecision yielded CVs between 1.7 and 7.3 % (one run/day, up to 17 days) using control sera (three levels, Roche Diagnostics). The recovery of target values in different control sera was determined performing triplicate measurements in three independent runs. The recovery of Cystatin levels in control sera from Roche Diagnostics and other manufacturers ranged between 86.0 - 107.8 % and 86.4 - 100.5%, respectively.

All method comparison experiments were designed in compliance with CLSI EP09-A3, performed using > 119 serum samples and analyzed with Passing-Bablok regression. Statistical analysis of method comparison experiments against the Roche Cystatin C Gen.1 yielded correlation coefficients > 0.990, slopes between 0.92 and 1.13 (0.89 - 1.16, 95 % confidence interval) and intercepts from -0.13 to 0.07 (-0.16 - 0.10) mg/L. Method comparison studies against other available ERM-DA471/IFCC standardized Cystatin C immunoassays (Siemens N Latex Cystatin C, Gentian Cystatin C) performed on different analyzer systems (Siemens BN II Nephelometer, Abbott Architect, Roche cobas c502) showed excellent correlation coefficients (r > 0.990). Calculated slopes ranged between 0.93 and 1.04 (0.91-1.06), and intercepts between -0.10 to 0.04 (-0.13 - 0.07) mg/L, respectively, confirming the successful standardization of the new Cystatin C Gen. 2 immunoassay to the ERM-DA471/IFCC reference material.

CONCLUSIONS Our study demonstrated an excellent technical performance of the new Roche Tina-quant® Cystatin C Gen. 2 assay. Method comparison results revealed a high degree of comparability to other available Cystatin C immunoassays that are traceable to ERM-DA471/IFCC. Due to the excellent study results the new Tina-quant® Cystatin C Gen. 2 assay is well-suitable for routine use.

Disclaimer: The study was sponsored by Roche Diagnostics GmbH, Germany. The Roche Tina-quant® Cystatin C Gen.2 assay is not yet cleared for use in the U.S.

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Effects of CH3CN-cosolvent and CuBr-catalyst on the synthesis of difluorophosphonates as chemical specialty

H. Tsai. *Department of Chemistry and Material Engineering, Chung Cheng Institute of Technology, National Defense University, Ta-hsi, Tao-yuan, Taiwan*

Background: Fluorophosphonates have found applications as hyperlipidaemic drugs, hormone substitutes, cancer chemotherapy and nervous chemical warfare. However there is generally a conspicuous lack of methods for the preparation of difluoromethanephos phonates. Effects of CH_3CN-cosolvent and CuBr-catalyst on the synthesis of difluoro phosphonates $(EtO)_2P(O)CF_2C(O)R$ were discussed in this research.

Methods: Synthesis of $(EtO)_2P(O)CF_2Br$: ^{19}F NMR: -62.0 ppm (d, J = 90.3); ^{31}P NMR: -0.65 ppm (t, J = 93.7); ^1H NMR: 4.42 ppm (4H, q, J = 7.1), 1.42 ppm (6H, t, J = 7.1). Procedure for Synthesis of $(EtO)_2P(O)CF_2C(O)CO_2Et$: (Method A): A three-necked flask was charged with 15.0 mL of a 2.0 M monoglyme solution of $(EtO)_2P(O)CF_2ZnBr$. 42.0 mmols of freshly distilled $ClC(O)CO_2Et$ was added and stirred at rt for 48 hours. The mixture was filtered, extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$, concentrated by rotary evaporation and vacuum distilled at 94-95°C/0.15 mmHg to give 54 % of the titled compound. ^1H NMR : 1.40 (9H, t, J = 7.1), 4.30 (6H, m, J = 7.1). ^{13}CNMR : 13.9 (s), 16.2 (d, J = 5), 63.5 (s), 65.8 (d, J = 7), 115.5 (t, d, J = 275, J = 199), 158.5 (s),181.8 (t, d, J = 16). ^{19}FNMR : -115 (d, J = 95). ^{31}PNMR : 4.5 (t, J = 95). (Method B): 7.5 mL dry CH_3CN, 0.45 mmol CuBr and 42.0 mmols $ClC(O)CO_2Et$ were added to a 15.0 mL of 2.0 M monoglyme solution of $(EtO)_2P(O)CF_2ZnBr$. Stirred at rt for 0.5 hour. 58 % of the titled compound was afforded. (Method C): 0.45 mmols CuBr and 42.0 mmols of $ClC(O)CO_2Et$ were added to a 15.0 mL of 2.0 M monoglyme solution of $(EtO)_2P(O)CF_2ZnBr$, Stirred at rt for 24 hours. The ^{19}F NMR spectrum indicated the presence of $(EtO)_2P(O)CF_2C(O)CO_2Et$, $(E,Z)-(EtO)_2P(O)$ CF=CFP(O)(OEt)$_2$ and $(EtO)_2P(O)F$.

Results: Reaction of triethylphosphite with dibromodifluoromethane *via* Michaelis-Arbuzov reaction gave 95 % yield of $(EtO)_2P(O)CF_2Br$. Subsequently reacted with acid washed zinc powder in the presence of monoglyme, $(EtO)_2P(O)CF_2ZnBr$ was obtained. It took 48 hours to synthesize $(EtO)_2P(O)CF_2C(O)R$ from the acylation of $(EtO)_2P(O)$ CF_2ZnBr with $ClC(O)R$ (R = CO_2Et, OEt, NEt_2) at rt However, this situation can be easily ameliorated upon addition of a catalytic amount of cuprous bromide and the addition of acetonitrile as cosolvent to the reaction mixture. This reaction is completed within 0.5 hour to yield $(EtO)_2P(O)CF_2C(O)CO_2Et$ in a 58 % isolated yield. Furthermore, if the reaction was carried out in the presence of 1.5 % CuBr-catalyst without the CH_3CN as cosolvent, in addition to the formation of 12 % of $(EtO)_2P(O)CF_2C(O)CO_2Et$, 18 % yield of (E) and (Z)-(1,2-difluoroethylenediyl) bisphosphonate $(EtO)_2P(O)CF=CFP(O)$ (OEt)$_2$ and 70 % of $(EtO)_2P(O)F$ at -82 ppm $(J_{P-F} = 972$ Hz) were observed in ^{19}FNMR spectrum.

Conclusion: In the presence of CH_3CN-cosolvent, monoglyme solvent and appropriate CuBr-catalyst, acylation of $(EtO)_2P(O)CF_2ZnBr$ with ethyl chloroformate, diethyl carbamoyl chloride or ethyl oxalyl chloride gave good yields of 2-oxo-1,1-difluorophos phonates $(EtO)_2P(O)CF_2C(O)R$ as chemical specialty. However, if these acylations were carried out without acetonitrile as cosolvent, the mixture products of $(EtO)_2P(O)CF_2C(O)CO_2Et$, $(E,Z)-(EtO)_2P(O)CF=CFP(O)(OEt)_2$ and $(EtO)_2P(O)F$ were observed.

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Neutrophil Gelatinase-Associated Lipocalin Levels in Patients with Thalassemia and Sickle Cell Disease: Correlation with Renal Injury

I. Papassotiriou[1], E. Voskaridou[2], A. Margeli[1], A. Kattamis[3]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Thalassemia Center, "Laikon" General Hospital, Athens, Greece, [3]Hematology-Oncology Divison, Department of Pediatrics, University of Athens Medical School, Athens, Greece*

Background and Aims: Neutrophil gelatinase-associated lipocalin (NGAL) is a protein belonging to the lipocalin superfamily initially found in activated neutrophils, in accordance with its role as an innate antibacterial factor. However, it subsequently was shown that many other types of cells, including in the kidney tubule, may produce NGAL in response to various injuries. The increase in NGAL production and release from tubular cells after harmful stimuli of various kinds may have self-defensive intent based on the activation of specific iron-dependent pathways, which in all probability also represent the mechanism through which NGAL promotes kidney growth and differentiation. NGAL levels clearly correlate with severity of renal impairment, probably expressing the degree of active damage underlying the chronic condition. For all these reasons, NGAL may become one of the most promising next-generation biomarkers in clinical nephrology and beyond. We aimed to investigate the clinical significance of NGAL levels and its correlation with renal function in patients with hemoglobinopathies.

Patients and Methods: 117 adult patients with hemoglobinopathies were included in the study divided in 3 groups. Group A: 30 patients with transfusion-dependent thalassemia major (TM); Group B: 29 patients with thalassemia intermedia (TI) and Group C: 58 patients with HbS/betathalassemia disease, while 20 apparently healthy individuals served as controls (Group D). In patients and controls along with standard blood and urine chemistry, measurements of serum Cystatin C and NGAL were performed. Estimated Glomerular Filtration Rate (eGFR) values were calculated with an unadjusted for body surface Cystatin C based equation: eGFR (mL/min)=77.24(Cys C)exp(-1.2623)

Results: The main results of the study showed that: a) NGAL levels were significantly higher in all the groups of patients compared to controls: Group A 95.0±45.0µg/L, Group B 139.1±86.1µg/L, Group C 117.8±37.3µg/L vs Group D 50.3±11.3µg/L (p<0.001), b) Cystatin C levels were significantly higher in patients of Group A 0.94±0.34mg/L and Group C 1.04±0.50mg/L compared to controls 0.75±0.09mg/L (p0.300), c) NGAL levels and eGFR values (Group A: 96.9±39.8, Group B: 117.0±26.0, Group C: 86.2±27.8 and group D: 109.6±15.0mL/min, respectively) correlated significantly in patients of Group A and Group C (r=-0.739, p<0.001 and r=-0.735, p<0.001, respectively), while NGAL values are independent from eGFR values in patients of Group B.

Conclusions: These findings illustrate the tubular-glomerular activation feedback mirrored by NGAL in patients with transfusion-dependent thalassemia major and HbS/betathalassemia disease, who suffer from renal injuries, indicating that tubular damage precedes GFR reduction. Upregulation of NGAL in patients with thalassemia intermedia independently of renal injuries may reflect the compensatory, protective role of NGAL in response to diverse cellular stresses, including inflammation and oxidative stress. However, recent reports have implicated NGAL upregulation as a mechanism that contributes to anemia in the setting of chronic low grade inflammation. In experimental models, systemic and medullary NGAL has been demonstrated to induce inhibition of erythropoiesis through induction of apoptosis and arrest of differentiation of erythroid progenitor cells.

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Evaluation of Dried Blood Spots for Use in Isoelectric Focusing Electrophoresis in Deficient Alpha-1-Antitrypsin Phenotype Interpretation

M. M. Duran. *Geonostics, Lincolnshire, IL*

Background: Laboratory diagnostics contribute significantly in the diagnosis of Alpha-1-Antitrypsin (AAT) deficiency, utilizing AAT serum concentration, AAT phenotype determination by isoelectric focusing (IEF) electrophoresis, and genotyping. Dried blood spots (DBS) are a potentially attractive sample type for IEF phenotype analysis on the Sebia Hydrasys because of the ease of sample collection. Previous work demonstrated that the common phenotypes of MM, MS, and MZ, as a DBS sample, were indistinguishable from their companion serum samples. In this current study, we present the novel methodology of DBS for the 3 most prevalent deficient phenotypes: SS, SZ, and ZZ.

Methods: Eighteen whole blood samples from known phenotypes SS, SZ, and ZZ

were spotted on filter paper using 50 uL of sample. The blood spots dried overnight and then the 50 uL dried blood samples were punched and rehydrated with a buffer/deionized water solution. Ten uL of each extracted DBS sample was applied to the IEF comb. Eighteen serum samples of the corresponding DBS phenotypes were also placed on a comb for comparison. IEF was performed on the Sebia Hydrasys using standard protocol and the resultant gel was stained, washed, and digitally scanned.

Results: In Figure 1, phenotype SS is on the left, phenotype SZ is in the center, and phenotype ZZ is on the right. For each phenotype, the DBS sample is on the left and the corresponding serum sample is on the right. All 18 DBS phenotype samples displayed the unique identifiable banding patterns present in the serum samples.

Conclusion: IEF of DBS samples adequately reveal the S and Z alleles, in the absence of the M allele, on the Sebia Hydrasys. Work is underway to validate the 100+ additional rare alleles and to establish AAT protein stability on filter paper
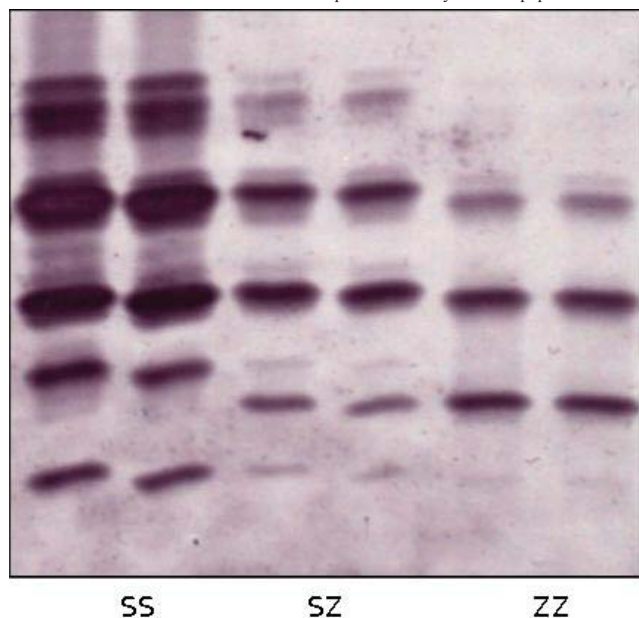

SS   SZ   ZZ

Figure 1

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Evaluation of INNOTEST® β-Amyloid (1-40) immunoassay and comparison with IBL™ Human Amyloid (1-40) immunoassay

J. Oudart, R. Mahmoudi, G. Fiabane, P. Arthuis-Demoulin, J. Novella, L. Ramont. *CHU de Reims, Reims, France*

Background: Combined assay of cerebrospinal fluid (CSF) biomarkers Tau protein, phosphorylated Tau protein and beta Amyloid peptide 1-42 (Aβ42) is commonly used in the diagnosis of Alzheimer's disease (AD). CSF biomarkers are now included in diagnosis criteria in addition to clinical evaluation and medical imaging. In discordant cases where an isolated reduction of Aβ42 peptide is found, the beta Amyloid peptide 1-40 (Aβ40) measurement associated with Aβ42/Aβ40 ratio allows better classification of patients. According to the literature, the clinical cut off for this ratio is less than 0.05 in AD.

The aim of this study was to evaluate the INNOTEST® β-Amyloid (1-40) immunoassay and compare it versus IBL™ Human Amyloid (1-40) immunoassay.

Methods: - Evaluation of Innotest® β-Amyloid (1-40) immunoassay (Fujirebio Europe, Belgium): intra-assay precision studies were evaluated on two CSF samples (5000 and 9800 ng/L respectively), 16 consecutive times. Inter-assay precision studies were evaluated at the 2 same levels of concentrations (n= 10). Dilutional linearity was evaluated by successive dilutions of a CSF sample in the sample buffer.

- Comparison between INNOTEST® β-Amyloid (1-40) immunoassay and IBL™ Human Amyloid (1-40) immunoassay (IBL, Japan): seventy four patients were included in this study. Aβ40 peptide concentration was assessed using the 2 immunoassays for all patients.

Differences between assays were analyzed by Pearson Test and the degree of agreement between measurements was evaluated using Bland-Altman analysis.

Results: For Aβ40 measurement with the INNOTEST® β-Amyloid (1-40) immunoassay, the intra-assay CVs were 1.29% and 2.68% at Aβ40 concentrations of 5000 and 9800 ng/L respectively (n= 16). The inter-assay CVs were 13.94% and 15.66% at the same Aβ40 concentrations (n = 10). The mean SD recovery of CSF Aβ40 immunoassay was 85% (slope: 1.0264; intercept: -328.83; R2= 99.8%).

The correlation coefficient between the 2 immunoassays (INNOTEST® vs IBL™ assay) was R2= 0.735. Interpretation of Aβ42/Aβ40 ratio was concordant between the 2 immunoassays at 92% (68/74) of the cases using a cut-off at 0.05.

Conclusion: This preliminary study showed that INNOTEST® β-Amyloid (1-40) immunoassay's intra-assay CVs, inter-assay CVs and linearity CVs were less than 20%. INNOTEST® β-Amyloid (1-40) immunoassay seems to correlate with IBL™ Human Amyloid (1-40) immunoassay which is commonly used in the laboratories.

B-375

Evaluation of a new liquid UIBC method on Architect c4000 analyzer

M. Gramegna, M. La Motta, R. Lucini. *Sentinel CH. SpA, Milan, Italy*

Objective The objective of this study is to evaluate the analytical and clinical performances of a new liquid UIBC Method on Architect c4000 analyzer.

Relevance: Transferrin is the principal plasma protein for transport of iron. One molecule of TRF binds two ferric ions and an associated anion, usually bicarbonate in vivo. Normally 30% of the iron binding sites of transferrin are occupied by Fe3+, the additional amount of iron that can be bound is the unsaturated iron-binding capacity (UIBC). The sum of serum iron and UIBC represents the total iron-binding capacity (TIBC). UIBC measurement is an iron panel parameter used in the diagnosis and treatment of anemia.

Methodology UIBC is determined directly by saturating the transferrin at an alkaline pH with a known excess amount of iron. The iron that remains free after transferrin saturation is reduced to ferrous state and then complexed by ferene-S to form a stable complex which colour intensity is measured at 580-600 nm. UIBC is therefore determined by subtracting the quantity of unbound iron from the total added quantity. The instrument used for this evaluation was an Architect c4000 analyzer, a random-access analyzer. To perform this evaluation, modified CLSI protocols were adopted. Acceptance criteria as total imprecision were ≤ 5% for samples ≤ 110 µg/dL and ≤ 2% for samples ≥ 250 µg/dL. LOD should be ≤ 41 µg/dL, The method should be linear up to 500 µg/dL. Claimed goal for on board calibration stability was ≥ 7 days and reagent on board stability was 35 days. Comparison to commercial methods had following acceptability: slope 0.90 - 1.10, intercept ≤ 15, r ≥ 0.975.

Validation: Total imprecision (21 days) gave CV% at 103 µg/dL lower than 5%, CV% at 136 µg/dL lower than 4% and at 269 µg/dL lower than 2%. LOD was 13.1 µg/dL. The test was linear from 19 µg/dL up to 500 µg/dL. On board reagent stability was up to 35 days and on board calibration stability was up to 21 days. Compared vs a commercial Ferrozine method (n = 82, samples between 6.5 and 486 µg/dL) linear regression gave y = 1.16x - 9.57 and r = 0.998. Compared versus Sentinel UIBC Liquid REF 17639 (n = 82, samples between 41.5 and 466 µg/dL) linear regression gave y = 1.10x - 10.3 and r = 0.997. Bilirubin (up to 66 mg/dL), hemoglobin (up to 100 mg/dL) and triglycerides (up to 1000 mg/dL) did not interfere.

Conclusions Analytical and clinical performances of new liquid UIBC method on Architect c4000 analyzer meets the acceptance criteria and it shows all the requirements for its use as routine clinical chemistry assay.

B-376

Performance characteristics of a cystatin C immunoassay on the Beckman Coulter AU5800, AU680 and IMMAGE 800 Systems

C. Townsley, T. Nilsen. *Gentian AS, Moss, Norway*

Background: Cystatin C is a biomarker of kidney function. Its measurement can be used to estimate glomerular filtration rate. The objective was to evaluate the performance characteristics of this assay on the Beckman Coulter AU5800, AU680 and IMMAGE 800 systems.

Methods: A cystatin C particle-enhanced turbidimetric immunoassay (Gentian, Norway) with avian antibodies is standardized against the international calibrator standard ERM-DA471/IFCC and holds a current FDA 510k for use on other platforms. Measurement was carried out on the Beckman Coulter AU5800, AU680

and IMMAGE 800 systems at different sites between 2008 and 2013 using a cystatin c immunoassay from Gentian, Norway. Different reagent lots were used and new serum samples and dilution series were made for each site study in the case of precision, linearity, security zone, interference, recovery and limit of quantification. Protocols based on CLSI guidelines were used.

Results: The measuring range is 0.45 - 8.0 mg/L, linearity was proven with a minimum range of 0.45-6.9 mg/L on all instruments with prozone of 32 mg/L. The total C.V. for precision ranged from 1.5 - 6.2% with total C.V. at 1 mg/L of ≤2.7%. LoQ for all instruments was observed to be <0.5 mg/L. Interference studies with potential interferents, Intralipid (≤10 g/L), hemoglobin (≤6 g/L) and bilirubin (≤200 mg/L) showed no interference. Total analysis time was 10 minutes.

Conclusion: The Gentian Cystatin C immunoassay is validated for use on the Beckman Coulter AU5800, AU680 and IMMAGE 800 systems. The assay shows acceptable performance characteristics for measuring cystatin C in human serum and plasma samples on these systems.

Summary of results for studies on AU5800, AU680 and IMMAGE 800

	AU5800	AU680	IMMAGE 800
Linearity (mg/L)	0.45 – 7.07	0.44 - 9.02	0.37 - 6.9
Recovery (%)	96 - 100	98 - 105	100 - 110
Security zone (mg/L)	32	40	40
Precision (total C.V.%)	1.62 - 3.42	1.51 - 2.44	2.7 - 6.2
Bilirubin interference testing	≤400 mg/L	≤200 mg/L	≤800 mg/L
Hemoglobin interference testing	≤6 g/L	≤8 g/L	≤8 g/L
Intralipid interference testing	≤10 g/L	≤16 g/L	≤16 g/L

B-377

Evaluation of new Lipase Color Liquid on Architect c4000 analyzer

M. Gramegna, L. Politi, R. Lucini. *Sentinel CH. SpA, Milan, Italy*

Objective The objective of this study is to evaluate the analytical and clinical performances of new Lipase Color Liquid reagent on Architect c4000 analyzer.

Relevance Lipase enzymes are produced in the pancreas and also secreted in small amounts by the salivary glands as well as by gastric, pulmonary and intestinal mucosa. Determination of lipase is used for diagnosis and treatment of diseases of pancreas such as acute and chronic pancreatitis and obstruction of the pancreatic duct.

Methodology The method for the determination of lipase is based on the cleavage of specific chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid (6'-methylresorufin)-ester emulsified in stabilized micro-particles. In the presence of specific activators of pancreatic lipase as colipase, calcium ions and bile acids, the substrate is converted in 1,2-O-dilauryl-rac-glycerol and glutaric acid-6'-methylresorufin-ester which decomposes spontaneously in glutaric acid and methylresorufin. The increase of absorbance at 580 nm, due to methylresorufin formation, is proportional to the activity of lipase in the sample. For the evaluation of this reagent, modified CLSI protocols were adopted. Acceptance criteria for total imprecision were ≤ 5% for lipase samples. LOD should be ≤ 3.0 U/L. LOQ was defined as the analyte concentration at which the % CV is less than 20%. The method should be linear up to 300 U/L. Method comparison was evaluated by comparing new Lipase Color Liquid reagent on Architect c4000 versus commercial method on Hitachi Modular P.

Validation Total imprecision (during 40 days on 3 level samples) gave CV% at 24 U/L (L1), 67 U/L (L2) and 157 U/L (L3) lower than 5%. LOD was 1.5 U/L. LOQ was 3.0 U/L. The test was linear from 0 U/L up to 600 U/L. On board calibration stability and reagent on board stability were up to 40 days. This lipase test (y) was compared with reference method (x) that uses the same substrate (methylresorufin) and gave the following results: $y = 1.07x + 5.78$; $r = 0.99$; $n = 74$. The test is not affected by the presence of hemoglobin up to 150 mg/dL, bilirubin (unconjugated and coniugated) up to 66 mg/dL. The test is affected by the presence of lipids up to 100 mg/dL.

Conclusions Analytical and clinical performances of new Lipase Color Liquid reagent on Architect c4000 analyzer meets the requirements for its use as colorimetric test to measure lipase in serum. Specificity and better precision make this assay very suitable for the routine measurement of this critical analyte.

B-378

New algorithm for alpha-1-antitrypsin (AAT) deficiency investigation including high resolution capillary zone electrophoresis (CZE-HR) for serum protein electrophoresis (SPE) for screening

J. P. Émond, C. Bergeron, M. Beaulieu. *University of Montréal Hospital Center (CHUM), Montréal, QC, Canada*

Background: AAT inhibits proteases such as neutrophil elastase. AAT deficiency is an underdiagnosed condition, which predisposes individuals to early onset of emphysema. We propose an algorithm in which 1st-step is based on AAT nephelometric measurement in conjunction with high resolution capillary zone electrophoresis (CZE-HR) SPE. CZE-HR SPE allows clear separation and quantification of AAT in an isolated fraction along with assessment of clinical conditions affecting the accuracy of AAT quantification. Genotyping and phenotyping are done in sequence only if required. We aim to review this algorithm performance after 14 month utilisation.

Methods: Implementation of a new algorithm using i) AAT measurement and CZE-HR SPE as screening and interpretation tools, and if required ii) genotyping in 2nd step, iii) addition of phenotyping in 3rd step. AAT concentration decision cut-off is 1.15g/L in normal CZE-HR SPE patients and 2.0g/L in inflamed ones. Integrated interpretation report is always provided. This algorithm replaces individual AAT quantification and/or genotyping requests. CZE-HR SPE was conducted on Sebia Capillarys 2 automated system with high resolution buffer. Retrospective 14 month utilisation study is based on 172 consecutive medical requests for AAT deficiency investigation.

Results: Our population consists of 172 individuals (83 men, 89 women; age (years; mean ± 2SD) of 51.3 ± 34.6 and 52.5 ± 27.4, respectively). CZE-HR SPE allows AAT detection of as low as 0.07 g/L (7 mg/dL). In our population, investigation was conclusive after 1st step in 58% of cases (n=99) where genotyping was not required. Genotyping lead to be conclusive in an additional 25% of patients (n=43), whereas phenotyping was required in 17% (n=30). Overall, detection rate of deficiency was 11%: i) deficiency associated with common variants (SS, SZ, ZZ) was 7.0% (n=12) and ii) additional 7 patients (4.0%) require SERPINA1 gene sequencing for confirmation of rare non-S or non-Z deficiency variants. We identified also 23 heterozygotes carriers (13.4%; 17 patients with S allele and 6 with Z allele). One case previously genotyped as "MS" was retested with the algorithm approach, and was correctly phenotyped as Pi*SNull. Despite adding phenotyping testing in our repertoire, the overall cost of investigation has decreased by 30% when using our new algorithm strategy as compared to our previous one (AAT measurement and genotyping for all).

Conclusion: High resolution automated SPE (CZE-HR) is a powerful tool to assess AAT and to determine many clinical states affecting accuracy of AAT quantification by common techniques. Those conditions may impact screening effectiveness. Implementation of our AAT deficiency screening algorithm has allowed us to increase the efficiency of investigation while eliminating genotyping in majority of cases. Moreover, we were able to perform expensive phenotyping testing in our laboratory repertoire while reducing overall testing cost by 30%. This allows us to selectively require outside sequencing services for only 4.0% of tested patients with unusual deficiency-associated variants.

B-379

Biochip Array Technology Rapidly Identifies a Platelet-Derived Alzheimer's Disease-Specific Phenotype

M. Veitinger[1], R. Oehler[2], E. Umlauf[1], R. Baumgartner[1], C. Gerner[3], R. Babeluk[2], J. Attems[4], G. Mitulovic[5], E. Rappold[1], J. Lamont[6], M. Zellner[1]. [1]Institute of Physiology, Medical University of Vienna, Vienna, Austria, [2]Surgical Research Laboratories, Medical University of Vienna, Vienna, Austria, [3]Department of Medicine I , Medical University of Vienna, Vienna, Austria, [4]Institute for Ageing and Health, Newcastle University, Newcaste upon Tyne, United Kingdom, [5]Department of Medical and Clinical Laboratory Diagnostics, Medical University of Vienna, Vienna, Austria, [6]Randox Laboratories Limited, Crumlin, United Kingdom

Background: Globally >35 million people have Alzheimer's disease (AD) or a related dementia. Current diagnostic tests use neuropsychological review and brain scan and a number of studies have reported AD-specific cerebrospinal fluid biomarkers but analysis requires invasive lumbar puncture. There is an urgent need to develop a new minimally invasive diagnostic procedure. In this study platelets, which share biochemical features with neurons, were used as a surrogate to characterize AD-modulated proteins. The combination of biochip array technology with a powerful biomarker algorithm is innovative and generates a reliable ante mortem AD test.

Methods: Ethics was granted and 62 patients with clinically suspected AD and 63 age/sex-matched control subjects were enrolled. 2D-DIGE was performed on gel-filtered platelets to resolve alkaline platelet proteins using pH6-9 strips and acidic proteins on pH4-7, subsequently separated in the

second dimension using 11.5%SDS-PAGE. DeCyder software was applied to detect spots and all sample gels were matched with the master gel. Tryptic digest identified peptides using Spectrum Mill MS Proteomics Workbench software and UniProt database. *APOE ε4* and *GSTO1*A140* genotyping was performed. Platelet rich plasma was derived from whole blood (20min, 120 x *g*) and stored at -80⁰C. After thawing, the platelets were isolated and lysed with SDS before addition of 2%BSA/PBS to bind excess SDS. This lysate was applied to the protein biochip employing immunoassay sandwich principles. The protein biochip was subsequently imaged on the Evidence Investigator analyser.

Results: Monoamine-oxidase-B displayed the most significant increase in spot density in AD patients.The decrease in ApoE spot density was attributed to isoform ε3 and accordingly, this spot exhibited lower detection in *ε4*-positive patients. A second ApoE spot exhibited increased density and was assigned to isoform ε4. The fourth strongest confirmed AD-related biomarker was identified as tropomyosin1 (Tm1) and adjacent spots were also identified as Tm1. Glutathione S-transferase omega1 (GSTO1) spots displayed a strong association with the *APOE*-genotype in AD patients. *APOE*-genotyping revealed significantly more AD *APOE ε4* carriers(66%)than the control group(11%). Genotyping revealed that exclusively two *GSTO1*A140* alleles were present in non-*APOE ε4* AD patients (*n*=20) relative to 38% in controls (30% in non-*APOE ε4* controls) and 32% in *APOE ε4*-positive patients. Biochip determination of the *GSTO1*A140* and *APOE ε4* allele count identified 98% of all samples correctly for the *GSTO1* SNP rs4925 genotype and 100% correct genotyping was achieved for *APOE ε4* by normalization with either ERK2 or panApoE concentrations. Biochip analysis with the cellular loading control replicated also the higher expression of both quantitative markers Tm1 and MaoB in patients relative to controls.

Conclusion: The most powerful AD biomarker algorithm was identified following combinatorial review of the proteins/isoforms that exhibited the most significant fold change. This study has demonstrated the utility of measuring multiple biomarkers from a single platelet preparation to aid the diagnosis of late-onset AD. The combination of biochip array technology with a powerful biomarker algorithm is innovative, generating a reliable ante mortem AD test. It offers the potential to rapidly detect an AD-specific phenotype using routine blood-based clinical screening.

B-380

The Value of Serum Dipeptidyl Peptidase-IV in Early Detection of Mucopolysaccharidosis

I. Kurt[1], E. Sertoglu[2], I. Okur[3], S. Tapan[1], M. Uyanik[1], H. Kayadibi[4], F. Ezgu[3], H. I. Aydin[5], A. Hasanoglu[3]. *[1]Gulhane School of Medicine, Department of Clinical Biochemistry, Ankara, Turkey, [2]Ankara Mevki Military Hospital, Anittepe Dispensary, Biochemistry Laboratory, Ankara, Turkey, [3]Gazi University Faculty of Medicine, Department of Pediatric Metabolism and Nutrition, Ankara, Turkey, [4]Adana Military Hospital, Department of Biochemistry, Adana, Turkey, [5]Gulhane School of Medicine, Department of Pediatric Metabolism, Ankara, Turkey*

Background/Aim: The mucopolysaccharidosis (MPS) are a group of eleven lysosomal storage disorders (LSDs) that result from the absence or malfunctioning of enzymes required for the breakdown of glycosaminoglycans (GAGs). Early recognition of MPS allows for immediate treatment, before the onset of irreversible impairment, and, in many cases, prevention of catastrophic health outcomes, including death. We aimed to investigate the diagnostic utility of serum dipeptidyl peptidase-IV (DPP-IV) enzyme activity, urinary GAG/Cre ratio, total adenosine deaminase (ADA) and ADA-1 isoenzyme activity in the diagnosis of MPS.

Material and Method: 31 MPS patients which were previously diagnosed by clinical and enzymatic analysis were included in the study along with 31 healthy controls matched with patients for age and gender (8 (4 - 14) years, 61% male for patients and 8 (5 - 11) years, 58% for controls). Serum DPP-IV enzyme activity was measured according to Beesley et al and reported as nmol/min/mL of plasma. Serum total ADA and ADA-1 isoenzyme activity was measured according to Guisty G, Galanti B colorimetric method and reported as U/L. Urinary GAG concentration was measured by dimethylmethylene blue method, creatinine was measured by the modified Jaffe method and results were reported as mg GAGs/mmol creatinine. All of the statistical analyses were carried out by using the SPSS (V15) package for Windows.

Results: Serum DPP-IV enzyme activity, urinary GAG/Cre ratio, total ADA and ADA-1 isoenzyme activity were significantly higher in patients than in controls (p< 0.001, p< 0.001, p= 0.038 and p= 0.006, respectively). There were significant correlations between serum DPP-IV enzyme activity and urinary GAG/Cre ratios,

ADA-1 activity, ADA-1/total ADA (r= 0.498, p< 0.001; r= 0.348, p= 0.006; r= 0.270, p= 0.034, respectively). Area under ROC curve for DPP-IV enzyme activity was 0.988, p< 0.001 and for urinary GAG/Cre ratio was 0.986, p< 0.001. DPP-IV enzyme activity and urinary GAG/Cre ratio were the most significant parameters according to the univariate logistic regression analysis (p= 0.001 and p< 0.001, respectively).

Conclusion: According to our results, measurement of plasma DPP-IV activity can be used as a biomarker for MPS screening. However, no prominent diagnostic value emerged (so far) for the soluble plasma DPP-IV, although methods for plasma DPP-IV activity measurements are available since the 1980s. Although many cases are needed to decide more precisely, the results of this study indicate that the measurement of serum DPP-IV activity can be used as first-line screening test complementary to urinary GAG/Cre ratio for MPS screening.

B-381

Macrophage Inflammatory Protein (MIP-1α), an Early Biomarker of Chronic Kidney Disease

M. Summers, T. McFadden, E. Healy, C. Richardson, J. Lamont, R. McConnell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: Chronic kidney disease (CKD) describes abnormal kidney function and/or structure. It is frequently unrecognised and presents with progressive decline of renal function leading to end-stage renal failure and death. The Modification of Diet in Renal Disease (MDRD) classification of renal disease describes the progressive stages of the disease (stages 1-5) with respect to the estimated glomerular filtration rate (eGFR). The complexity in diagnosing a patient with CKD at an early stage of disease has led to most patients not receiving a diagnosis until the disease has progressed to an advanced stage. Currently there are no reliable methods for assessing early stage kidney disease. Inflammation plays a key role in the developmental progression of CKD and identification of inflammatory biomarkers in patients suspected of having CKD would aid early diagnosis. This study aimed to investigate the potential of MIP-1α as an early biomarker of CKD, which would aid the diagnosis of early stage CKD.

Methods: MIP-1α was assessed in serum samples from a total of 202 subjects: 152 CKD patients (51 Stage 1, 50 Stage 2 and 51 Stage 3) and 50 healthy controls. The analysis was performed with a biochip based immunoassay applied to the Evidence Investigator analyser. Statistical analysis was performed using MedCalc v12.5, all data represented as median [95% CI].

Results: Differences in concentration of MIP-1α across the disease groups and controls were initially assessed using the Kruskal-Wallis test and MIP-1α displayed significant difference between the diseased subjects and the healthy controls (significance determined as p<0.05). Post-hoc analysis was performed comparing CKD groups with controls using Mann-Whitney (with Bonferroni correction). This showed that MIP-1α displayed significantly higher median concentrations at all CKD stages (Stage 1-3) compared to control. (10.39 [9.0-13.77], 8.7 [7.29-11.24], 11.9 [9.89-14.23] pg/ml respectively; p<0.0001 for all) compared to control (5.36 [4.89-5.81] pg/ml). Receiver Operating Characteristic (ROC) curve analysis was conducted to assess diagnostic performance of diseased versus healthy subjects. Area under the curve was determined as 0.856(95% CI: 0.800-0.901).

Conclusion: The results indicate increased median concentration of MIP-1α in the serum of CKD patients (stages1-3) compared to controls. This study highlights the potential utility of MIP-1α in diagnosing early stage CKD. Early identification will enable effective clinical management and prevent progression to a later stage.

B-382

FIA-MS/MS multiplex enzyme assay for screening oligosaccharidoses

K. K. Nickander[1], S. E. Hofherr[2], M. J. Magera[1], D. Matern[1]. *[1]Mayo Clinic, Rochester, MN, [2]Children's National Medical Center, Washington, DC*

Background: Oligosaccharidoses are autosomal recessive lysosomal storage disorders of glycoprotein catabolism. Current clinical testing for oligosaccharidoses is performed by a thin layer chromatography (TLC) screen lacking sensitivity and specificity. A positive TLC screen is followed by individual fluorometric enzyme assays in leukocytes or fibroblasts, which although specific, address only one enzyme at a time. We addressed this widespread deficiency by developing a multiplexed enzyme assay to screen for several oligosaccharidoses, including α-mannosidosis, β-mannosidosis, Schindler disease, sialidosis, galactosialidosis, GM1 gangliosidosis, Morquio B disease, fucosidosis, and mucolipidoses II α/β, III α/β, and III γ, utilizing flow injection analysis and tandem mass spectrometry (FIA-MS/MS).

Methods: Leukocytes or fibroblast lysates (100 μL) prepared in 0.1 M citrate

phosphate buffer, pH 4.5, are incubated overnight with 3 different substrate mixes (10 µL) then extracted using solid phase extraction and FIA-MS/MS (API 3200; AB Sciex, Framingham, MA). Substrate mix 1 was prepared with 0.2 mM 4-methylumbelliferyl α-D-mannopyranoside, 0.8 mM 4-nitrophenyl β-D-mannopyranoside, and 0.8 mM 4-methylumbelliferyl N-acetyl-α-D-galactosaminide, substrate mix 2 was prepared with 0.4 mM 4-methylumbelliferyl-N-acetyl-α-D-neuraminic acid sodium salt hydrate and substrate mix 3 was prepared with 0.4 mM 4-methylumbelliferyl-β-D-galactopyranoside and 0.4 mM 4-methylumbelliferyl α-L-fucopyranoside. All mixes also included 0.2 mM umbelliferone as an internal control. Linearity was assessed by 1:2 dilutions of substrate starting at 4 times the normal starting concentration and processed with heat inactivated matrix. Reference intervals were determined from 157 leukocyte and 253 fibroblast specimens. Accuracy was determined from 15 known deficient specimens.

Results: Precision (inter-assay and intra-assay) performance was within acceptable limits ($\leq$20% CV). Method response for the individual substrates demonstrated acceptable linearity from 6.25-400% the normal starting concentration, with R^2 values ranging 0.982-0.999. Acceptable clinical utility was demonstrated by correct identification of 100% (N=15) of specimens with decreased or minimal residual enzyme activity.

Conclusions: The FIA-MS/MS method provides a reliable alternative to TLC, improving sensitivity and specificity without the need for follow-up assays with individual enzymes. Six different enzymes are assayed screening for 9 different oligosaccharidoses.

B-383

Assessment of Albuminuria using High Performance Liquid Chromatography in Diabetic and Nondiabetic Patients

R. Prusa, M. Fortova, E. Klapkova, K. Kotaska. *Faculty Hospital Motol, Prague 5, Czech Republic*

Background: Many studies have found higher values of urinary albumin reported using high performance liquid chromatography (HPLC) in comparison with immunochemical methods. Two opposite hypotheses explaining the difference between these methods are as follows: 1) the presence of immunounreactive albumin in urine that is not detected using immunochemical methods, 2) the presence of co-eluting proteins that falsely overestimate the results on HPLC. The aim of our study was the implementation of HPLC method for albuminuria, testing the hypothesis about co-eluting proteins, and comparison of albuminuria assessed using HPLC and immunoturbidimetric methods in diabetic and nondiabetic patient samples.

Methods: We developed the HPLC method for detection of albuminuria under these chromatographic conditions: multilinear gradient consisting of water (solvent A), acetonitrile (solvent B), 100 mM NaH_2PO_4 (solvent C), 1 % trifluoracetic acid (solvent D), flow rate 2 mL/min, temperature 22 °C, UV detection in the wavelength 280 nm, chromatographic column Zorbax 300 SB-C3, liquid chromatograph Agilent 1200 (Agilent Technologies, USA). We analyzed two mixtures of urine. The first one was prepared from 30 patient urine samples with immunoturbidimetrically physiological albuminuria, to which we added albumin standard. The second mixture was prepared from 30 patient urine samples with mild albuminuria. We compared albuminuria assessed using HPLC with the immunoturbidimetric method (automatic analyser Cobas Integra 400, Roche Diagnostics GmbH) in two patient groups: 636 diabetics [345 males and 291 females, mean age 50.1 years (1-97)] and 456 nondiabetics [250 males and 206 females, mean age 33.9 years (0.1-91)].

Results: Transferrin, α-1-acid glycoprotein, α-1-antitrypsin, α-1-antichymotrypsin and hemopexin do not interfere with albumin in HPLC method. The elution curve of prealbumin splits into several peaks, of which a few interfere with albumin. This interference has no clinical importance. In mixture 1 we did not find a significant difference between the albuminuria assessed using both methods (79 mg/L vs. 82 mg/L), while in mixture 2 we measured over 26 % higher albuminuria using HPLC (79 mg/L vs. 100 mg/L). These results suggest the existence of immunounreactive albumin. We found a statistically significant difference between the methods in both patient groups (14.6 ± 19.3 mg/L immunoturbidimetrically vs. 25.3 ± 21.1 mg/L HPLC in diabetics, 30.1 ± 23.6 mg/L immunoturbidimetrically vs. 41.2 ± 27.6 mg/L HPLC in nondiabetics, median ± standard error of the mean, p < 0.0001, Mann-Whitney test).

Conclusion: Our results prove that the HPLC method for albumin detection is more sensitive than immunoturbidimetry. We did not confirm nonspecificity of the HPLC method. We found statistically significant higher concentrations of urinary albumin using HPLC in both diabetic and nondiabetic patients.

Acknowledgment: Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague,Czech Republic).

B-384

Performance Evaluation of the AnshLite Myelin Basic Protein Chemiluminescent Immunoassay Using Cerebrospinal Fluid

J. Lu[1], D. G. Grenache[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT*

Background: Myelin is the insulating sheath surrounding neurons. Myelin basic protein (MBP) accounts for about one-third of total central nervous system (CNS) myelin protein. The cerebrospinal fluid (CSF) concentration of MBP increases in response to neuronal damage allowing the measurement of CSF MBP to be used clinically as a nonspecific marker of CNS inflammation. The objective of this study was to evaluate the performance characteristics of the AnshLite™ MBP chemiluminescent immunoassay (CLIA) for the quantitative determination of MBP in CSF.

Methods: MBP was measured using the AnshLite MBP CLIA (Ansh Labs, Webster, TX, USA). Performance characteristics including precision, linearity, analytical sensitivity, recovery, method comparison, MBP stability, the effects of freeze/thaw cycles, and the reference were evaluated using residual CSF specimens sent to ARUP Laboratories. The University of Utah's Institutional Review Board approved the project.

Results: Precision was determined using CSF pools with MBP concentrations of 3.51 and 1.54 ng/mL assayed in three replicates once each day for ten days. Within-run and total CVs were 9.6 and 14.8%, respectively. Linearity was determined by serially diluting a high-MBP CSF sample with the zero calibrator to create six samples each tested in two replicates. The assay was linear within the measuring range to 11.25 ng/mL (linear regression y=0.98(x)+0.05, R^2=1.00). The limit of blank (LOB) and limit of detection (LOD) were determined by testing the zero calibrator ten times and 0.45 ng/mL calibrator seven times. The LOB was 0.01 ng/mL calculated as the mean concentration added to 3 SD. The LOD was 0.08 ng/mL calculated as the LOB added to 3 SD. Accuracy was determined by recovery studies performed by adding volumes of one of two calibrators (6 and 14 ng/mL) to two CSF samples with MBP concentrations of 1.18 and 0.34 ng/mL. Calculated recovery ranged from 86 to 109%. A method comparison using 42 samples with an MBP concentration range of 0.30-58.57 ng/mL was performed using the Beckman MBP ELISA (Beckman Coulter, Inc., Brea, CA) as the comparator method. Deming regression yielded y=2.75(x)-2.68, R^2=0.95, Sx/y=4.78. MBP stability was determined by storing two CSF specimens with MBP concentrations of 6.77 and 1.49 ng/mL at room temperature for two days, 4°C for two weeks, and -20°C for three weeks. MBP decreased <12% relative to time zero under all conditions. The effects of up to three freeze/thaw cycles were evaluated by testing two CSF samples with MBP concentrations of 7.02 and 1.59 ng/mL after each freeze/thaw cycle. The MBP concentration changed by -12.6, +6.94, and -18.0% relative to time zero after each cycle, respectively. The reference interval was established using 130 CSF specimens that were negative for oligoclonal bands. Using non-parametric analysis, this was determined to be 0.0 to 5.5 ng/mL.

Conclusions: The AnshLite™ MBP CLIA demonstrates acceptable performance characteristics for quantifying MBP in CSF. MBP is stable for two days at room temperature, two weeks at 4°C and three weeks at -20°C. Freeze/thaw cycles of CSF samples for MBP testing should be avoided.

B-385

Rationalising not rationing: the case of serum protein electrophoresis (SPE)

R. M. Dorizzi, V. Zanardi, P. De Vita, R. Agnoletti, A. Alberelli, S. Babini. *Corelab, Laboratorio AVR, Pievesestina di Cesena, Italy*

Background Serum protein electrophoresis (SPE) is highly requested in Italy. The AVR laboratory, which serves 1,125,000 inhabitants, runs about 300,000 tests/year while e.g. in Kent 10,557 SPEs are requested in the same period for a catchment area of 759,000 inhabitants. Since research/monitoring of monoclonal components is the only appropriate indication for this test, the AVR Laboratory carried since 2010 several actions to increase the appropriateness of the requests: adoption of automated alerts in the CPOE, benchmarking of the requesting modes of GPs, frequent meetings with GPs, changes in the report format. Since October 1st 2013 the report contains the various fractions only when a monoclonal component (MC) is present; in the other cases only the comment "Monoclonal components are not noticeable" is reported. In January 2014 we carried out the first audit of the effect of this action in the fourth trimester after its implementation.

Materials and methods We extracted the results of the SPEs carried in the fourth quarter of the years 2009-2013 stored in the LIS (Noemalife, Bologna, Italy). All the SPEs were performed using Capillarys (Sebia, Marcy l'Etoile, France). We assessed

the tests carried out in inpatients and outpatients and the percentage of detected MCs.

Results The results summarized in the table shows a steady decline of the demand for SPE since 2009 and a constant increase in the percentage of detected MCs consistent with a progressive increase of the appropriateness of the request. This trend is more evident in inpatients and it could be suggested that a MC is more frequently detected in patients with symptoms requiring hospitalization and undergoing more accurate investigation.

Conclusions 1) The laboratory can and should promote the appropriateness of laboratory tests; 2) appropriateness can improve in relatively a short time; 3) effectiveness of the actions can be measured with simple tools.

OUTPATIENTS			
Trimester 4	Tot. SPE	Detected MCs	MCs %
2009	63,474	3,333	5.3
2010	62,076	3,608	5.8
2011	54,182	3,602	6.6
2012	53,296	3,718	7.0
2013	45,736	3,542	7.7
INPATIENTS			
Trimester 4	Tot. SPE	Detected MCs	MCs %
2009	16,924	1,612	9.5
2010	14,123	1,365	9.7
2011	12,760	1,232	9.7
2012	10,356	1,055	10.2
2013	8,606	1,091	12.7

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Performance Evaluation of a Polyclonal Based Fecal Alpha-1-Antitrypsin ELISA

J. A. Erickson[1], R. A. Jensen[2], D. G. Grenache[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT, [2]Parasitology and Fecal Testing Technical Section, ARUP Laboratories, Salt Lake City, UT, [3]University of Utah School of Medicine, Department of Pathology, Salt Lake City, UT*

Background: Alpha-1-antitrypsin (A1A) is a serum protease inhibitor that inhibits a wide range of proteases. A1A resists degradation by digestive enzymes and therefore, can be used to detect the presence of serum proteins in the gastrointestinal tract. The primary causes of protein-losing enteropathy can be divided into erosive gastrointestinal disorders, non-erosive gastrointestinal disorders, and disorders involving increased central venous pressure or mesenteric lymphatic obstruction. The diagnosis of protein-losing enteropathy is most commonly based on the determination of fecal A1A clearance. The purpose of this study was to assess the performance characteristics of a polyclonal antibody based A1A ELISA for the measurement of A1A in stool and to determine reference limits for A1A in stool and A1A clearance.

Methods: Stool samples included deidentified residual random stool specimens sent to ARUP Laboratories for routine testing, and timed stool specimens and paired serum obtained from healthy volunteers. A1A was extracted from stool and measured using the ImmuChrom Human A1A ELISA (ImmuChrom, GmbH, Heppenheim, Germany) according to the manufacturer's instructions. Serum A1A was measured using the Tina-quant® A1A assay (ver.2) on a cobas® 8000 modular analyzer (Roche Diagnostics, Indianapolis, IN). The performance characteristics evaluated for the stool ELISA were analytical sensitivity, linearity, accuracy, precision, and A1A stability in stool. The University of Utah's Internal Review Board approved the project.

Results: The analytical sensitivity was 0.14 ng/mL (0.002 mg/g stool) determined from 23 replicates of the sample diluent (mean + 3SD). Linearity was evaluated using diluted stool extracts with elevated A1A concentrations (range, 1.5 - 90.0 ng/mL). Linear regression produced results of $y = 1.02x - 3.15$ ($r^2 = 0.986$). Accuracy was determined from analyte recovery studies by adding sera of known A1A concentration into previously measured stool extracts. Recovery (measured ng A1A / expected ng A1A) ranged from 95.2 to 118.4%. Precision was determined from ten stool extracts obtained from each of two random stool specimens, with one extract analyzed in five

replicates each day for ten days. Repeatability and within-laboratory CVs were 5.4 and 6.5% at 17.3 ng/mL (0.21 mg/g) and 13.8 and 14.5% at 66.9 ng/mL (0.84 mg/g), respectively. A1A was stable in stool for a minimum of 2 days, 7 days and 3 months at room temperature, 4 - 8 °C and -20 °C, respectively. A1A measured in timed stool obtained from 45 healthy volunteers (21 males, 24 females, ages 21 - 61) ranged from <0.002 to 0.59 mg/g stool. Using a robust skewed method, the reference limits for A1A in stool and A1A clearance were ≤0.47 mg/g and ≤45 mL/day, respectively.

Conclusions: The ImmuChrom Human A1A ELISA demonstrates acceptable performance for quantifying A1A in stool. The assay can also be used in conjunction with the Roche Diagnostics Tina-quant A1A (ver.2) assay for assessing A1A clearance.

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Lipolysis Suppresses Insulin Signaling and Glucose Uptake

G. R. Mullins, L. Wang, T. E. Harris. *University of Virginia, Charlottesville, VA*

Background: Acute hyperglycemia often develops after trauma or major surgery, particularly surgery within the abdominal cavity such as coronary bypass procedures. This condition, termed stress-induced hyperglycemia, contributes to mortality and delays healing in post-surgery and ICU patients and is a result of systemic insulin resistance (IR) caused by the adaptive stress response. Although intensive insulin treatment reduces hyperglycemia and mortality in some patients, insulin therapy after surgery can result in hypoglycemic incidents, emphasizing the need to develop new treatment strategies. Despite its clinical significance, the pathophysiology of post-surgery acute IR is largely unknown. Understanding the contributing molecular mechanisms will provide new insights in developing therapeutic approaches to improve patient outcomes after major surgery. It is known that β-adrenergic stimulation by catecholamines will inhibit insulin stimulated glucose uptake in adipose tissue. This catabolic signaling leads to the release of stored fat through lipolysis, which may play a role in the observed stress-induced IR and hyperglycemia. Our objective was to investigate a possible mechanistic link between lipolysis and catecholamine-induced IR in adipose tissue, which would likely contribute to stress-induced IR after surgery.

Methods/Results: We have used biochemical assays to investigate the effect of catecholamine-induced lipolysis on insulin signaling and glucose uptake in adipocytes. Activation of the cyclic AMP/Protein Kinase A pathway acutely inhibits insulin signaling by disrupting mammalian Target of Rapamycin (mTOR) complexes 1 and 2 in adipocytes. Activation of lipolysis is required for this PKA-induced inhibition of mTOR. The effect of lipolysis is also transferrable in vitro, suggesting a lipid product of lipolysis may mediate mTOR complex dissociation. In addition, the signaling lipid, referred to as the mTOR Dissociative Factor (mDF) is downstream of Adipose Triglyceride Lipase (ATGL) and depleted by Diacylglycerol Lipase (DAGL), suggesting it is likely a diacylglycerol released during lipolysis. Here we summarize our progress in our effort to isolate and identify the mDF through reverse-phase high-performance liquid chromatography (RP-HPLC). We show that activation of the PKA pathway inhibits mTOR activity and adipocyte glucose uptake by 40-50%, as measured by phosphor-specific immunoblots and radiometric glucose uptake assays, respectively. Although significant, this inhibition is not seen when lipolysis is genetically or pharmacologically blocked. This suggests that inhibition of mTOR by lipolysis is a potential mechanism of dampened glucose uptake during catecholaminergic signaling and provides new insight into stress-induced hyperglycemia.

Conclusion: This study demonstrates a novel intracellular signaling mechanism where activation of lipolysis controls the PI3K-Akt-mTOR pathway to inhibit insulin signaling. Understanding this mechanism provides new insight into insulin resistance that is concurrent with elevated lipolysis found during obesity or after major surgery. Understanding the molecular mechanism of stress-induced insulin resistance will lay the groundwork for future therapeutic treatments and improve patient outcomes after surgery. In addition, identification of the mDF may provide a new biomarker for predicting the risk of insulin resistance and hyperglycemia.