Wednesday, August 3, 2016

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

Proteins/Enzymes

B-283

Validation of a Serum S-100B ELISA for Measuring S-100B in Cerebrospinal Fluid

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Background: S-100B is a calcium binding protein with a molecular weight of approximately 22 kD. The protein has a homodimeric ß,ß-chain structure and is found in astroglial and Schwann cells. S-100B is highly specific for tissue of the nervous system and also for malignant melanoma cells. As with other S-100 proteins, it is suggested that S-100B serves as a calcium sensor regulating the function and subcellular dissemination of specific target proteins. Various studies have reported cerebrospinal fluid (CSF) S-100B concentrations to be valuable prognostic markers for patients suffering traumatic brain injury and may have diagnostic potential for Creutzfeldt-Jakob disease. The purpose of this study was to assess the performance characteristics and validate the CanAg® S100 EIA (Fujirebio Diagnostics, Inc., Gothenburg, Sweden), an ELISA intended for and previously validated for serum, for use in quantifying S-100B in CSF.

Methods: Residual CSF specimens sent to ARUP Laboratories were used for this study. S-100B was measured according to the test kit manufacturer's protocol. The performance characteristics evaluated were analytical sensitivity, linearity, recovery, precision and S-100B stability. CSF specimens used for the reference limit study were selected based on results that were within the reference limits for all the following tests: albumin, IgG, IgG albumin ratio, IgG index, albumin index, CSF IgG synthesis rate, and oligoclonal bands. The University of Utah's Institutional Review Board approved this study.

Results: The parametric limit of blank was 11 ng/L (zero calibrator, 20 replicates). Dilution of an elevated S-100B CSF specimen with the zero calibrator to 11 samples with different concentrations, and each measured in triplicate, produced linear regression results of $y = 0.999x + 1.34$, $r^2 = 0.996$. Adding aliquots of a serum specimen with a high S-100B concentration to five different CSF specimens (maximum ratio 1:10) generated a mean recovery of 92% (84 - 103%). Precision was determined from two CSF pools tested over 20 days in triplicate. Repeatability and within-laboratory CVs were 3.5 and 4.0% at 140 ng/L, and 2.3 and 3.1% at 1177 ng/L, respectively. S-100B was stable in CSF for 4 hours at room temperature, and minimums of 7 days and 4 weeks at 4 - 8 °C and -20 °C, respectively. The analyte was also stable over a minimum of three freeze/thaw cycles. S-100B measured from 141 CSF specimens (69 males, 72 females, ages 3 - 77 years) generated an upper reference limit of 692 ng/L (nonparametric analysis, 95th percentile). A slight increase was observed with age but was considered clinically insignificant because of the large confidence interval of the slope (slope:1.963 ± 0.957; 95% CI, 0.08744 - 3.838). No significant difference between genders was observed (p = 0.542).

Conclusions: The CanAg S100 EIA demonstrates acceptable performance for quantifying S-100B in CSF. A CSF reference interval has been established for the ELISA which potentially may be of value in assessing and/or monitoring traumatic brain conditions.

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Evaluation of Performance of the New VITROS® LDHI (IFCC) Slide Assay (In development)

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VITROS Chemistry Products LDHI Slides quantitatively measure lactate dehydrogenase (LDH) activity in serum and plasma using VITROS 250/350/5,1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System. Lactate dehydrogenase is an enzyme with a widespread tissue distribution and increased levels reflect tissue damage. Causes of elevated LDH include neoplastic states

(i.e. leukemia, lymphoma, solid tumors), anemia (i.e. megaloblastic, hemolytic), inflammatory and infectious states, disorders of the lung and muscle, renal and myocardial infarctions, hepatic disease, trauma and shock[1-4]. The VITROS LDHI Slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Lactate dehydrogenase catalyzes the conversion of pyruvate and NADH to lactate and NAD+. The oxidation of NADH, which is monitored by reflectance spectrophotometry, is used to measure lactate dehydrogenase activity. LDHI measurements using the slide pyruvate to lactate reaction are traceable to results from the *IFCC primary reference procedure for LDH* via commutable calibrators. We evaluated the accuracy of 90 patient serum samples (90 - 851 U/L) on the VITROS 350 System compared to the IFCC comparative method. The VITROS LDHI Slides assay showed excellent correlation with the IFCC method. VITROS 350 System = 1.01 * IFCC - 7.5; (r) = 0.997. A 10-day precision study conducted on four VITROS Systems showed excellent precision. Mean LDHI concentrations of 195 U/L and 627 U/L resulted in within-laboratory percent coefficient of variation (%CV) of 3.5% and 1.3% respectively on the VITROS 350 Chemistry System, 3.0% and 1.5% on the VITROS 5,1 FS Chemistry System, 3.1% and 1.5% on the VITROS 4600 Chemistry System, and 3.2% and 2.0% on the VITROS 5600 Integrated System. The Limit of Blank (LoB) is 27.6 U/L based on 72 determinations with 4 blank samples. The VITROS LDHI Slides assay has exhibited good correlation with serum samples across a broad measuring range compared to the IFCC comparative method. In addition excellent precision and good low end sensitivity has been observed on the VITROS 350, VITROS 5,1 FS, VITROS 4600, and VITROS 5600 Systems. 1. Tietz Fundamentals of Clinical Chemistry, Seventh Edition, Saunders, 2015 2. Quist J, Hill AR. Serum lactate dehydrogenase (LDH) in *Pneumocystis carinii* pneumonia, tuberculosis, and bacterial pneumonia. Chest. 1995;108:415-418. 3. Winzelberg GG, Hull JD, Agar JW, Rose BD, Pletka PG. Elevation of serum lactate dehydrogenase levels in renal infarction. JAMA 1979; 242: 268-269. 4. Zein JG et al., Prognostic Significance of Elevated Serum Lactate Dehydrogenase (LDH) in Patients with Severe Sepsis; *Chest.* 2004;126(4_MeetingAbstracts):873S

B-285

An Acute Phase Response Score from Serum Protein Capillary Electrophoresis Analysis

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Many types of tissue injury, from many sources, provoke an acute phase response (APR), also called a systemic inflammatory response. Common APR cellular and molecular markers are the white blood cell count, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). All APR markers reflect an injury, but their responses may differ greatly from each other in intensity and time course, and may also vary according to the source of injury. Due to marker variability, more than one is often used to test for injury. Marker results may also be combined into a score for a more reliable diagnosis or prognosis. Most of the proteins that form the peaks of a serum electrophoresis (SELP) have been used as APR markers. Increases in alpha-1-antitrypsin (AAT), in the alpha-2 proteins, haptoglobin and alpha-2-macroglobulin, and in C3 and C4 complement, which form the beta-2 peak; and decreases in prealbumin (PA) and in transferrin (TRF), the beta-1 peak, have served as APR markers. OBJECTIVES: (1) To determine whether the total protein of an SELP zone, or the peak protein, is a better APR measure; and (2) to create a score from the peak or zone protein values that is a superior APR measure. METHOD: CRP and ESR are common measures of the APR. A CRP or ESR test, or both, were ordered on 200 recent SELP tests. For each SELP the protein in the PA, alpha-1, alpha-2, beta-1, and beta-2 zones, and in the peaks of these zones, was calculated from the total protein and the SELP curve data from a CapillaryS(tm) capillary electrophoresis analyzer. Subtraction of the peak protein from its zone protein yielded the zone's non-peak protein. These three protein measures from each zone were correlated with the corresponding ESRs and CRPs. The peak and zone proteins from each SELP were also combined in various ways to create a score which was correlated with the CRP and ESR and analyzed by multilinear regression for the contribution of its components. SELP curve analysis was by our Python programs and statistical analysis was via "R". RESULTS: In every case the peak proteins produced far better correlation coefficients than the non-peak zone proteins. Only the alpha-1 zone non-peak protein produced a good, but smaller, correlation coefficient. The alpha-1 zone is known to contain other APR proteins. Several linear combinations of the five peak proteins were formed into APR scores. The simple sum of the peak z-values (the number of standard deviations of a peak from the peak mean), with negation of PA and TRF z-values, was one of the best scores, correlating much better with the ESR and somewhat better with the CRP than the individual peak values. For correlation with CRP, the alpha-1 peak protein made a

dominant contribution. For correlation with ESR, the alpha-2 and beta-1 peaks made major contributions and alpha-1, beta-2, and PA peaks made smaller contributions. Combination SELP scores can be excellent APR metrics.

B-286

Clinical Validation and Identification of Non-Protein Solute Interferences for Serum Total Protein Measurement by Digital Refractometry

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OBJECTIVES:

Clinical refractometers are exempt from FDA premarket notification as Class 1 devices. As such, there are few comprehensive clinical validations of refractometers in the literature or available from manufacturers. A subset of laboratories (based on proficiency testing self-reporting) still use refractometry as their method for serum total protein (TP) measurement in conjunction with protein electrophoresis. The primary objective of this study was to conduct a clinical validation of a refractometer for serum TP measurement. A digital refractometer was chosen to eliminate subjectivity involved in manual refractometry methods. A secondary objective was to identify and characterize potential interference of non-protein dissolved solutes on TP measurement.

METHODS:

Experiments were designed in adherence to Clinical and Laboratory Standards Institute® (CLSI)-based protocols. A preliminary evaluation of the Palm Abbe™ model PA202X (MISCO) refractometer was conducted through device familiarization and carryover studies. Precision was measured over 20 days with 2 runs daily at 2 concentrations of quality control (QC) materials (Bio-Rad Liquid Unassayed Multiqual®). Accuracy was assessed by testing TP in 50 clinical serum specimens over 5 days on the Palm Abbe digital refractometer, a manual refractometer, and the cobas c502 chemistry analyzer (Total Protein Gen. 2, Roche Diagnostics). Linearity was evaluated using dilutions of high TP sample pools with either low TP sample pools or ddH_2O. An interference screen was conducted using low concentration (TP=6 g/dL) and high concentration (TP=8 g/dL) analyte pools, spiked with hemoglobin, bilirubin, glucose, sodium chloride, or Intralipid®. Characterization of interference was then evaluated through serial dilutions when required. Reference interval (RI) verification studies were conducted using 20 male and 20 female serum samples from healthy donors.

RESULTS:

Carryover of 8.5% was lowered to acceptable levels (<2%) when a ddH_2O wash step was included between specimen measurements. Precision studies demonstrated overall repeatability of 0.8% CV (low QC, TP=2.4 g/dL) and 0.3% CV (high QC, TP=10.6 g/dL). Accuracy studies demonstrated a correlation to both manual refractometry (Deming regression, y=1.1148x-1.039, r^2=0.97) and the Roche TP assay (Deming regression, y=1.008x+0.356, r^2=0.93), although a negative bias was noted at lower concentrations of TP (versus manual refractometry) and an overall positive bias (+5.8%) was observed versus the Roche chemistry assay. Linearity was verified using a) low and high concentration patient pools (TP range tested, 4.4-10.8 g/dL; slope 1.009), and b) ddH_2O and high concentration patient pools (TP range tested, 1.7-13.9 g/dL; slope 0.950). A trend toward decreased recovery was observed when using ddH_2O as a diluent at lower TP concentrations. Interference (>3.63%) was observed at high concentrations of glucose (>267 mg/dL) and triglycerides (>580 mg/dL). Our current laboratory adult male and female RIs for TP (6.3-8.2 g/dL) were verified on the Palm Abbe refractometer.

CONCLUSIONS:

The performance characteristics of the Palm Abbe digital refractometer using a human TP scale were validated in a clinical laboratory setting. TP results were in general comparable to those obtained using manual refractometry and an automated chemistry analyzer. Refractometer results for TP measurement, however, may be falsely elevated in the presence of high concentrations of non-protein solutes such as glucose and triglycerides.

B-287

Study of reference materials suitable for the IFCC method of ALP measurement

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Background: Reference procedures for the measurement of catalytic activity concentrations of alkaline phosphatase (ALP) were recommended in 2011 by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The reaction principle of the IFCC procedure is based on the use of 2-amino-2-methyl-1-propanol (AMP) and 4-nitrophenyl phosphate (4-NPP) . This procedure will be used as a reference measurement system for the certification of primary reference materials. However, there is no current certified reference material for ALP by this method.

In the IFCC procedure for ALP presented in 1983, AMP concentration in the reaction mixture was 350 mmol/L. In the current primary reference method, the AMP concentration increased to 750 mmol/L for pH stability and improvement. Currently, three types of commercial kits of AMP are available; these are at final concentrations of 350, 750, and 900 mmol/L.

Objective: Primary reference materials must be essential for traceable data for the primary reference IFCC methods. Therefore, we examined raw materials suitable for this purpose.

Methods: Patient serum, liver, bone, small intestine, and placenta-derived ALP isozyme (Joko Ltd. Japan) were used as samples. Freeze-dried control material was prepared using human liver type recombinant ALP (Asahi Kasei Pharma Ltd. Japan). Reagents were prepared using the IFCC procedure and various AMP concentrations (125-1250 mmol/L). Three types of commercial kits with different AMP concentrations were also prepared. Using these reagents, ALP activity of above-mentioned samples were measured, and each isozyme sample reactivity was compared with that of the patient serum.

Results: For placenta-derived ALP, a significant inhibition was confirmed at AMP concentration >250 mmol/L. The liver type ALP demonstrated similar reactivity to human serum and to recombinant ALP. The reactivity of the placental type differed from patient serum in the measurement using the commercial kits.

Conclusion: Previously, BCR371 (porcine kidney origin), from the Institute for Reference Materials and Methods (IRMM), and SRM 909b, from the National Institute of Standards and Technology (NIST), were used as reference materials of ALP. However, these are no longer available. For the past several years, new reference materials have been investigated by the IRMM and IFCC. The placental type ALP is not suitable as a reference material because it causes inhibition by AMP. The IFCC has recommended that the focus is on the measurement of liver type ALP, which is abundantly present in human serum.

The control material of recombinant ALP had similar properties to the human liver type ALP and would be precisely applied to a reference material for the primary reference method of ALP activity measurement.

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Paraoxonase 1 status (Arylesterase, Homocysteine Thiolactonase activities and Q192R polymorphism) : contributors of Diabetic Retinopathy.

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BACKGROUND: The high density lipoprotein (HDL) associated enzyme paraoxonase-1 (PON1), antioxidant and anti-inflammatory, destroys the proinflammatory molecules involved in the initiation and progression of microvascular complications in diabetes. OBJECTIVE: To determine the role of Serum Paraoxonase 1 (PON 1) status i.e - Arylesterase and Homocysteine thiolactonase activities, Paraoxonase 1 Q192R polymorphism, Monocytic Paraoxonase 2 (PON 2) Lactonase activity, and Serum Advanced Oxidation Protein Products (AOPP) levels in cases of diabetic retinopathy. MATERIAL AND METHODS: The study group consists of 40 type 2 DM patients with retinopathy, having diabetes more than 5 years, and 40 healthy individuals selected randomly having no retinopathy aged 30-65 years. Exclusion criteria included smoking, alcohol abuse, post menopausal women,

ischemic heart disease, liver or kidney disease, patients on lipid lowering drugs and having diabetes less than 5 years. Serum PON 1 Arylesterase activity was measured by using phenylacetate as substrate and PON1 polymorphism was done by using double substrate method (phenylacetate and p-nitro phenyl acetate). Serum PON1 Homocysteine Thiolactonase activity was measured using a modification of the Ellman cholinesterase method and Monocytic PON 2 lactonase activity by using DHC (Dihydrocoumarin) as substrate. Serum Advanced oxidation protein products (AOPP) was done by Witko-Sarsat et al method. RESULT: Statistical data was analyzed with SPSS version 21. Normality of distribution was checked with Shapiro-Wilk test. Logistic regression was done to determine the contribution of various parameters towards presence or absence of disease (diabetic retinopathy). Results are presented as Mean±SD. PON1 Arylesterase (130.568 ± 45.52 vs 169.495 ± 41.06 kU/L), PON1 Homocysteine thiolactonase activities (0.029 ± 0.013 vs 0.038 ± 0.0124 mmol/L) and PON 2 lactonase activity (2.791 ± 1.006 vs 5.249 ± 2.259 U/mg protein) were significantly decreased in cases than controls. AOPP levels were significantly increased in cases than controls (42.717 ± 8.953 vs 32.206 ± 5.865 µM/L of Chloramine-T Equivalents). Significant association was seen of PON1 Arylesterase and PON2 lactonase activities with AOPP levels (negative correlation) and PON1 homocysteine thiolactonase activity (positive correlation). Logistic regression studies, along with known risk factors shows significant independent contribution towards the presence of diabetic retinopathy with PON1 Arylesterase activity (Nagelkerke's $R^2 = 0.905$, p<0.001) and PON1 homocysteine thiolactonase activity showed contribution towards presence of diabetic retinopathy (Nagelkerke's $R^2 = 0.853$, p<0.001). Significant difference was found for phenotypic distribution by chi-square test. (p value = 0.006). PON1 RR phenotype is at increased risk of the disease. CONCLUSION: The findings of the study demonstrate that the estimation of PON1 arylesterase and homocysteine thiolactonase activities provides more valuable information for prediction of risk of diabetic retinopathy apart from known risk factors and can be used in diagnosis and management of diabetic retinopathy. The observed frequency of R allele is more in cases than in controls, showing that RR phenotype is at increased risk of the disease and QQ phenotype is protective suggesting that more definitive treatment is required in diabetic patients with PON1 RR phenotype or an individual with PON1 RR phenotype with diabetes is more prone for diabetic complications.

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Prediction of Pressure Ulcer Based on the Protein Fraction Waveform Data

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Background: The development of pressure ulcer is influenced by the patient's nutrition status and nursing care. However, the Braden scale, generally used for NST (Nutrition Support Team) activities, is limited by its lack of objectivity.

Objective: The aim of this study was to establish the indices for early detection and intervention of bedsore development by using protein fraction waveform data.

Method: Raw electrophoresis waveforms obtained by Sebia CAPILLARYS2 PROTEIN(E)6 were normalized as previously described. Positive group consisted of 64 patients with bedsore development and a negative group consisted with 7642 patients without pressure ulcer during hospitalization. Waveform data one week before the development of pressure ulcer were used in the positive group and wave form data at admission was used for the negative group. Raw waveform data were normalized without eliminating the information between the ALB and α1 fractions. Mobility data was corrected by positioning the peak at 75, and DMF at 300 and β1 peak position at 203. ROC analysis was conducted at each 300 point of the normalized mobility. Logistic regression analysis was performed using these data as explanatory variables and a prediction formula was obtained to detect bedsore risks.

Results: The tail position of the albumin in the β1 fraction (mobility 200) dropped, which was strongly correlated with Cu and inflammatory proteins (AUC = 0.814). The tail position of the prealbumin (mobility 69) strongly correlated with C4 (AUC = 0.789). The tail position of on the α2 albumin (mobility 164) negatively correlated with Zn (AUC = 0.753). The logistic regression formula was found to be -4.471+0.0251 * age+0.0526 * P114+0.00244 * P163-0.0177 * P199+0.00233 * P216 (AUC = 0.876).

Conclusion: A high diagnostic characteristic to detect bedsore by the protein fraction pattern existed at the mobility 200, 69 and 164. By combining these results with that of logistic regression analysis, had a high performance to predict the development of pressure ulcer.

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Evaluation of the Kappa Freelite and Lambda Freelite assays on The Binding Site Optilite turbidimetric analyser using urine samples

D. G. McEntee, J. Chen, B. A. Johnson-Brett, L. Southan, D. J. Matters, P. J. Showell, S. J. Harding. *The Binding Site, Birmingham, United Kingdom*

Elevated levels of free light chains in urine maybe indicative of kidney disease or a malignant lymphoproliferative condition such as malignant melanoma, lymphocytic neoplasms, Waldrenströms macroglobulinemia, AL amyloidosis or light chain deposition disease. In a healthy kidney, the tubular cells selectively absorb free light chains so very low levels are found in urine of a healthy individual. There are differing glomerular filtration rates for free kappa (κ-FLC) and free lambda (λ-FLC) light chains and hence differing measuring ranges and reference intervals. Samples of unknown antigen concentration are assayed on the Optilite and the results are read from a calibration curve. The κ-FLC assay has a measuring range of 2.9 - 127mg/L at the standard 1/10 sample dilution (reference interval 1.35 -24.19mg/L). The λ-FLC assay has a measuring range of 5.2-139mg/L at the standard 1/8 sample dilution (reference interval 0.24 - 6.66mg/L). High κ-FLC samples are automatically re-measured with an upper measuring limit of 127000mg/L and high λ-FLC samples are automatically re-measured with an upper measuring limit of 139000mg/L. Low samples are measured at 1/2, with sensitivities of 0.58mg/L and 1.30mg/L respectively. Correlation to the Binding Site Freelite assays for the Siemens BN™II was performed for κ-FLC using 228 urine samples including 20 from normal subjects (Range <0.31 - 207000mg/L). Using a medical decision point of the top end of the normal range, this gave 2x2 table agreement of 100% positive, 87.91% negative and 95.22% overall. For lambda, the comparison comprised 146 urine samples including 23 from normal subjects (Range 1.25 - 47600mg/L), this gave 2x2 table agreement of 100% positive, 96.7% negative and 97.95% overall. Precision was assessed using a method based on CLSI (EP05-A2), measuring samples at two concentrations for κ-FLC and λ-FLC on a single analyser over 5 days. Precision results produced total precision CVs of 8.6% (8.2mg/L κ-FLC), 6.5% (54.0mg/L κ-FLC), 9.8% (8.9mg/L λ-FLC) and 5.7% (43.1mg/L λ-FLC). Interference was tested by running interferents; ascorbic acid (342umol/L) and urobilirubin (45mg/L) and negative controls in urine samples. Urobilirubin interference was 4.13% at 9.08mg/L κ-FLC, 2.61% at 100.0mg/L κ-FLC, 3.85% at 10.6mg/L λ-FLC and 4.95% at 134.5mg/L λ-FLC. Ascorbic acid interference was -2.82% at 4.38mg/L κ-FLC, -5.78% at 85.2mg/L κ-FLC, -4.49% at 10.3mg/L λ-FLC and -3.1% at 102.7mg/L λ-FLC. We conclude that the Kappa Freelite and Lambda Freelite assays for the Optilite provide a reliable, accurate and precise method for quantifying free light chains in urine and would be useful in identifying patients with kidney disease or a malignant lymphoproliferative condition.

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Development of a Multi-Analyte Biochip Array for the Early Diagnosis of Ischaemic Stroke

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Background: Stroke is the second leading cause of death globally. Following acute ischaemic stroke, thrombolytic therapy can be administered and early administration (within 3 hours of symptoms onset) can help limit stroke damage and disability. This therapy is not applicable to patients with haemorrhagic stroke. There is an unmet clinical need for the development of rapid assays to complement existing medical examination for the early diagnosis of ischaemic stroke and to discriminate between ischaemic and haemorrhagic stroke. Studies reported Glutathione S-Transferase-Pi (GST-Pi), Nucleoside Diphosphate Kinase A (NDKA), Parkinson Protein 7 (PARK7) as plasma markers for early diagnosis of ischaemic stroke; Glial Fibrillary Acidic Protein (GFAP) was found to be a biomarker enabling differentiation between ischemic and haemorrhagic stroke.

This investigative study aimed to develop a biochip array for the simultaneous determination of these four biomarkers from a single sample to facilitate the identification of ischaemic stroke patients that qualify for thrombolytic treatment within the first hours post-symptoms onset.

Methods: Simultaneous chemiluminescent sandwich immunoassays were developed on the Evidence Investigator analyser, the capture antibodies being immobilised on the biochip surface at discrete test sites. Serum samples from stroke patients on admission (within 6 hours of onset of neurological symptoms) and day 4 (ischaemic

n=26, haemorrhagic n=6) and healthy control samples (n=9) were assessed. The Mann-Whitney t-Test was applied to determine statistical significance (p<0.05) of the results.

Results: All assays presented clinically relevant assay ranges with sensitivities <1.0 ng/ml and within-run precision CV(%)<10. Results from ischaemic stroke patients revealed that median concentration levels on admission were significantly increased for GST-Pi (12.93 ng/ml; p=0.0005), NDKA (102.79 ng/ml; p<0.0001), PARK7 (10.75 ng/ml; p=0.0004) and GFAP (0.16 ng/ml; p<0.0001) when compared to control levels [GST-Pi: 1.17 ng/ml, NDKA: 13.77 ng/ml, PARK7: 1.9 ng/ml and GFAP: 0.00 ng/ml]. Four days after admission, levels decreased for GST-Pi (5.58 ng/ml; p=0.0293), NDKA (75.06 ng/ml; p=ns) and PARK7 (5.80 ng/ml; p=ns), whereas GFAP levels continued to increase (0.65 ng/ml; p=ns). Results from haemorrhagic stroke samples revealed that on admission, GFAP levels were significantly elevated (4.06 ng/ml; p=0.0008) when compared to controls, the GST-Pi levels were also elevated (3.94 ng/ml; p=ns), however the difference from control values was not significant. Levels of GFAP (14.31 ng/ml; p=ns) and GST-Pi (20.9 ng/ml; p=ns) were increased at day 4 when compared to levels on admission but the difference was not significant. As expected, on admission serum levels of GFAP were significantly higher in haemorrhagic (4.06 ng/ml; p=0.0004) than in ischaemic stroke patients (0.16 ng/ml).

Conclusion: The developed biochip array allowed simultaneous determination of GST-Pi, NDKA, PARK7 and GFAP from a single serum sample. These biomarkers were significantly elevated in serum samples from ischaemic stroke patients when compared to controls. Furthermore, serum levels of GFAP on admission were significantly higher in haemorrhagic than in ischaemic stroke patients and controls. The results suggest that this array will complement existing imaging techniques in the identification of ischaemic stroke patients that qualify for thrombolytic treatment within the first vital hours post symptoms onset.

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Evaluation of the IgA CSF assay for use on the Binding Site Optilite turbidimetric analyser

D. G. McEntee, J. Chen, D. J. Matters, P. J. Showell, S. Kausar, M. Solanki, S. J. Harding. *The Binding Site, Birmingham, United Kingdom*

The measurement of IgA in cerebrospinal fluid (CSF) and paired CSF and serum samples aids the assessment of the body's ability to resist infectious disease in conjunction with other clinical and laboratory findings. An increased CSF protein level can be indicative of barrier dysfunction and/or intrathecal synthesis of immunoglobulin (Ig) within the central nervous system (CNS). More specifically, an increase in intrathecal IgA in CSF can be indicative of bacterial infections such as tuberculous meningitis. Calculation of CSF/serum ratios and comparison of the Ig ratios to the albumin CSF/serum value can differentiate between the serum-derived Ig and intrathecal Ig synthesis. Here we describe the evaluation of the IgA CSF assay for use on The Binding Site Optilite® analyser. The measuring range of the assay for CSF are 1.65-40 mg/L at the 1/2 (standard) analyser dilution and 0.91-20mg/L at the 1/1 analyser dilution. For serum, the assay range is 330-8000mg/L at the 1/400 analyser dilution. Correlation to the Binding Site IgA CSF assay for the SPAPLUS® was performed using 98 CSF samples (range 0.959-36.079mg/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; y=1.10x + 0.12. Correlation to the Binding Site IgA serum assay for the SPAPLUS® was performed using 85 serum samples (range 620-4750mg/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; y=0.92x + 79.02. The precision study was based on CLSI EP5-A2. The study was performed over 5 working days for CSF samples and 21 days for serum samples, with 2 runs per day, where each sample was run in duplicate within each run. 3 CSF levels (3.53, 4.59 & 28.9mg/L) and 7 serum levels (557, 687, 1142, 2995, 3797, 6204, 7463mg/L) were assessed on 3 different analysers using 3 different reagent lots for serum and two reagent lots for CSF. All levels gave total precision values of <9% CV. A linearity study was performed following the CLSI EP6-A. The linearity has been confirmed using a serially diluted CSF sample over the range of 1.446-44.745mg/L and a serially diluted serum sample over the range of 258-8930mg/L with deviation from linearity <10%. Interference testing followed CLSI EP7-A2. Serum and CSF samples close to the medical decision points were tested. No significant assay interference effects were observed in serum when tested with triglyceride (500mg/dL), Intralipid (2000mg/dL), bilirubin (100mg/L) or haemoglobin (5g/L). No significant assay interference effects were observed in CSF when tested with haemoglobin (2.5g/L), bilirubin (200mg/L), acetaminophen (1324μmol/L) or acetylsalicylic acid (3.63mmol/L). A limit of quantitation (LoQ) study based on CLSI EP17 was also carried out and gave a limit of 0.91mg/L. In conclusion, the IgA CSF assay for the Optilite® analyser provides

a reliable, accurate and precise method for quantifying IgA in CSF and if abnormal levels of IgA are detected it can be useful in identifying patients who have a variety of CNS disorders.

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Evaluation of the serum IgG Hevylite Kappa and IgG Hevylite Lambda immunoassay on the Optilite® turbidimetric analyser

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Elevated serum concentrations of monoclonal protein are indicative of an underlying abnormality such as monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma and other lymphoproliferative disorders. International guidelines recommend serum protein electrophoresis (SPE) densitometry is performed to quantify monoclonal proteins. Turbidimetry can also be used in these instances to measure total IgG but this will include non-tumour immunoglobulin, and measurement of either IgGκ or IgGλ may give a more accurate representation of tumour production. Here we describe a polyclonal antibody immunoassay for IgG heavy/light chains on the Binding Site Optilite® analyser. The measuring ranges are 2.3 - 30g/L and 1.5 - 17.5g/L at the 1/20 standard analyser dilution for the IgG Kappa assay & IgG Lambda assays respectively. Correlation to the Binding Site IgG heavy/light chain assays for the Siemens BN™II analyser was carried out using 117 samples for IgG Kappa (range 0.810 - 26.5g/L) and Lambda (range 0.186 - 14.300g/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; kappa y= 1.01x - 0.19, lambda y=0.93x - 0.03. Precision studies were run based on the CLSI approved guideline EP5-A2. The precision was tested using 7 targeted levels covering the medical decision point, pathological concentrations, the reference interval and the minimum dilution, on 3 kit lots and 3 analysers over 21 days. All levels gave a total precision result of <10%. Interference testing was carried out using bilirubin (200mg/L), haemoglobin (5g/L), intralipid (2000mg/dL), triglyceride (1000mg/dL) and 15 other common drug and metabolite interferents. The interferents were spiked into serum base pools targeted at 6 levels for both specificities. The difference between the interferent spiked base pools and the negative control showed no significant interference at any level with <10% interference seen at any level. The linear range was established for both the IgG Kappa and IgG Lambda assay by analysing a serially diluted spiked sample covering the measuring range at the standard 1/20 analyser dilution. Both assays passed with an acceptance criteria of <10% for the recovery at each dilution upon expected versus observed calculated results. We conclude that the Hevylite IgG Kappa and IgG Lambda assays for the Optilite turbidimetric analyser provide a reliable, accurate and precise method for quantifying intact IgG immunoglobulins in human serum and show good agreement with existing assays.

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Performance of IgM CSF assay for use on the Binding Site Optilite® turbidimetric analyser

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The measurement of IgM in cerebrospinal fluid (CSF) serum samples aids the assessment of the body's ability to resist infectious disease. An increase in CSF levels can be indicative of barrier dysfunction and/or intrathecal synthesis of immunoglobulin (Ig) within the central nervous system (CNS). Here we describe the evaluation of an IgM CSF assay for use on the Binding Site's Optilite® analyser. The measuring range of the assay for CSF samples is 0.11-40.0mg/L. For serum, the range is 60-3200mg/L. Correlation to the Binding Site IgM CSF and serum assays for the SPAPLUS was performed using 97 CSF samples (range 0.29 - 4.418mg/L) and 50 serum samples (range 402 - 4152mg/L). This demonstrated good agreement when analyzed by Passing-Bablok regression; y=0.97x + 0.03 for CSF and y=0.99x - 0.01 for serum. The precision study was based on CLSI approved guideline EP5-A2 and ran over 5 working days for CSF samples and 21 days for serum samples, with 2 runs of duplicate testing per day. 3 pooled CSF samples and 5 pooled serum samples were assessed on 3 analysers using 3 reagent lots. Results are shown in table 1. A linearity study was performed following the CLSI approved guideline EP6-A. and demonstrated linearity over the range 0.109 - 5.227mg/L for CSF and 50.175 - 2639.351mg/L for serum. Interference was tested by spiking base pools at one CSF level (1.30mg/L) with 200mg/L bilirubin and 2.5g/L hemoglobin and at two serum levels (310.01 & 2492.43mg /L) with 200mg/L bilirubin, 5g/L hemoglobin, 2000mg/dL intralipid and 1000mg/dL triglycerides and comparing with a negative control.

Interference at all analyte concentrations of <7% was detected. We conclude that the IgM CSF assay for the Optilite® analyser provides a reliable, accurate and precise method for quantifying IgM in CSF and shows good agreement with existing assays.

Table 1: Precision of IgM CSF Optilite assay

Analyte concentration	Total precision (%CV) (Acceptance <10%)	Within run precision (%CV) (Acceptance <5%)	Between run precision (%CV) (Acceptance <8%)	Between day precision (%CV) (Acceptance <8%)
0.4382mg/L CSF	5.90%	2.80%	2.50%	4.60%
1.201mg/L CSF	3.60%	2.20%	2.60%	1.30%
3.0237mg/L CSF	4.20%	2.30%	2.90%	2.00%
242.51mg/L serum	6.40%	1.70%	1.90%	5.90%
394.93mg/L serum	4.40%	1.80%	1.70%	3.70%
1492.86mg/L serum	5.80%	2.00%	3.70%	4.00%
1882.43mg/L serum	5.00%	2.30%	2.20%	3.90%
2576.82mg/L serum	4.90%	2.70%	2.40%	3.30%

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Performance of a low level IgG assay for use on the Binding Site Optilite® turbidimetric analyser.

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Serum is the predominant source for proteins present in CSF, the levels of which are regulated by the permeability of the blood-CSF barrier and CSF flow rate. An increase in CSF protein levels can indicate barrier dysfunction and/or local (intrathecal) synthesis of immunoglobulin (Ig) within the central nervous system (CNS). Albumin in CSF originates exclusively from blood, therefore the albumin CSF/serum ratio provides a measurement of barrier function. The assessment of barrier function, intrathecal synthesis and other variable CSF analytes can be useful in the diagnosis of a variety of CNS disorders. Here we describe the performance of a low level IgG assay for measurement of Serum, CSF and Urine samples on the Binding Site's Optilite analyser. Precision was verified using a protocol based on CLSI (EP05-A2). Samples were spiked with purified IgG to give serum levels of 13 mg/L, 25.5 mg/L, 42.5 mg/L, 80 mg/L, 114.25 mg/L and 1000mg/L; CSF levels of 37.24 mg/L, 116.75 mg/L and 1114.44 mg/L and urine levels of 13 mg/L, 70mg/L and 110 mg/L. To validate the serum precision, samples were tested in duplicate, twice per day on 1 kit lot over 3 analysers for 21 days. The CSF and Urine studies were carried out testing samples in duplicate twice per day, on one batch for 5 days. CSF was tested across 3 analysers and urine on one. All matrices and analyte concentrations gave total precision CVs of <10 %. Linearity was verified by assaying serially-diluted serum and CSF samples across an extension of the reportable measuring range and comparing expected versus observed results. Linearity was validated for CSF over the range of 3.67-154.50mg/L and 486.59-31310.42mg/L for serum. This provides a measuring range of 4.2-135mg/L at neat for CSF and urine and of 75-1350mg/L for serum, with an upper limit of 1350mg/L utilizing auto-dilutions. Interference was validated by spiking serum pools between 2300- 22950 mg/L with 200 mg/L Bilirubin, 5 g/L Haemoglobin, 1000 mg/dL Intralipid and 500 mg/dL Triglyceride; Urine pools of 12.559 and 108.064 mg/L with 45mg/L urobilinogen and 1000mg/L Ascorbic Acid; and CSF pools between 9.65-827.56mg/L with 50mg/L Bilirubin, 0.625 g/L Haemoglobin, 200mg/L Acetominophen and 600mg/L Acetylsalicylic Acid. All data was compared to a negative control. Interference at all analyte concentrations was ≤ 9.24%, for all substances tested. Correlation to the Binding Site SPAPLUS IgG assay for the serum comparison study was performed using 70 normal serum samples (Total range 5398 - 22249 mg/L). Analysis by Passing-Bablok regression (y=1.01x + 255.01) demonstrated acceptable agreement. Comparison to the Binding Site SPAPLUS IgG CSF assay using 66 clinical CSF samples (Total range 7.78 - 761.22 mg/L) established acceptable agreement when analyzed by Passing-Bablok regression; y=0.96x + 4.48. In conclusion, the low level IgG assay for the Binding Site Optilite analyser is reliable, accurate, precise and shows good agreement with existing assays.

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Performance of Rheumatoid Factor assay for use on the Binding Site Optilite® protein analyser

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Measurement of Rheumatoid Factor (RF) in serum has been shown to be of use in the detection and monitoring of Rheumatoid Arthritis (RA).Rheumatoid factors are antibodies directed against the Fc portion of IgG. Most factors are IgM antibodies, but may also be IgG or IgA. Rheumatic conditions and chronic inflammatory processes give rise to Rheumatoid Factors, which are produced by plasma cells present at sites of tissue injury. Between 60 and 80% of patients with active RA possess the RF protein in their blood or joint fluid, and therefore its detection is of great value in the diagnosis and monitoring of the disease. Turbidimetry is used in this assay to determine the protein concentrations. The measuring range at the standard 1/1 analyser sample dilution is 7 - 100 IU/mL. The lowest medical decision point for this assay is 12.5 IU/mL. The performance characteristics of the RF assay for use on The Binding Site Optilite® analyser assessed in the following manner. Comparison to the Randox RF assay on the Randox Imola analyser was performed using 20 patient samples (range = 8.18 - 402.01 IU/mL). Good agreement was seen with a Passing-Bablok regression slope of y = 0.95x + 2.97. Precision was assessed according to CLSI (EP05-A2), measuring samples at 5 concentrations, on 2 kit lots and 3 analysers over 21 days. Precision acceptance was ≤5% within-run CV, ≤8% between-day and between-run CV and ≤10% total CV. All %CV for the precision study came within the acceptance criteria (total precision results: 9.894 IU/mL - 9.30%, 16.587 IU/mL - 5.70%, 37.113 IU/mL - 3.90%, 76.501 IU/mL - 3.50%, 144.453 IU/mL - 7.00%). Linearity was assessed by assaying a serially-diluted sample pool across the width of the measuring range and comparing expected versus observed results. The acceptance criteria was nonlinearity ≤10% of the low medical decision point up to and including the low medical decision point. Above this point, acceptance criteria was nonlinearity within ± 10% for each dilution. CV acceptance criteria was <8.0% at each point in the series. Linearity passed these criteria across the range 6.23 - 111.627 IU/ml. Interference was tested by running triglyceride (10g/L), bilirubin (0.2g/L), haemoglobin (5.0g/L) and 13 other potential interferents at 3 levels, acceptance being ≤10% difference to a negative control. No significant interference was observed at any level with the interferents studied. In conclusion, the RF Optilite assay for the Optilite analyser provides a reliable, accurate and precise method for quantifying RF in serum and correlates well with existing methods.

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Alanine Aminotransferase activity in plasma as a marker of severity of liver disease

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BACKGROUND: Alanine aminotransferase (ALT) activity is an important screening, diagnostic, and monitoring test for liver disease. High levels of ALT can be a marker of liver disease with variable aetiologies and prognosis.

Several studies showed that the majority of cases with ALT concentrations >1000 IU/L are generally due to acute ischaemia, acute drug-induced liver injury (usually paracetamol) or acute viral hepatitis.

AIM: The aim of this study was to correlate the ALT activity > 1000 IU/L with aethiology and clinical outcome as a marker of severity of illness.

MATERIALS AND METHODS: This single-centre, retrospective, observational study was conducted in the Emergency laboratory of a tertiary referral university hospital over an 18 month period.

Using the Laboratoy Informatic System (LIS), all patients with ALT plasma concentrations > 1000 IU/L from January 2014 to June 2015 were selected. Clinical data for the final diagnosis and outcome were obtained from the medical electronic record.

ALT was measured by IFCC method in a Dimension Vista (Siemens HD).

RESULTS: During the 18-month study period a total of 207 patients with ALT levels > 1000 IU/L were included. Eleven patients were excluded because of their inconclusive diagnosis. The final study cohort consisted of 196 patients (100 males, 96 females), median age: 49 years (range 0-92).

Mean ALT activity was 2458±3152 IU/L in patients who died (n=74), and 2069±2342 IU/L in survivors (n=122) (p=0,001).

AETIOLOGY	N		%		%MORTALITY		MEAN ALT +/- 2DS
	<14 years	>14 years	<14 years	>14 years	<14 years	>14 years	
Ischaemic liver injury	5	41	11	27	60	66	2528 +/- 3330
Cholestasis	2	26	4	17	0	4	1586 +/- 1248
Hepatobiliar malignancy	5	14	11	9	0	43	1722+/- 1542
Cardiogenic Multiorgan failure	1	15	2	10	100	100	2112+/- 2828
Septic shock	1	14	2	9	0	71	2289+/- 2098
Drug induced	4	11	9	7	0	18	2777+/- 3142
Viral hepatitis	3	10	7	7	0	30	2282+/- 1896
Idiopathic	4	9	9	6	25	22	2150+/- 2022
Autoimmune hepatitis	6	3	13	2	0	0	2368+/- 2676
Other causes (osteosarcoma, biliary atresia, transplant,···)	14	8	31	5	10	8	1579+/- 1834

CONCLUSIONS: ALT activity in plasma above 1000 IU/L is not directly related to mortality in all aetiologies so it cannot be considered an independent factor for illness severity. When interpreted together with the patient diagnosis it may play a role as a prognostic factor of mortality, with the highest rates linked to cardiogenic shock, septic shock and ischaemic liver injury and the better prognosis in autoimmune hepatitis, cholestasis and hepatobiliar malignancy patients.

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Evaluation of CKD-EPI equations using cystatin c in elderly population

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Background: the assessment of renal function with the 24 hours urine creatinine clearance (Cl-Crea) in elderly population leads to a wrong urine collection. Besides the muscle mass frequently decreased in these patients do not reflect the values of serum creatinine. Recently, cystatin C (cysC) has become an important marker of kidney damage. This peptide is synthesized in all cells regardless of body muscle mass. It is freely filtered at the glomerulus and it is completely catabolized at the renal tubules which allow its use as a marker for renal filtration. Equations were proposed for estimating Glomerular Filtration Rate (GFR) using both cysC or creatinine (Crea) and cysC. Aim: To evaluate the use of CKD-EPI^{cysC} and CKD-EPI$^{cysC/crea}$ equations in an elderly population. Methods: we studied 140 patients with a mean age 71±11. We measured creatinine (compensated Jaffe IFCC-traceable) in serum and 24 hours urine and cystatin C (Immunoturbidimetry) in serum. Cl-Crea was calculated and patients were divided by GFR <60, 60-89 and > 90 ml/min/1.73m². We applied in these ranges the CKD-EPI^{cysC} and CKD-EPI$^{cysC/crea}$ equations. Results: the Cl-Crea correlated with both equations (r=0.80 and r=0.85, respectively; p <0.001). Cl-Crea and formulas in the range below 60ml/min/1.73m² were not different. In the 60-89 range, the Cl-Crea had higher values than CKD-EPI^{cysC} (77±9 vs 64±15; p=0.003), whereas Cl-Crea had no differences with CKD-EPI$^{cysC/crea}$ (p=0.17). Above 90 ml/min/1.73m², Cl-Crea had higher values respect to CKD-EPI^{cysC} and CKD-EPI$^{cysC/crea}$ (112±15 *vs* 71±17 and 112±15 *vs* 87±19, respectively; p<0.0001). Conclusion: both equations CKD-EPI^{cysC} and CKD-EPI$^{cysC/crea}$ have a good correlation with Cl-Crea considered as the standard method. It would be advisable and acceptable to use the CKD-EPI$^{cysC/crea}$ in elderly population up to the range of 90 ml/min/1.73m² corresponding to the clinical criteria that GFR below 90ml/min/1.73m2, is associated with impaired renal function.

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Performance Evaluation of an Albumin BCP Assay on the Atellica CH Analyzer*

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Introduction: Albumin measurements are used in the diagnosis and treatment of numerous diseases primarily involving the liver or kidneys. The Atellica™ CH Albumin BCP (Alb_P assay)* from Siemens Healthcare is intended for the quantitative measurement of albumin in human serum and plasma. In this assay, serum or plasma albumin quantitatively binds to bromocresol purple (BCP) dye to form an albumin/BCP complex that is measured as a bichromatic endpoint reaction at 596/694 nm.

The objective of this study was to evaluate the performance of the Alb_P assay on the Atellica CH Analyzer.

Methods: Assay linearity was evaluated according to Clinical and Laboratory Standards Institute (CLSI), protocol EP06-A. Limit of quantitation (LoQ) was evaluated according to CLSI protocol EP17-A2. Precision was evaluated according to CLSI protocol EP05-A3. Three levels of commercial control and a human serum pool were tested with albumin concentrations ranging from 2.7 to 7.1 g/dL. Each sample was assayed two times per run, two runs per day, for at least 20 days. A method comparison study (n = 130 serum samples) was conducted between the Alb_P assay and the ALBP assay on the ADVIA® 1800 Clinical Chemistry System according to CLSI protocol EP09-A3.

Results: The Alb_P assay is linear from 0.5 to 8.0 g/dL (5–80 g/L). LoQ was determined to be 0.4 g/dL (4 g/L) based on 225 determinations, with an interassay precision of ≤10%. Repeatability ranged from 0.73 to 1.73% CV, with within-lab precision of 1.02 to 1.79% CV. The method comparison study yielded a regression equation of y = 0.99x - 0.01 g/dL, with r of 0.996, versus the ALBP assay on the ADVIA 1800 system. No significant interference (bias <10%) was observed from 600 mg/dL hemoglobin, 30 mg/dL conjugated bilirubin, 30 mg/dL unconjugated bilirubin, or 500 mg/dL lipemia (Intralipid).

Conclusion: The Albumin BCP assay for the Atellica CH Analyzer enables measurement of albumin in human serum with excellent precision and accuracy.

*Under development. Not available for sale.

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Can supplementation of aspartate aminotransferase (AST) assays with pyridoxal-5'-phosphate (P5P) be useful in identifying macroenzyme aspartate aminotransferase (macroAST)?

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Background: Serum macroenzyme aspartate aminotransferase (macroAST) is a complex of AST and immunoglobulin that exhibits reduced clearance from blood due to its large size and results in increased serum AST activity. Confirmation of macroAST, which is benign, is critical to reduce unnecessary and invasive diagnostic procedures. Most methods used to identify macroAST involve precipitating or selectively removing large immunocomplexes from the serum sample. These methods require manual sample manipulation and the use of specialized reagents. Several case studies, including cases from our laboratory, have documented that macroAST samples show dramatic increases in AST activity upon assay supplementation with pyridoxal-5'-phosphate (P5P) suggesting this may be a unique characteristic of macroAST. **Objective:** To determine if the % increase in AST activity upon P5P supplementation can be used to identify macroAST in patient serum samples. **Methods:** Residual waste serum samples from physician-ordered macroAST or AST testing were used. AST activity was measured on a Roche Cobas® 6000 (Roche Diagnostics, Inc.) using both P5P-supplemented AST reagent (ASTLP, reference #:04467493190) and unsupplemented AST reagent (ASTL, reference #:20764949322). MacroAST was identified using a clinically validated polyethylene glycol-precipitation method developed at the Mayo Clinic (Rochester, MN). Three cohorts of samples were investigated: 11 positive macroAST samples, 20 samples from patients with isolated elevations of AST and negative for macroAST (AST >100 U/L and total bilirubin, alkaline phosphatase, and alanine aminotransferase within the reference interval), and 456 samples that had physician-ordered AST testing performed but no suspicion of macroAST. The % increase in AST activity upon P5P supplementation was calculated for each sample and the mean, median and distributions of the % increase in AST activity in each cohort were compared. Statistical significance was determined using the Student's t-test as well as the non-parametric wilcoxon test. Receiver operator characteristic (ROC) curve analysis was performed to determine the optimal % increase in AST activity cut-off for detecting macroAST. **Results:** The mean % increase in AST activity upon P5P supplementation in samples with confirmed macroAST was 223% (range 8-1590%; median 46%). In samples with isolated increases in AST but without macroAST, the mean % increase in AST with P5P supplementation was 29% (range 3-142%, median 24%). The cohort of samples with physician-ordered AST and no suspicion of macroAST had a mean increase in AST activity of 15% (range 0-125%, median 11%) upon P5P supplementation. The macroAST cohort showed a significantly larger % increase in AST activity upon P5P supplementation than the other two groups (p <0.0001). ROC curve analysis indicated the optimal diagnostic cut-off for identifying macroAST was 216% increase in AST activity which achieves a sensitivity of 27% and a specificity of 100%. **Conclusions:** In cases of confirmed macroAST, we have demonstrated that the AST activity associated with macroAST increases significantly upon P5P supplementation of reagent compared to non-macroenzyme AST. An increase in AST activity of ≥216% upon P5P supplementation

is 100% specific for macroAST. However, given significant overlap across cohorts and poor sensitivity, it is unlikely this approach will have clinical utility as diagnostic test for macroAST.

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Neoptrin and BACE1 as Novel Biomarkers in Alzheimer Disease

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Dementia is a disorder characterized by impairment of memory and at least one other cognitive domain (aphasia, apraxia, agnosia, executive function) .Dementia is one of the major causes of disability in later life. Alzheimer disease is a progressive neurodegenerative disorder of the brain that causes dementia, which is a gradual loss of memory, judgment, and ability to function, it is the most common dementia affecting elderly people .As our population continues to age, the increasing prevalence of Alzheimer disease will have an even greater impact. Neopterin is a catabolic product of guanosine triphosphate (GTP), a purine nucleotide; it is a sensitive marker for monitoring Th1-cell immune response. BACE1 (β-site APP Cleaving Enzyme 1) that initiates Aβ formation and increase is presentyears before AD symptoms arise, suggesting that Aβ42 is likely to initiate AD pathophysiology. It was addressed whether the BACE1 elevation observed in AD as an end product of advanced neurodegeneration and cell death or whether it is actively involved in disease progression. **Aim:** The aim of the present work was to study the use of serum neopterin concentration and Serum BACE 1 as new diagnostic markers in cases of Alzheimer's disease and correlate their concentration with the severity of Dementia of the Alzheimer type (DAT).**Patients**: One hundred subjects were enrolled in this study subclassified into fifty Alzheimer disease patients and fifty normal subjects. **Methods:** The concentration of neopterin was measured using DRG® Neopterin ELISA kit. BACE1 is measured derived from Immuno-Biological Laboratories Co., Ltd. **Results:** In the present study there was a significant positive correlation between Neopterin as well as BACE1 and Alzheimer disease, AUC was 0.926 with P<0.001for Neoptrin and 0.79 with P<0.01 for BACE1.The results suggest that Neopterin levels increase with increasing disease severity . Therefore, Neopterin levels may be a useful marker to follow progression of AD. However, itremains undetermined as to which is the initiating event, Aβ elevation and deposition or increased BACE activity, however As the rate-limiting enzyme in Aβ generation, BACE1, in principle, is an excellent therapeutic target for strategies to reduce the production of Aβ in AD.

Key words : AD , BACE1, Neoptrin, DAT(Dementia of Alzeimer type).