Abstracts of Scientific Posters

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Tuesday, July 27, Poster Sessions

Wednesday, July 28, Poster Sessions

Thursday, July 29, Poster Sessions

Tuesday AM, July 27

Poster Session: 10:00 am – 12:30 pm
Animal Clinical Chemistry

A-01

Toxicity Assessment Of The Aqueous Extract Of Calotropis Procera In Rabbits

J. D. Mbako. *University of Yaounde I, Yaounde, Cameroon,*

Calotropis procera has been reported to possess medicinal properties but equally pose deleterious effect on animals. To investigate the extent of damage, a toxicological evaluation of the aqueous extract of fresh leaves of the plant was conducted, with main objective being to identify target organs and no effects groups. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, cardiac glycosides, and flavonoids while elemental analysis showed traces of iron, lead, sodium, and potassium in concentrations of 0.23, 0.03, 0.82 and 9.5 mg/g respectively. Acute toxicity study was carried out with oral administration of 200, 400, 800, and 1600mg/kg of the extract once to groups I, II, III and IV respectively within a 24 hours observation period, with Groups V, (controls) given water. Four rabbits died within 24 hours and LD_{50} was estimated (940mg/kg). 80, 40 and 20mg/kg of the extract were administered daily to groups I, II, and III respectively during sub-acute toxicity study for 14 days, with Groups IV, given water.
Statistical analysis of Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Albumin and protein showed no significant changes at $P<0.05$. Changes in Packed Cell Volume (PCV), White Blood Cells (WBC), Haemoglobin (Hb), Platelets, and Differential Leucocyte Count (Lymphocytes, Monocytes, Eosinophils, Heterophils/Neutrophils and Basophils) were equally statistically insignificant at $P<0.05$. However, gross and histopathological examination of some organs and tissues (heart, liver, kidney, brain, small intestine and lungs) revealed lesions. It was concluded that the extract had no significant effect on blood parameters when administered orally at tolerable doses since controls were also affected but have lethal effects at higher doses since the effect was found to be dose-dependent.
Keywords: *Calotropis procera*, Toxicity, Histopathology, haematology, Serum Enzymes and Protein.

A-02

RNase L is involved in hematopoiesis and iron retention

X. Yi, A. Zhou. *Cleveland State University, Cleveland, OH,*

Background: RNase L is an interferon (IFN) inducible enzyme that plays an important role in IFN functions against viruses and cellular proliferation. Previous studies in our lab have demonstrated that RNase L mediates the expression of proinflammatory genes such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which have been well demonstrated involved in iron deficiency.
Objective: The aim of this study was to evaluate the effect of RNase L on hematopoiesis and iron deficiency in the RNase L deficient mouse model.
Methods: RNase L wild type and null mice with C57BL/6 background in both genders (2 weeks) were used in the *in vivo* experiments. Initially, different groups of the animals were treated with 20ug Lipopolysaccharide (LPS) every other day for one week (three times in total) by an intraperitoneal route (i.p.). Total RNAs in the liver and kidney were isolated and the expression of erythropoietin (EPO) and hepcidin genes, which predominantly regulate iron homeostasis, was determined by RT-PCR. These animals were fed with an iron-deficient diet for 20days and blood was collected for hemotologic profile analysis.
Results: Light-colored kidney and liver were observed in RNase $L^{-/-}$ mice. The latter was more severe in pregnant mice. The expression of EPO gene in the kidney was increased in RNase $L^{-/-}$mice after LPS treatment and lack of RNase L suppressed the induction of hepcidin by LPS. Complete blood cell profile showed no obvious difference in red blood cells (RBC), hemoglobin (Hgb) between RNase L wild type and null mice, but a significant decrease of mean corpuscular volume (MCV), a surrogate marker for detecting early iron deficiency, and white blood cells (WBC) in RNase $L^{-/-}$ mice.
Conclusion: Our data suggest that RNase L may be involved in hematopoiesis through modulating iron homeostasis by regulating the expression of certain genes, such as EPO and hepcidin.

A-03

The effects of nitric oxide and asymmetric dimethylarginine in the rat endometriosis model

T. Cayci, E. O. Akgul, Y. Gulcan Kurt, T. S. Ceyhan, I. Aydin, O. Onguru, H. Yaman, E. Cakir, C. Bilgi, K. M. Erbil. *Gulhane Military Medical Academy, Ankara, Turkey,*

Background: The aim of study was to investigate the effects of nitric oxide (NO) and asymmetric dimethylarginine (ADMA) in endometriosis model.
Methods: Forty-one rats in which endometriotic implants were divided into four groups, including Group 1 (infliximab), Group 2 (etanercept), Group 3 (letrozole), Group 4 (control). There were 11 rats in Group 5 (normal). Implants' size, plasma ADMA and nitrate/nitrite (NO_x) levels and histological score were assessed.
Results: In the groups of 1, 2 and 3, plasma ADMA levels were higher than groups of 4 and normal, 296.8 ± 66.2, 285.9 ± 35.7, 200.3 ± 41.0, 125.3 ± 16.7, 111.3 ± 6.5 µmol/L, while NO_x levels lower than groups of control and normal 19.6 ± 3.8, 19.8 ± 4.4, 39.3 ± 6.1, 80.5 ± 5.3, and 91.1 ± 5.0 µmol/L, respectively.
Conclusion: Infliximab, etanercept and letrozole have regressed endometriotic implants, decreased plasma NO_x levels, and increased plasma ADMA levels.

A-04

Pentraxin 3 as a Potential Biomarker of Acetaminophen-Induced Liver Injury

E. Cakir, E. O. Akgul, I. Aydin, O. Onguru, T. Cayci, Y. Gulcan Kurt, M. Agilli, F. N. Aydin, M. Gulec, N. Ersoz, T. Turker, H. Yaman, C. Bilgi, K. M. Erbil. *Gulhane Military Medical Academy, Ankara, Turkey,*

Background: Overdose of acetaminophen can lead to severe liver injury in humans and experimental animals. Pentraxin-3 is produced and released by several cell types. In this study, we aimed to evaluate whether pentraxin-3 is a potential biomarker in the identification of acetaminophen-induced liver injury.
Methods: Thirty adult Wistar rats were randomly divided into three groups: control, acetaminophen-1 and acetaminophen-2 groups. Acetaminophen-1 and acetaminophen-2 group rats were given as a single dose 1 and 2 g/kg body weight of acetaminophen by gastric tube, respectively. Liver tissues and blood samples were obtained for biochemical and histopathological analysis. Biochemical parameters, plasma and liver pentraxin-3 levels and degree of liver necrosis were comparable in the control, acetaminophen-1 and acetaminophen-2 group animals.
Results: Acetaminophen treatments caused necrosis in the liver after 48 h. In rats of group of acetaminophen-1 and acetaminophen-2 when compared with rats of control group (7.5 ± 3.3 ng/mg protein), mean liver pentraxin-3 concentrations were 14.1 ± 3.0 ($p = 0.032$) and 28.5 ± 8.2 ($p < 0.001$) ng/mg protein, respectively. The degrees of liver necrosis of the acetaminophen-1 and acetaminophen-2 groups were higher than the group of control ($p < 0.001$ and $p < 0.001$, respectively). Increased serum alanine aminotransferase, aspartate aminotransferase activities in acetaminophen-1 and acetaminophen-2 rats were in good agreement with histopathological injury.
Conclusion: This study suggests a role of pentraxin-3 in the acetaminophen-induced liver injury in the rats. The elevated liver pentraxin-3 in the acetaminophen-induced hepatic necrosis might be a marker of acute histological liver damage.

A-05

The Association between Neopterin and Acetaminophen-Induced Nephrotoxicity

E. Cakir, E. O. Akgul, I. Aydin, T. Cayci, Y. Gulcan Kurt, O. Onguru, F. N. Aydin, M. Agilli, H. Yaman, N. Ersoz, A. Guven, T. Turker, C. Bilgi, K. M. Erbil. *Gulhane Military Medical Academy, Ankara, Turkey,*

Background: In large dosages, acetaminophen (APAP) produces acute kidney necrosis in most mammalian species. High neopterin levels has been accepted as strong indicator for the clinical severity of some diseases. In this study, we aimed to evaluate whether neopterin is a biomarker in the identification of APAP-induced nehrotoxicity.
Methods: Thirty adult male Wistar rats were randomly divided into three groups: control, APAP-1 and APAP-2 groups. APAP-1 and APAP-2 group rats were given as a single dose 1 and 2 g/kg body weight of APAP by gastric tube, respectively. Kidney tissues and blood samples were obtained for biochemical and histopathological analysis. Biochemical parameters, serum and kidney neopterin levels and grade of tubular injury were comparable in the control, APAP-1 and APAP-2 group animals.
Results: APAP treatments caused tubular necrosis in the kidney and increase in serum creatinine concentrations accompanied by elevated serum and kidney neopterin levels. In rats of group of APAP-1 and APAP-2 when compared with rats of control group (109.1

pmol/mg protein), median kidney neopterin concentrations were 162.1 ($p = 0.089$) and 222.2 ($p < 0.001$) pmol/mg protein, respectively. The grade of tubular injury of the APAP-1 and APAP-2 groups was higher than the group of control ($p < 0.001$ and $p < 0.001$, respectively).

Conclusion: Serum and kidney neopterin levels could be sensible alternative to evaluate the risk to have nephrotoxicity due to overdose of APAP. The elevated serum and kidney neopterin in the APAP-induced tubular necrosis might be a marker of acute histological kidney injury.

A-06

Measurement of TS-131, a New Monopyridinium Oxime, by High Performance Liquid Chromatography in Rat Plasma Samples

Z. I. Kunak[1], E. O. Akgul[1], H. Yaren[1], T. Cayci[1], Y. Gulcan Kurt[1], I. Aydin[1], H. Yaman[1], L. Kenar[1], T. Subasi[2], E. Cakir[1], E. Macit[1], A. S. Demir[2], K. M. Erbil[1], J. Z. Karasova[3]. *[1]Gulhane Military Medical Academy, Ankara, Turkey, [2]Middle East Technical University, Ankara, Turkey, [3]Faculty of Military Health Sciences, Hradec Kralove, Czech Republic,*

Background: TS-131 is a monopyridinium aldoxime-type cholinesterase reactivator developed as a potential alternative to commercially available oximes.

Methods: Male Spraque Dawley rats were treated intramuscularly with TS-131 and the samples were collected 30 min later. Separation was carried out by HPLC using octadecyl silica stationary phase and a mobile phase consisting of 92% 0.1 M ammonium acetate and 8% methanol. Measurements were carried out at 40 °C. Quantitative absorbance was monitored at 242 nm.

Results: The calibration curve was linear through the range of 0.78-3200 µmol/L, which is well beyond the detected plasma level range of TS-131. Limit of quantitation was 0.39 µmol/L. Intra-day and inter-day precisions of the HPLC determinations gave standard deviations as 1.94 and 1.22%, respectively. After spiking, average spike recoveries ranged from 99.2% to 100.4% and, overall mean recovery of 99.8% was found.

Conclusion: A sensitive, simple and reliable high performance liquid chromatography (HPLC) method with diode array detector was developed for the measurement of TS-131 concentrations in rat plasma samples.

A-07

Efficacy of Hyperbaric Oxygen Therapy and S-Methylisothiourea in Experimental Acute Necrotizing Pancreatitis

O. Altinel, N. Ersoz, B. Uysal, M. Ozturk, T. Cayci, B. Kurt, I. H. Ozerhan, Y. Gulcan Kurt, H. Yaman, A. Guven, A. Korkmaz, M. Yasar. *Gulhane Military Medical Academy, Ankara, Turkey,*

Background: Acute pancreatitis is a disease where the trigger mechanisms of the inflammation and thus also the influence of cytokines are very closely associated and are therefore apparent. Neopterin (NP) has been recognized as a valid marker for cellular immune activation. The aim of this study was to investigate the individual and combined effects of HBO and S-methylisothiourea (SMT) therapies on biochemical and histopathological changes, oxidative stress, and bacterial translocation (BT) in an experimental rat model of acute necrotizing pancreatitis (ANP).

Methods: Fifty Sprague-Dawley rats were randomly divided into five groups: SHAM, ANP, HBO, SMT, and HBO+SMT groups. Rats in all groups expect sham group received sodium taurocholate while rats in sham group received normal saline injection into the common biliopancreatic duct. HBO or SMT was applied to rats in the HBO, SMT, and HBO+SMT groups after induction of pancreatitis. All surviving animals were killed at the 4th day after induction of pancreatitis. Their tissue and blood samples were obtained for biochemical, microbiological and histopathological analysis.

Results: Serum amylase and NP, oxidative stress parameters, histopathologic score, BT, and survival rates were better in the therapy groups than in the ANP group. Histopathologic injury scores [median (min-max)] of the HBO+SMT group [8 (7-9)] were lower than the group of ANP [18 (15-23)] ($p<0.01$). NP levels were significantly higher in the ANP group than the sham group ($p<0.001$). There was no statistically correlation between serum amylase and NP levels in the HBO group ($r=0.611$; $p=0.08$) and the SMT group ($r=-0.094$, $p= 0.797$). The number of infected rats in the HBO+SMT group was significantly lower compared to the ANP group. Oxidative stress parameters improved in all treatment groups when compared with the ANP group.

Conclusion: This study showed that activation of cellular immunity is implicated in the pathogenesis of ANP and may be a main contributory factor to severity of pancreatitis. NP may be considered a reliable prognostic indicator. Additional evaluation of the actions of HBO and SMT treatments in ANP is warranted to improve treatment results, possibly by development of synergistic therapies. Further research studies are required to investigate future therapeutic opportunities in the ANP.

A-08

Cardioprotective activity of Terminalia *arjuna* in Isoproterenol induced Myocardial ischemia in rats

S. K. Shukla, S. Dwivedi, U. R. Singh, S. B. Sharma. *University college of medical sciences and GTB Hospital, Delhi, India,*

Cardiovascular diseases are one of the leading cause of morbidity and mortality in developed as well as in developing countries, including India too and their prevention are a major public health challenge[1]. Oxidative stress plays a potential role in CAD and it has been suggested to accelerate atherosclerosis[2]. *Terminalia arjuna* is traditionally used for the treatment of heart diseases[3,4,5]. The present study demonstrates the effect of hydroalcoholic extract of *Terminalia arjuna* bark on lipid peroxidation (MDA) , reduced glutathione (GSH) and superoxide dismutase (SOD) to evaluate their antioxidant activity and then sacrificed for histopathological investigation.24 male albino rats weighing 200-250g were divided into three experimental groups (n=8 in each groups). Normal healthy rats were given normal saline for 30 days. Control group was given normal saline for30 days, Isoproterenol (85mg/kg b.w) administered on 29th and 30th day[6]. In drug treated group hydroalcoholic extract of T. *arjuna* (HETA), orally administered for 30 days at a dose of 100 mg/kg b.w, Isoproterenol (85mg/kg b.w) administered on 29th and 30th day. Fasting blood samples were taken prior to and after given the treatment to estimate MDA, GSH and SOD, and then sacrificed for histopathological examination.

Results- HETA produced significant depletion in MDA with a concomitant elevation in activity of SOD, GSH level was also significantly increased by HETA ($p<0.001$). On staining with Haematoxylin and eosin normal-Clear integrity of myocardial cell membrane, normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils were seen in normal group while in ISP control -patchy areas of necrosis, hyalinization of myofibrils with focal cellular infiltrations were seen, the myofibrils showed vacuolar changes with fragmentation suggestive of necrosis. Animals pre-treated with T.A 100mg/kg b.w ,the morphology of the myocardium was almost similar to that observed in normal animals.

Conclusions- Hydroalcoholic extract of T. *arjuna* bark was found to be more effective in producing antioxidant response and also restore the morphology of myocardium towards normal. The results suggest that crude bark of TA augments endogenous antioxidant compounds of rat heart[7] and also prevents oxidative stress associated with Isoproterenol induced ischemia in rats heart.

References

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A-09

Evaluation of Species Specific Calibrations for Measuring Albumin in Urine of Dogs and Monkeys using an Automated Human Immunoturbidometric Assay

D. F. Adams, K. M. Lynch, T. S. Sellers, D. Ennulat. *GlaxoSmithKline, King of Prussia, PA,*

The Siemens human immunoturbidometric microalbumin assay for the ADVIA® 1650 Chemistry System is an immunological method used to assess and/or monitor renal disease in clinical medicine. Application of this method for nonclinical species could provide a reliable and automated method for assessing kidney injury in nonclinical safety studies. However, the anti-human antibody used in this assay demonstrated only partial cross-reactivity to dog and cynomolgus monkey albumin. Here we describe the use of commercially available dog and cynomolgus monkey albumin to create species-specific calibrations for accurately measuring albumin in urine from beagle dogs and cynomolgus monkeys using the Siemens assay on the ADVIA 1650 Chemistry System. Stock solutions of dog and cynomolgus monkey albumin diluted in diH_2O to concentrations of 500 mg/L were used to prepare multi-point calibration curves. Intra- and inter-assay precision was acceptable (CVs < 5%) using commercially available human controls (albumin concentrations of 30-40 mg/L and 150-200 mg/L) and dog and monkey urine. Linearity was demonstrated in both species across the calibration range of 5 to 500 mg/L (dog: R^2

= 0.9999, monkey: R^2 = 0.9997). Additionally, the stock 500 mg/L calibrators were stable at -70°C for 2 months. Spike/recovery evaluations demonstrated acceptable recovery (87-104%) in both species. Mean (min-max) urinary albumin (normalized to creatinine) values in clinically healthy beagle dogs and cynomolgus monkeys were 0.43 (0.03-2.80) mg/mmol and 1.47 (0.41-8.69) mg/mmol, respectively (n = 24/species). Additionally, urinary albumin concentrations for 20 clinically healthy beagle dogs correlated well (y = 2.87x + 12.93, R^2 = 0.9488) with albumin concentrations measured using a dog specific microalbumin ELISA method. Species-specific calibration of the Siemens human immunoturbidometric microalbumin assay provides an easily adaptable automated method for measurement of urinary albumin concentrations in dogs and monkeys.

A-10

Effect of Vortexing Blood on In Vitro Hemolysis Assay Results

D. W. Sprenger, H. L. Jordan. *GlaxoSmithKline, Research Triangle Park, NC,*

Background: The assessment of the hemolytic potential of parenteral drug formulations is essential for safe administration of intravascular therapeutics. Drug safety laboratories continue to develop better methods for assessing hemolytic potential. Experiments with canine and human blood were conducted.
Methods: To simulate intravascular administration, in vitro hemolytic studies combine test articles with whole blood or washed cells using rapid mixing at physiological temperature and pH. The objective of this experiment was to determine the extent to which vortexing speed and duration contribute to the disruption of human red blood cells (RBC) in vitro as measured by release of hemoglobin (Hgb) and potassium (K^+). Washed RBCs from a healthy human volunteer were mixed with either normal saline or a 20% cyclodextrin vehicle (Captisol®) in citrate buffer and maintained at 37°C. Five mixing levels increasing in speed and duration, 1 to 60 seconds, were evaluated using the Scientific Industries Vortex Genie2 Model G-560. RBCs suspensions were incubated with an equal volume of test article in each tube. One ml of 5% dextrose was added to stop the lytic reaction. Cells and debri were removed after a 5 minute incubation at 37 °C. Hgb and K^+ concentrations were determined with the Olympus AU640^e and expressed as a percent of positive control.
Results: Saline: Hgb release ranged from 0.0% to 0.2%; K^+ release ranged from 0.0% to 0.3%. Cyclodextrin/citrate: Hgb release ranged from 0.1% to 1.2%; K^+ release ranged from 2.4% to 3.4%. The greatest release was found at the maximum mixing levels (40s at speed 5 or 60s at speed 6).
Conclusion: Vortexing human RBCs in normal saline up to 60 seconds at medium speed did not cause hemolysis. 20% cyclodextrin did not cause hemolysis at low mixing speeds, however vortexing at longer times and higher speed (i.e., 40s at speed 5 or 60s at speed 6) caused slight to moderate hemolysis.

A-11

Effects of phytic acid and exercise on some serum analytes in rats orally exposed to diets supplemented with cadmium

T. Daley, S. Omoregie, V. Wright, F. Omoruyi. *Northern Caribbean University, Mandeville, Jamaica,*

Cadmium is a ubiquitous environmental pollutant of increasing worldwide concern. The uptake of this element is mainly through ingestion of food crops grown on cadmium containing soil. Cadmium has been reported to be high in the soil where food crops are grown in some parishes of Jamaica. Cadmium is a well-known human carcinogen and a potent nephrotoxin. Phytic acid is a storage form of phosphorus which is found in significant quantities in plant seeds, roots and tubers and has been shown to be high in some food crops grown in Jamaica. The anti-nutrient property of phytic acid is based on its strong ability to chelate multivalent metal ions, including cadmium, precipitate and decrease the availability of these minerals as a result of the formation of very insoluble salts that are poorly absorbed from the gut. In this study, we determined the effects of phytic acid and exercise on the metabolism of cadmium in rats.
Five groups of rats were fed as follows: Group 1 was fed control diet, group 2 was fed control diet supplemented with cadmium and subjected to exercise, group 3 was fed control diet supplemented with phytic acid plus cadmium and subjected to exercise. Group 4 was fed control diet supplemented with cadmium and phytic acid and group 5 was fed control diet supplemented with cadmium. The animals were fed for four weeks and then sacrificed. Blood samples were collected for some cardiac markers, electrolytes, liver enzymes, electrolytes, lipid profile and some renal function evaluation.
The group that was fed control diet supplemented with cadmium displayed increased electrolytes, liver enzymes and cardiac markers compared to other test groups. Similarly, Blood urea nitrogen, uric acid and phosphate were increased in group 5 rats compared to other test groups. Increased alkaline phosphatase activity was observed in group 2 rats while amylase activity increased in groups 2 and 4 rats compared to other test groups. There was decrease in the weights of the liver and kidneys of rats fed cadmium only compared to the other groups. These observations suggest that consumption of diet high in phytic acid with relatively high physical activity may be protective against the adverse effects of cadmium.

A-12

Effect of sulfite on antioxidant enzymes and lipid peroxidation in normal and sulfite oxidase-deficient rat erythrocytes

O. Ozturk[1], S. Oktar[2], M. Aydin[3], V. Kuçukatay[4], N. Yilmaz[1], Z. Yonden[1], S. Sogut[1]. *[1]Mustafa Kemal University, Faculty of Medicine, Department of Biochemistry, Hatay, Turkey, [2]Mustafa Kemal University, Faculty of Medicine, Department of Pharmacology, Hatay, Turkey, [3]Mustafa Kemal University, Faculty of Medicine, Department of Physiology, Hatay, Turkey, [4]Pamukkale University, Faculty of Medicine, Department of Physiology, Denizli, Turkey,*

Background: Sulfite and related chemical such as sulfite salts and sulfur dioxide has been used a preservative in food and drugs. This molecule has also been generated from the catabolism of sulfur-containing amino acids. Sulfite is a very reactive and potentially toxic molecule and has to be detoxified by the enzyme sulfite oxidase (SOX). The aim of this study was to investigate the effects of ingested sulfite on erythrocyte antioxidant/oxidant status.
Methods: Rats were assigned to four groups (n=10 rats/group) as follows; control (C), sulfite (CS), deficient (D) and deficient + sulfite (DS). SOX deficiency was established by feeding rats a low molibdenyum diet and adding to their drinking water 200 ppm tungsten (W). Sulfite (25 mg/kg) was administered to the animals via their drinking water. Antioxidant status was evaluated by measuring activity of glucose-6-phosphate dehydrogenase (G-6-PD), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities and oxidant status by measurig thiobarbituric acid reactive substances (TBARS) in normal and SOX deficient rats.
Results: The results are given in the table below:

Parameters	Control Group (C)	Sulfite Treated Group (CS)	SOX Deficient Group (D)	SOX Deficient Sulfite Treated Group (DS)
Hepatic SOX (unite/mg protein)	6.1±2.5	5.9±1.8	0.30±0.09[a]	0.19±0.08[a]
G-6-PD (IU/gHb)	11.1±1.06	16.4±8.06[b]	28.8±4.7[a]	31.2±8.5[a]
Cu.Zn-SOD (U/gHb)	4234±290	5115±132[a]	5467±153[a]	6599±51[c]
GPx (U/gHb)	2.4±0.2	2.8±0.6[b]	3.9±0.48[a]	3.8±0.47[a]
CAT (k/gHb)	120±13	134±9.6	130±9	121 ±17
TBARS (nmol/gHb)	0.66±0.21	0.72±0.37[d]	0.5±0.23[c]	0.9±0.48[c]

Values are expressed as mean ± S.D. P < 0.05 was considered as significant.
a: p<0.001 compared with control group, b: p<0.01 compared with D and DS groups.
c: p<0.001 compared with the other groups, d: p<0.05 compared with control group.
Conclusion: Erythrocyte G-6-PD, SOD and GPx but not CAT activities were found to be significantly increased with and without sulfite treatment in SOX deficient groups. TBARS levels were found to be significantly increased in CS and DS groups and decreased in D group. These results suggest that erythrocyte antioxidant capacity where defense mechanism against the oxidative challenge may be up regulated by both endogenous and exogenous sulfite due to its oxidating nature. Altough this kind up regulation is also observed in DS groups, it seems to be insufficient.

A-13

Evaluation of an Electrochemiluminescent Immunoassay for Measurement of Insulin in Rat Plasma

R. McGeary, T. Sellers, D. Ennulat. *GlaxoSmithKline, King of Prussia, PA,*

Insulin is a peptide hormone secreted by the pancreatic β-cells of the islets of Langerhans that is involved in maintenance of blood glucose levels and regulation of carbohydrate, fat and protein metabolism. Inhibition of select protein kinases in the insulin signaling pathway is increasingly targeted for the development of new anti-cancer therapies, however pharmacologically-mediated inhibition of this pathway may result in altered insulin levels or function. Therefore, insulin may be a useful biomarker of pharmacologically-mediated effects of certain candidate anti-cancer drugs in nonclinical safety studies. We evaluated the Meso Scale Discovery® (MSD, Gaithersburg, MD) rat electrochemiluminescent immunoassay for the measurement of insulin in plasma from Sprague-Dawley rats. Advantages of this methodology for rodent species include the small sample volume

requirement (20 uL/duplicate analyses) and broad dynamic range, thus limiting the need for repeat analyses. Intra- and inter-assay precision was acceptable (CVs ≤6% and ≤15%, respectively) for commercially available rat insulin controls (Linco, Millipore Corporation, St. Charles, MI) and pooled rat plasma samples. Linearity/recovery was demonstrated up to 40000 ng/L (y=9622.3X - 8931, r^2=0.9999) using insulin calibrator spiked into pooled rat plasma. Plasma insulin concentrations for clinically healthy fasted rats (combined sexes) ranged from 21 to 741 ng/L. Plasma insulin concentrations were stable for up to 6 months at -80°C. To assess the utility of this assay in a drug safety study, plasma insulin was measured at 30 minutes, 1-, 2-, 4-, 8- and 24 hours post-dose on Day 1 and Day 28 of a 4-week study in rats given a compound known to inhibit components of the insulin signaling pathway. Dose-dependent increases in plasma insulin concentrations were observed over the 24-hour timecourse in drug-treated rats compared to control. Peak insulin concentrations were generally observed between 4 and 8 hours post-dose and progressed in magnitude in high dose rats by Day 28. In conclusion, the MSD rat insulin assay is a sensitive and precise micronized assay for measurement of plasma insulin in rats with demonstrated utility in the detection of drug-induced insulin increases in rats on a nonclinical drug safety study.

52BZC-2
K1C-2

A-14

Apoptozis after acute spinal cord injury in rats and the effect of Erythropoietin(EPO)

Y. E. Doventas[1], S. Yigit[1], M. Koldas[1], B. Ozdemir[2]. *[1]Haseki Edu.and Res. Hospital,Clinical Biochemistry, Istanbul, Turkey, [2]Haseki Edu.and Res. Hospital,Neurosurgery, Istanbul, Turkey,*

Erythropoietin(EPO) is a hematopoietic growth factor that stimulates proliferation and differentiation of erythroid precursor cells and is also known to exert neurotrophic activity in the central nervous system. The purpose of this study was to investigate the effectiveness of recombinant human EPO in attenuating the severity of experimental SCI.

A total of 32 spraue-dawley rats underwent clip-compression induced SCI .They were opereted on with posterior laminectomy.Spinal cord trauma produced by extradural placement of the anevriysm clip ,for 1 min.Animals were divided into four groups. The first group only were operated on with posterior laminektomy,second group were underwent clip-compression induced SCI. The third group received a low total dose. (EPO -L).(2 doses of 1000 IU each i.p) The fourth group received a high total dose (EPO-H) early post-op 5000iu/kg i.p. , in postop 24 h. 3000iu/kg ip and in post-op 48 h.1000iu/kg i.p. (3 doz-9000iu/kg). Follow-up was for 6 weeks. Estimation of the functional progress of each rat was calculated using the locomotor rating scale of Basso et al, with a range from 0 to 21.

RESULTS: After surgery the animals suffered paraplegia with urinary disturbances. Rats that received EPO demonstrated statistically significant functional improvement compared to the Control group, throughout study interval. On the last follow-up at 2 weeks the EPO-L rats achieved caspase 3 0,54 , the EPO-H 0,41 , and the control group 0,21 .Comparison between the two EPO groups reveals superior final outcome of the group treated with lower total dose.

CONCLUSION: Our study supports current knowledge, that EPO administration has a positive effect on functional recovery after experimental ASCI. These data reflect the positive impact of EPO on the pathophysiologic cascade of secondary neural damage.

A-15

Lateral flow immunochromatographic assay using europium chelate-loaded silica nanoparticle labels for highly sensitive detection of chloramphenicol

W. Yang, X. Xia, H. Zhang, Y. Xu, Q. LI. *XMU, Xiamen, China,*

Background: Due to its well-known highly toxic effects on humans, Chloramphenicol (CAP) is prohibited from use in food-producing animals in many countries. However, illegal use of CAP still remains due to its potency, availability and low-cost. CAP adulterated in animal supplies is one of essential global concerns in the past 30 years. A permanent control of CAP levels in foodstuffs of animal origin is indispensable and a simple, rapid and yet sensitive method is highly needed. A competitive lateral flow immunochromatographic assay using fluorescent europium chelate-loaded silica nanoparticle labels for highly sensitive detection of chloramphenicol is developed.

Methods: CAP specific polyclonal antibody was raised in rabbits against synthesized chloramphenicol-keyhole limpet hemocyanin conjugates. Chloramphenicol-bovine serum albumin conjugates were prepared and used as coating antigen. Detection results could be either qualitatively assessed by visual observation or quantitatively performed with a digital camera and Adobe Photoshop software owing to the unique ultra bright nanoparticle reporters.

Results: A detection limit of 0.1 ng/mL and a linear relationship from 0.2 to 4.0 ng/mL were achieved. Nanoparticle labels led to a significant improvement in sensitivity, which is at least 50 times that of colloidal gold immunochromatographic assay and comparable with that of enzyme linked immunosorbent assay. Applicability of the developed method was confirmed with spiked milk samples.

Conclusions: Combining the advantages of rapidness, ease of use, and high sensitivity of fluorescence detection with signal amplification of europium chelate-loaded silica nanoparticles, the described assay format is suitable for quantitative testing of chloramphenicol on site.

A-16

Fructosamine Assay for the Veterinary Laboratory

L. Leon. *Catachem Inc., Bridgeport, CT,*

In diabetes monitoring, to determine the effectiveness of treatments, veterinarians evaluate serum fructosamine (glycated serum protein) to determine the average glucose level being experienced by the animal over a 2-3 week period. This study compares a single liquid reagent test from Catachem Inc. with an established fructosamine test already on the market. Non-enzymatic glycation of blood proteins has been reported to occur through formation of ketoamines in a two step reaction: 1. Formation of a Schiff base by reversible coupling of glucose to protein. 2. Non-reversible Amadori rearrangement to the corresponding ketoamine or fructosamine. The amount of fructosamine in serum is increased in diabetes mellitus owing to the high concentration of glucose in the animals's blood. Catachem's kinetic fructosamine test is based on the ability of ketoamines to reduce nitro blue tetrazolium in alkaline conditions to form a purple colored formazan complex. This liquid stable, single reagent test is linear to 1000 µmol/L, has reduced interferences as it is run kinetically and can be applied to most automated instruments. A comparison of the data demonstrates the reagent's precision and shows its performance against an existing method.

Accuracy and Precision: Using an automated analyzer, correlation studies were carried out between Catachem's fructosamine procedure (Y) and a reference procedure based on the reduction of nitro blue tetrazolium (X). Serum samples were assayed and the results compared by the least squares regression. The following statistics were observed for Accuracy and precision:

Catachem's Fructosamine Precision						
Fruct.	**within-run**		**day-day**		**Total**	
Mean	SD	CV	SD	CV	SD	CV
µmo/L	µmo/L	%	µmol/L	%	µmol/L	%
215	9.6	4.45	15.9	8.04	15.98	7.76
513	14.29	2.78	22.37	4.68	25.91	5.23
830	19.34	2.78	27.17	3.44	30.81	3.81
Catachem's Fructosamine Accuracy						
Y=1.06 + 8.55 r = 0.995						

A-17

The Determination of Enzygnost Thrombin Anti-Thrombin III Complex in Pig Citrated Plasma to Support Pre-Clinical Toxicology Studies.

C. Starks, B. Litzenberger, M. Genato, J. Dharmadhikari, C. McDonough. *Huntingdon Life Sciences, East Millstone, NJ,*

Objective: The purpose of this project was to determine if the performance of the Siemens Diagnostics EIA test kit Enzygnost Thrombin Anti-Thrombin III Complex (TAT) (product #OWMG-15 or equivalent) was fit-for-purpose when analyzing pig citrated plasma to support pre-clinical safety assessment toxicology studies. Since the method is specific for human, matrix effect interference was evaluated.

Methods and Materials: The Siemens Diagnostics EIA test kit Enzygnost Thrombin Anti-Thrombin III Complex (TAT) (product #OWMG-15 or equivalent) is a sandwich enzyme immunoassay. TAT concentrations are quantified by measuring the test sample solution absorbance at a specified wavelength and comparing the values with those from a standard curve. The color intensity is proportional to the concentration of TAT. The calibration range was 2 to 60 ug/L. The Spectra Max 340 PC 384 Microplate Reader version 1.0 was used for testing. There were no design modification made to this assay, however a second control was made from the two highest standards provided in the kit. in order to demonstrate adequate analytical quality control over the calibration range (kit only offers 1 QC material).

Results: The Intra-assay precision and accuracy was conducted by analysing QCs and a pooled pig sodium citrated plasma in duplicate replicates of 5 within one run. The Intra-assay precision measurements ranged from 2.6% to 4.2 % RSD for QC samples and 8.4% RSD for Pig sodium citrated plasma pool. The recovery of the QCs ranged from 84.1%to 86.9 %.

The Inter-assay precision and accuracy was performed by analysing QCs and pool pig sodium citrated plasma in separate runs against at least three independent standard curves. The Inter-assay precision measurements ranged from 7.2 to 8.1 % relative standard deviation (RSD) for QC samples and 10.7 % RSD for the pig sodium citrated plasma pool. Matrix Effect was performed by preparing spiked samples, which consist of mixing each QC level with the pooled pig sodium citrated plasma at a 1:20 dilution. The mean recovery of the spikes samples ranged from 95.6% to 99.2 %.
The assay was used on a study to evaluate treatment effects of a test device applied to a coagulopathic swine model. The group mean values for pretest and two post treatment timepoints were 25.42 ug/L, 30.67 ug/L and 41.79 ug/L respectively.
Conclusion: The quantitative method for the measurement of Thrombin Anti-Thrombin in pig sodium citrated plasma using the Siemens Diagnostics EIA test kit Enzygnost Thrombin Anti-Thrombin III Complex (TAT) (product #OWMG-15 or equivalent) is fit-for-purpose when analyzing pig citrated plasma. The pre-clinical safety trial results demonstrates that the assay translates from human to pig and can serve as a biomarker for hemostasis evaluations.

A-18

Evaluation of newly developed ELISAs for rat, mouse, dog and pig NGAL

K. Bangert, J. O. Eriksen, L. O. Uttenthal. *BioPorto Diagnostics A/S, Gentofte, Denmark,*

Background Acute kidney injury (AKI) is a frequent complication of critical illness and major surgery. However, clinicians have had few possibilities of improving the outcome of AKI, which still carries a high mortality. AKI in humans can now be diagnosed at a very early stage by means of NGAL measurements. To facilitate the use of animal models in AKI research and toxicological studies, we have developed specific ELISAs for determining rat, mouse, dog and pig NGAL and here report their initial evaluation.
Methods NGAL was captured with a species-specific NGAL monoclonal antibody in precoated microwells and detected with another species-specific biotinylated monoclonal antibody. The specificity of the antibodies was ascertained by positive reaction with the peak of recombinant NGAL and the 25-kDa peak of native NGAL from plasma and urine subjected to molecular size exclusion chromatography. The assays were calibrated with recombinant full-length NGAL diluted in buffer. Normal plasma and urine samples (n = 2-5) were obtained from commercial sources. The intraassay (n = 6) and interassay (n = 4) variation was determined by repeated measurements of normal plasma and urine samples. The detection limit was defined as the NGAL concentration corresponding to the OD signal of the blank + 2 standard deviations (SD). Linearity was considered acceptable if the serial dilutions of plasma and urine did not deviate more than 15% from the expected values. Analytical recovery of recombinant NGAL added to plasma, urine or buffer was determined at 4 different levels covering most of the calibration curve.
Results

	Variation		Detection limit	Acceptable linearity	Analytical recovery	Normal levels Mean ± SD (ng/mL)	
	Intraassay	Interassay	pg/mL			Plasma	Urine
Rat	0.9-2.6%	2.9-5.6%	0.23	Yes	91-104%	233 ± 82	847 ± 584
Mouse	1.9-5.9%	3.3-6.0%	0.70	Yes	93-103%	235 ± 82	137 ± 23
Dog	2.3-4.5%	2.8-5.8%	0.56	Yes	97-106%	13.5 ± 0.4	7.6 ± 2.5
Pig	1.6-3.0%	0.8-3.3%	0.81	Yes	90-101%	162 ± 212	134 ± 48

Conclusions The newly developed ELISAs have an acceptable performance and can be used to test whether NGAL responses in animals are similar to those in humans. The ELISAs may prove to be valuable tools for investigating AKI in drug discovery, toxicology, experimental surgery and disease models.

A-19

The Effect of Chronic Hypoxia and Carbon Monoxide on Oxidative Stress in Rat Vascular Tissues

J. Kalra[1], H. Neufeld[2], C. Vandier[3], R. Wang[4]. *[1]Royal University Hospital, Saskatoon, SK, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada, [3]Universite Francois Rabelais de Tours, Tours, France, [4]Lakehead University, Thunder Bay, ON, Canada,*

Objectives: It has been suggested that the oxidative stress can be compounded by hypoxia and can contribute to the apoptotic process. We have previously suggested an important role for oxidative stress in heart failure and atherosclerosis. We studied the biochemical changes and resulting enzymatic oxidative stress level in vascular tissues of rats due to chronic hypoxia (HC) or carbon monoxide (CO) inhalation.
Methods: Adult female wisteria rats (220-270g) were divided into three groups: Group A rats (control group, n=5) lived under normal laboratory conditions. Group B rats ("CO" group, n=8) lived for 3 weeks with chronic CO inhalation (530 ppm). Group C rats ("HC" group, n=10) lived under chronic hypoxia conditions (hyperbaric chamber @0.5 atm (50.5 KPa)) for 3 weeks. Their aortic tissues were analyzed for expression of apoptotic proteins (Bcl-2, Bax and Mcl-1) and hypoxia related heme oxygenase proteins (HO-1 and HO-2). Tissues were also assayed for levels of lipid hydroperoxide (maldondialdehyde (MDA)) and activities of lipid hydroperoxidase (FOX2 assay) and antioxidant enzymes (superoxide dismutase, SOD), catalase (CAT) and glutathione Peroxidase (GSH-Px).
Results: Our study indicated significant (<0.05) changes in the following groups (as measured by 1 tailed t-test): CO animals showed an increase in MDA ($p=0.016$) and decrease in HO-1 ($p=0.046$) and SOD ($p=0.024$) in comparison to the control animals. HC animals showed increased expression of lipid hydroperoxidase ($p<0.001$) and catalase ($p<0.001$), and decreased expression of GSH-Px ($p=<0.001$) in comparison with control animals. It is important to note that the CO and HC animals were significantly different in their stress responses in a number of ways: HC animals had the highest increases in MDA ($p=0.025$) and lipid hydroperoxidase ($p<0.001$), while decreases in HO-1 ($p=0.004$) and SOD ($p=0.043$) were noted in both CO and HC with the levels decreasing more in HC groups. CO rats also had higher levels of catalase ($p<0.001$) and GSH-Px ($p<0.001$) than the control animals while HC was much higher than the control animals.
Conclusions: These findings strengthen the current theory that the heme oxygenase protein family plays an important role in the response to hypoxia and related oxidative stress, and suggest that hypoxia and carbon monoxide affects oxidative stress differently.

Tuesday AM, July 27

Poster Session: 10:00 am – 12:30 pm
Automation/Computer Applications

A-20

Creating Informative, Useful, and Eye-Appealing Laboratory Reports Using Excel™ Software

F. H. Wians. *Lone Star Pathology Laboratory and Scientific Writing Consultants, Inc, Helotes, TX,*

Excel™ spreadsheets can be used to provide highly informative, eye-appealing, patient-specific laboratory reports that combine the power of the calculational and graphics features in this powerful software program. Moreover, the logical function arguments in Excel can be used to provide automated interpretive information and the rationale for the interpretation. These advantages are especially desirable when significant amounts of laboratory data are used to calculate various simple or complex indices and these indices are used routinely to provide important diagnostic and/or prognostic information with significant clinical impact. Six Excel-based patient-reports were developed for calculating, graphing, and/or interpreting the laboratory data used in the assessment and/or monitoring of: 1) cerebrospinal fluid oligoclonal bands and indices; 2) adrenal gland lateralization; 3) intra-operative parathyroid hormone (PTH) testing; 4) the location of an ectopic PTH-secreting tumor based on selective venous sampling for PTH; 5) the source of elevated serum adrenocorticotropic hormone (ACTH) in patients who have undergone inferior petrosal sinus sampling for ACTH due to a suspected pituitary ACTH-secreting tumor; and, 6) a plasma cell dyscrasia based on serum levels of kappa- and lambda free light chains. **Input** data for each of the Excel-based reports consists of the results for all individual laboratory tests used to calculate the required indices and/or ratios for each of the aforementioned six reports. The **output** reports contain graphs of patient data and interpretive information, along with, where appropriate, colorful anatomic figures that identify the type of report. The accuracy and reliability of all calculations and interpretive information performed or provided on each report were validated using three separate approaches: 1) by comparing the results of manual and Excel-based calculations using the same data; 2) by comparing the results of Laboratory Information System-programmed and Excel-based calculations using the same data; and, 3) validation of the interpretive information by an appropriately qualified and experienced, board-certified physician. All Excel-based patient reports containing interpretive information were reviewed and endorsed by such a physician, prior to release to the requestor. Using real-world patient data, examples of all six Excel-based, patient reports will be shown, including the formulas used to calculate all parameters and the logic rules used to provide interpretive information. The principal advantages of Excel spreadsheet-based patient reports include the ability to prepare rapidly patient-specific laboratory reports using a single software program that is readily available in most clinical laboratories and provides error-free (provided the raw data are entered correctly) automated calculation, interpretation, and/or graphical display of large amounts of simple or complex data in a comprehensive, highly informative, and useful report that can be inserted into the patient's medical record.

A-21

Retrospective analysis of serum creatinine patient data from before and during a calibration failure interval to evaluate sensitivity and specificity of a simple running means algorithm for patient-based quality control (QC)

A. J. Horn, J. D. Landmark, D. F. Stickle. *University of Nebraska Medical Center, Omaha, NE,*

Background: Our laboratory recently experienced a 12-hour interval of undetected miscalibration for serum creatinine (-35% proportional bias) due to a procedural error utilizing the Beckman DxC analyzer. We examined error-associated patient data and 1-month's prior patient data to evaluate whether simple monitoring of running means of patient data could have succeeded in early detection of this particular error with reasonably high specificity.

Methods: Error-interval data comprised 86 serum creatinine results over approximately a 12-hour interval that had a proportional bias of -35% (corrected result = 1.53 x reported result; r^2 > 0.99). The preceding month's patient data for serum creatinine (21,713 results) was obtained as a control dataset, which was assumed to have zero bias. A restricted results interval of 0.3-1.5 mg/dL was selected as the basis for calculation of running means, which represented approximately the central 95% bounds of the best-fit normal curve associated with the lower half of the control dataset's cumulative results distribution. The restricted error dataset overall mean (70 results, average 0.67 mg/dL) differed from that for the restricted control dataset (17,980 results, average 0.87 mg/dL) by -0.20 mg/dL (delta value). Using running means calculated as a function of the sample size, *n* (from *n*=10-70), we tabulated the number of discrete intervals of QC failures that would occur for the control dataset (i.e., the number of false positives that would occur during one month) for assumed delta value QC limits. For the same assumed delta value QC limits, running means as a function of *n* were determined for 1000 replicates of the error dataset in which the order of the 70 results was randomized, and the percentage of replicate error datasets with QC failures was tabulated. The results (with parameters of delta value QC limits and sample size *n*) were evaluated for sensitivity (to flag the error datasets) and for specificity (to not flag data within the control dataset).

Results: Delta values less than the observed delta value were needed in order to maintain high sensitivity for detection of error in all replicates of the error dataset, while using *n* large enough to avoid an unacceptably high rate of false positives in the control dataset. Using *n*=40, a delta value of -0.17 mg/dL (-20% bias) had 100% sensitivity for detection of error within the error datasets, with an average of 42 samples needed for error detection. These parameters had a borderline-acceptable false-positive rate (flagging of data in the control dataset) of 4/month (1/week). For $n \leq 35$, the false positive rate in the control dataset was greater than 2/week. Bias correction in the error datasets produced no false positives. Bias reduction in error datasets to -20% reduced sensitivity for error detection to 95%, with average length to detection of 47 samples.

Conclusions: A QC algorithm using simple running means of patient data for *n*=40 would have identified this particular serum creatinine calibration error at a sample interval that was less than half of the undetected error interval, but with penalty of an anticipated false positive rate of 1/week.

A-22

Goal-based Quality Control: Exploring Use of Standardized Control Charts and Procedures

D. M. Parry. *St Boniface General Hospital, Winnipeg, MB, Canada,*

Detecting unacceptable error or change in assay performance is a universal quality control challenge and current wisdom is that control charts and control procedures should be based on actual performance data. Unfortunately, this approach to setting quality control practices is seriously undermined by the difficulty, variability and inconsistency in obtaining representative performance data.

In goal-based quality control, performance goals are used instead of performance data to determine the precision and accuracy that are required for acceptable assay performance. This approach uses a tolerance budget for bias (bias budget) instead of measured bias. Measured bias is subject to change over time and therefore control practices based on measured bias require ongoing adjustment, whereas bias budget requires only monitoring measured bias to ensure that it falls within budget.

The objective of this project is to illustrate use of performance goals (instead of performance data) for setting control limits (standard deviation) of control charts.

A goal of 3.5 sigma for assay performance and a goal of 15% of its performance standard (PS) for bias budget were selected to determine to the control limits (SD_{cc}) for three different automated assays by the following formula:

$SD_{cc} = [(PS - 0.15PS)/3.5] \times MEAN_{QC} \times 1/100$

Data to illustrate derivation of goal-based quality control limits.

ANALYTE	Performance Standard	Performance Goal (Sigma)	Bias Budget Goal	$Mean_{QC1}$	SD_{QC1}	CV_{QC1}	n_{QC1}	Goal-based QC Limits (SD_{cc})
Glucose	10%	3.5	1.5%	3.4	0.08	2.5	798	0.08
CK	30%	3.5	4.5%	93.9	4.06	4.3	997	6.84
Sodium	4 mmol/L	3.0	0.6 mmol/L	110.8	1.31	1.2	791	1.26

For maximum error detection, the multi-rule control procedure (1-2s/1-3s/2-2s/R4s/4-1s/10x) can be used. This quality control strategy is considered exploratory until empirically validated.

It is concluded that goal-based quality control has a number of potential consequences: 1) standardized control charts (Levey-Jennings charts) simplifying implementation across laboratories, 2) minimal lag period to obtain control means, 3) consistency across labs in a region and 4) provides a point of reference to labs for comparing actual performance.

A-23

BD Vacutainer® Rapid Serum Tube and Siemens Dimension Vista® 1500 Intelligent Lab System - Improvements in Laboratory Turnaround Time Metrics

J. C. Wesenberg, K. L. Ost. *David Thompson Health Region, Red Deer, AB, Canada,*

The BD Vacutainer® Rapid Serum Tube (RST) contains thrombin and gel barrier. The thrombin promotes rapid clotting allowing centrifugation within 5 minutes to provide serum within a timeframe comparable to plasma. The Siemens Dimension Vista® 1500 Intelligent Lab System (Vista) incorporates four technologies to enable analyzer consolidation and enhanced workflow.

This study determined improvements in laboratory turnaround time (TAT) metrics in a Lean laboratory environment for all specimens collected at Red Deer Regional Hospital following replacement of two chemistry and two immunoassay analyzers with two Vista systems and replacement of BD Vacutainer® SST™ Tube (SST) and BD Vacutainer® PST™ Tube (PST) with RST.

Baseline metrics were determined in June 2009 for creatinine (SST, chemistry analyzer), troponin (PST, chemistry analyzer) and β-hCG (SST, immunoassay analyzer). Metrics included the daily mean for the number of specimens, average TAT from received in laboratory to result verification and the percentage of results verified within 30, 40, 50 and 60 minutes. Metrics were reassessed in September after Vista implementation in July and then again in November after RST implementation in October.

Test	Creatinine			Troponin			β-hCG		
Month	Jun	Sep	Nov	Jun	Sep	Nov	Jun	Sep	Nov
Analyzer	Chem	Vista	Vista	Chem	Vista	Vista	Imm	Vista	Vista
BD Tube	SST	SST	RST	PST	PST	RST	SST	SST	RST
	Daily Mean			Daily Mean			Daily Mean		
# Specimens	189	192	191	40	46	44	6	6	6
Average TAT min	51	48	39	48	41	40	88	64	50
% Verified									
< 30 min	6	8	25	1	9	9	0	2	6
< 40 min	26	28	58	21	51	53	3	9	31
< 50 min	53	55	79	55	77	78	12	25	62
< 60 min	74	77	89	78	90	90	27	53	83

The overall improvement in average TAT for creatinine (24%) was contributed to by Vista (6%) and RST (18%). For troponin, Vista provided a 17% improvement in average TAT. Since PST was used previously (to avoid latent clot formation common in cardiac patient serum specimens), there was no further improvement with RST. Overall improvement in average TAT for β-hCG (43%) was contributed to by Vista (27%) and RST (16%). Improvements in the percentage of tubes verified at each time interval were also observed for each test.

Significant improvements in laboratory turnaround time metrics were achieved by analyzer consolidation and enhanced workflow with Vista and by enhanced workflow through the replacement of SST with RST.

A-24

Comparison of Manual versus Automated On-board Dilutions on the Beckman Coulter UniCel® DxI 800 Immunoassay System

S. P. Wyness[1], W. L. Roberts[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]University of Utah, Department of Pathology, Salt Lake City, UT,*

Background: Automation in the clinical laboratory can increase laboratory efficiency while maintaining or improving quality. The on-board dilution capability on the Beckman Coulter UniCel DxI 800 analyzer was compared to manual dilutions.

Methods: The study was performed with 5 different immunoassays available on the DxI 800 platform (BR Monitor, GI Monitor, insulin, myoglobin and Ostase). Manual dilutions to on-board dilutions were compared in terms of recovery and labor savings. The amount of time saved per month was estimated based on hypothetical monthly test volumes.

Results: A summary of the overall correlation between manual versus on-board dilutions is shown (Table 1). Mean recoveries of automated versus manual dilution ranged from 95.0-102.7%. On average the amount of time saved with on-board dilutions was 4 minutes per sample. Hypothetical monthly volumes used were 100, 144, 310, 343, and 875, for myoglobin, Ostase, BR Monitor, insulin, and GI Monitor, respectively. We examined our actual lab results to determine the percentage of samples that required dilution. These percentages were 0.95, 2.01, 2.83, 2.96, and 9.16, for insulin, Ostase, myoglobin, BR Monitor, and GI Monitor, respectively. The monthly time savings arranged in order of greatest to least impact are GI Monitor 321 minutes, BR Monitor 37 minutes, insulin 13 minutes, Ostase 12 minutes and myoglobin 11 minutes.

Conclusion: The new automated dilution capability on the UniCel DxI worked well for all five assays tested. This capability can be applied to any assay that requires dilutions offered on the platform. Implementation of the on board dilution capability on the DxI 800 platform is a valuable feature that should improve efficiency and maintain or improve quality.

Table 1 Method comparison

Analyte	N	Mean % Recovery ±SD	Slope	R
BR Monitor	36	102.7 ± 5.5	1.02	0.996
GI Monitor	26	101.1 ± 8.2	0.95	0.999
Insulin	27	97.3 ± 5.2	0.94	0.999
Myoglobin	27	99.4 ± 6.5	0.98	0.997
Ostase	27	95.0 ± 5.6	0.93	0.993

A-25

Consolidation And Discrete Automation Of The University Malaya Medical Centre (Ummc) Hospital Laboratory With Siemens Vista, Centaur Xp And Streamlab

M. Thevarajah[1], Y. Chew[2], Y. Chan[2]. *[1]University Malaya, Kuala Lumpur, Malaysia, [2]University Malaya Medical Centre, Kuala Lumpur, Malaysia,*

Background: Our laboratory is integrated in as a tertiary academic hospital of more than 700 beds. Per year about 4 million clinical chemistry, hematology and immunodiagnostics results are reported. Targeted automation is an essential element of laboratory workflow with the drive towards economic efficiency and improved service quality. Our aim was to assess the impact of the installation of a Siemens Diagnostics Dimension Vista, Centaur XP, Easy Link Connectivity and StreamLab on the workload flow within our facility.

Methods: Siemens Diagnostics was awarded the automation tender in January 2009 in our facility after complying with the tender requirements set out in January 2008. After a reconstruction phase of 3 months to achieve a central core lab, the clinical chemistry and immunodiagnostics methods were consolidated on a new automation solution which included the addition of sample manager, on-line centrifugation, extended robotic track and a capping/de-capping module.

Results: Analytical systems could be decreased from the previous 4 RxL Dade Dimensions to 2 Dimension Vista systems. The 2 Centaur XP linked on the automation track allowed the laboratory to consolidate the clinical chemistry and immunoassay testing more efficiently. Time consuming steps like decapping and aliquoting were eliminated resulting on increased productivity per technician, a decreased TAT for most parameters and therefore a more rapid reporting of patient results. The StreamLab preanalytical sample processor further enhanced tube management, sorting and processing.

Conclusion: By use of the new Siemens automation solution, we could achieve a highly consolidated sample throughput for a broad spectrum of chemistry and immunodiagnostics methods. This in turn has resulted in immediate marked improvements in turnaround times for both clinical chemistry and immunoassay based analytes.

A-26

Development of an Excel based tool for Hepatitis B Virus genotyping and detection of antiviral resistance patterns

R. Sitnik, R. A. F. Santana, O. S. Ramos, L. Oyakawa, G. T. F. Dastoli, R. C. Petroni, V. F. D. Castro, C. B. Moyses, C. L. P. Mangueira, J. R. R. Pinho. *Hospital Israelita Albert Einstein, São Paulo, Brazil,*

Background: Hepatitis B virus (HBV) is a major cause of chronic liver disease worldwide. This virus has been classified into eight genotypes (A-H) with distinct geographic distribution worldwide. Molecular characterization of HBV is a useful tool in the management of HBV infected patients and many laboratories throughout the world are using it for genotyping and drug resistance mutations detection. To access these data, we have developed a method to allow identification not only of HBV genotypes and subgenotypes, but also of drug resistance mutations to major drugs in only one sequence reaction. The use of this new system improved the information obtained in only one PCR product, but sequence analysis was very laborious and manual.

Methods: To optimize this analysis we have developed an Excel application linked to a database with different mutation combinations and the resistance pattern related to different drugs based on international guidelines (Lamivudine - LMV, Adefovir, Entecavir,

Emtricitabine, Tenofovir, Telbivudine and Clevudine). With this application, all the analysis can be done by copying or exporting data from the sequencer to a specific cell on the Excel sheet. After this, the program performs the analysis and releases the patient report that includes different patterns to each drug: resistance, partial resistance and other relevant observation and/or references.

Results: Using this application, we have analyzed retrospectively 324 patients that had been tested in the Clinical Laboratory from a Private Hospital for HBV genotyping from March 2006 to December 2009. Previous reports were compared with the results obtained using the Excel application and it was able to correctly identify the resistance pattern found, in a much faster and friendly manner than with manual analysis. Also, it was possible to create a customizable database with all patient data, including genotypes, age, gender, or other relevant information. This allowed the analysis of genotypes and resistance patterns distributions among our population. Frequencies of the most common genotypes were: A - 43%; D - 29% and F - 14%. The number of patients with any known drug resistance mutations changed from 29.3% in 2006 to 32.5% in 2007, to 50% in 2008 and, in 2009 to 44.1%, suggesting an increase tendency. The most frequent mutations were related to LMV resistance, which was present in 18.9% of the patients in 2006 and in 36.6% in 2009. Although our results reflects only a small sample of patients, we have found genotypes A, D and F as the more frequent ones, although in a large country such as Brazil even rare genotypes in South America can be found, such as genotypes E and G. Our results also showed that there is an increasing number of patients resistant to many drugs, especially to LMV that has already been used for many years.

Conclusion: All resistance patterns were correctly detected by the developed tool and a database with patient data was created allowing further epidemiological studies. Furthermore, this tool allows results interfacing, automated report generation and may be very helpful for laboratories with a large demand of this assay.

A-27

Impact of Result Auto-verification (Software Automation) in Hematology

K. Hoi. *Abbott Diagnostics, Singapore, Singapore,*

Changi General Hospital (CGH) is an 800-bed acute-care general hospital in Singapore with CAP accreditation. Samples are received from clinics and wards via pneumatic tubes. Upon arrival, an order is entered in the LIS. Post-analysis, results are reviewed and released back to the LIS. Turnaround time (TAT) is measured from the time of sample reception to result availability in the LIS.

In 2007, one of the laboratory's objectives was to improve TAT for full blood count (FBC) testing while maintaining high quality standards and current staffing levels. This was despite expected growth in test volume. CGH implemented the following initiatives:

•Streamlined pre-analytic workflow by processing all samples as soon as received

•Installed two identical CELL-DYN Ruby hematology instruments for real-time backup and parallel analysis instead of one large routine and one smaller backup analyzer

•Test results auto-verified via Accelerator Instrument Manager middleware (Abbott) to improve TAT, reduced manual slide reviews and eliminated errors

•Placed LIS printers in all wards and clinics to eliminate time and labor of delivering printed reports

Auto-verification algorithms were adapted from International Society for Laboratory Haematology rules. Additional rules were added including i.e. hold-back rules for numeric-parameters include:

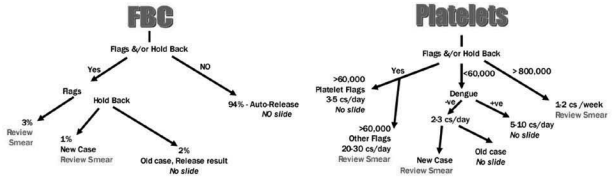
•Hb: <7.0 or > 18.4 g/dL

•Hct: <20.0 or >55.0%

•WBC: <2.0 or >30.0 x10^3/uL

•Platelets: <60 or >800 x 10^3/uL

Some decision tree-graphs are shown below:



Auto-verification helped the laboratory achieve:

•Consistent and standardized way of sample processing, analysis and reporting

•Improved TAT from 25 minutes to 17 minutes

•Increased TAT consistency

•Auto-verification of 92% - 95% of all results

•Maintained two technologists despite workload increasing from 300 to 400 daily samples

•Reduced daily manual film review rates from 90 to 20 per day

Auto-verification via middleware has been a major benefit to CGH.

A-28

A comparison of UF-1000i and manual microscopy for urine sediment analysis

G. Oh. *Eone Reference Laboratory, Seoul, Korea, Republic of,*

Background: Manual microscopic urine sediment evaluation is essential and provide important information but it is hard to standardize and requires significant labor. Recently, several automated analyzers were developed and have been introduced. We compared the performance of Sysmex UF-1000i with manual microscopy in urine sediment testing.

Methods: Seven hundred and eight samples were collected. The urine sediments were examined by manual microscopy and Sysmex UF-1000i automated urinalysis system.

Results: The within-run CVs for urine control samples ranged from 1.14% to 15.95% for UF-1000i. The agreement rates between methods were 92.0% for red blood cells, 85.4% for white blood cells, 92.6% for epithelial cells and 94.6% for bacteria. Microscopic review rate were 4.0% for instrumental reason, 20.5% for flags and 2.3% for both reasons. Total review rate was 29.0%. Most common cause of manual microscopic revisions was flag of crystals.

Conclusion: This automated urinalysis system demonstrated good concordance with microscopy. Using the automated process as a screening test can reduce workload, but additional microscopic evaluation is necessary.

A-29

Assessment of Turn Around Time for Add-on Tests by Using Automated Refrigerated Storage

G. Uppal, M. Szydlowska, M. Jin. *Temple University Hospital, Philadelphia, PA,*

Introduction: The laboratory receives high volume of add-on requests. Searching the specimen manually is often time consuming. Sometimes, it is time consuming in searching for a specimen. The turn around time (TAT) for the add-on tests is variable depending on the time spent in searching the specimen. We installed the Beckman automation analytical system. With the new automated system with automated refrigerator storage (stockyard), we expect to improve the TAT, sample storage and reduce both pre and post-analytical errors.

Objective: This study was to evaluate the effectiveness of Beckman Coulter automated refrigerator storage (stockyard) in reducing the TAT for the add-on tests and evaluation of other benefits.

Methods: We analyzed add-on tests in a selected two weeks period. All the tests are performed on Beckman automation system (with the automation line, auto-centrifuge, DxI, DxC and LX20 analyzers, and refrigerated stockyard). The refrigerated stockyard is integrated to analyzers via a sample power processor. The specimen can be automatically delivered to the stockyard and retrieved back to analyzer. Since the analytical time is the same, we compared the automated pre-analytical time for the add-on tests with the manual pre-analytical process for the add-on tests. The overall difference in the TAT for the add-on tests is attributed to the difference in the pre-analytical time.

Results: There were total 458 add-on tests in the two weeks period. Most add-on tests were liver function tests (107), cardiac markers including cTnI, CK, and CK-MB (70), amylase and lipase (39), and vancomycin (36). The major time for these requests was from 7:00 am to 7:00 pm with the peak time from 11:00 am to 12:00 pm. We compared the pre-analytical time for the add-on tests by the automation stockyard and manually. The average pre-analytical time for the sample retrieved from stockyard is 11 minutes (10-12 minutes). The average pre-analytical time for the manual process (searching the sample, loading the sample etc.) is 17 minutes (2-32 minutes). 86% of the 458 add-on tests were processed automatically on the automation line; the other 14% add-on tests are processed manually due to different tube size and hence cannot be stored in the refrigerated stockyard (Beckman automation system requests only one tube size).

Conclusions: The automated refrigerated storage (stockyard) has improved the average TAT for the add-on testing. The stockyard system has other benefits in the reducing number of errors, minimizing the exposure to the specimen, and providing the technologists time to focus on value-added tasks.

A-30

A format for presenting cumulative antibiogram using a novel in-house software ABSOFT®

G. WILSON, S. Badarudeen. *Hamad Medical Corporation, Doha, Qatar,*

Background: When antibacterials are selected for the empiric treatment of any infections, the knowledge locally of the most likely causative organisms and the prevalence of resistance of pathogens to antibacterial agents are essential. This involves generating a cumulative antibiogram. The paper highlights real time analysis of the cumulative

antibiogram additionally suggesting the format for its presentation using novel in house software ABSOFT®. To our knowledge till date, no format has been recommended for the presentation of cumulative antibiograms.

Methods: All microbiology samples coming to the microbiology laboratory from January 2008 to December 2008 were included in the study and the data (patient ID, Sample Number, Sex, Age, Sample type, Location, Organism and Antibiotics tested) was entered into an in-house software, ABSOFT® developed by the laboratory. Data was validated using pre-encoded algorithms; however the data entry process was manual. The presentation of *Streptococcus pneumoniae antibiogram* data was selected as a quality indicator because of emerging antimicrobial resistance and changes in the interpretive criteria for susceptibility testing.

Results: For our sample size it took an average of ~ 3 minutes daily which included the validation and correlation of the organism with the antibiotic using expert rules which added a safety component for patient care. Meaningful results were easily generated in color coded graphical format and a printed report received in ~ 3 minutes of query. The visual clarity and uniformity of the reports allowed writing of a report in a day.

Conclusion: ABSOFT® provides a simple, cost effective, reproducible, accurate, reliable, solution through innovative data entry, analysis and real-time presentation of the cumulative antibiogram. Yearly surveillance data forming part of data studies on a larger scale, collected and presented in a common format can be used to monitor any changes in epidemiology of infectious bacteria and the sensitivity pattern of pathogens causing infection. Trends in resistance of organisms to common medications can help in modifying the hospital antibiotic policy and can have a tremendous impact in general practice providing accurate, safe, effective and economical antibiotics for the patient.

A-31

GLM and Multivariable Statistical Comparisons of Means for Analysis of Mutiple Variables in QC Practices. A practical example.

V. M. Genta[1], R. Murray[1], D. Greiber[1], Y. Shen[1], D. Cline[2], S. Spingarn[2].
[1]Sentara Virginia Beach General Hospital, Virginia Beach, VA, [2]Sentara Norfolk General Hospital, Norfolk, VA,

Introduction Recently, the SVBGH physicians requested that the TSH assay be performed in house to improve the turn-around time. Consequently, the precision of the method was evaluated to verify its acceptability and to establish the QC parameters.

Materials and Methods Two COBAS 6000® (Roche Diagnostics) were employed by the chemistry department of Sentara Virginia Beach General Hospital Laboratory. After the method was installed by the manufacturer's representative according to his specifications, the precision of the method was verified with a short-term protocol. In brief, five independent assays were performed each day for five consecutive days with two levels of control material (IAP1® lot# 40721 and IAP3® lot# 40723, Bio-Rad) with two COBAS 6000 instruments. Each COBAS 6000 instrument performs immunoassays with two independent cells.The observations for each cell of each instrument and for each day of operation were recorded and transferred to Minitab® (Minitab, Inc.) statistical software for statistical analysis.

Results Descriptive statistics for each level of control showed: IAP1, mean=1, s =0.03, CV=3%, min=0.96, Q1=0.98, median=1, Q3=1.03, max=1.09; IPA3, mean=25.7, s=0.7, CV=2.3%, min=24.4, Q1=25.2, median=25.5, Q3=26.0, max = 27.6. The histogram of the observations for each level of control showed a quasi-normal distribution. The homogeneity of variances by day, instrument, and cells was confirmed by Levene's statistical test (P = 0.03 for IAP1, and P = 0.68 for IAP3) and Bonferroni's multiple 95% CI. This was optimal for analysis with the GLM and Bonferroni's multiple comparisons of means. Analysis with the GLM showed statistically significant differences (F<0.001) for instruments cells and days for both IAP1 and IAP3. However, Bonferroni's multiple comparisons of means and their 95% simultaneous intervals showed that these differences were minimal (IAP1, maximum difference = 0.06 mIU/mL; IAP3, maximum difference = 0.6 mIU/mL). These differences were not significant for either QC or clinical practices. The statistical significance was due to the very small variance for the GLM (MSE for IAP1 = 0.0004, for IAP3 = 0.28). Furthermore, the parallel box plots by date, instrument and cells clearly illustrated in one graphical representation the quasi-normal distribution of the observations, the homogeneity of means and variances, and the absence of either shifts or trends.

Discussion and Conclusions These results clearly indicated that the GLM, Bonferroni's multiple comparisons of means and the parallel box plots allow the comparison of multiple means with one statistical technique and one graphic representation. This is a definitive advantage over univariate methods (e.g. univariate t-test, one way-ANOVA, and Levey-Jennings charts). Furthermore, the multivariable techniques can quickly identify the major components of variability and assign a cause to an out-of-control situation. Finally, this example clearly shows that well designed statistical software packages, which offer a rich menu of statistical analysis techniques (e.g. Minitab, SAS®, SPSS®), are versatile and powerful for analyzing observations generated by complex QC designs dictated by the realities of laboratories with multiple instruments. Consequently, they are preferable to more limited software packages which offer only a limited menu of univariate statistical techniques.

A-32

Performance Evaluation of the Roche cobas® 8000 modular analyzer series*

S. Preston[1], R. Glover[1], R. Ryder[1], M. Elam[2], D. Bruton[2], J. Layton[2]. *[1]Huntsville Hospital, Huntsville, AL, [2]Roche Diagnostics, Indianapolis, IN,*

Objectives: The **cobas** 8000 modular analyzer series was evaluated by Huntsville Hospital Laboratory with selected general chemistry methods. The **cobas** 8000 modular analyzer series is a selective (random) access automated analyzer module for large volume laboratories with the capability of determining concentrations or activities of various substances in body fluids like enzymes, substrates, electrolytes, and specific proteins. The **cobas** 8000 core and dual **cobas c** 701 modules are the configurations used for this evaluation.

Methods: The system and selected assays were evaluated for Repeatability (within-run precision over different pipetting units and modules), Method Comparison, On-board QC stability, Reagent Drift Study, and Recovery of Daily QC. The core assays evaluated were: AST, GGT, CHOL, CREA, GLUC, BUN, CA, Na, K, CL, and CRP.

Results: Repeatability was well within acceptance criteria of ≤2% from one pipetting unit and ≤3.2% from multiple pipetting units. Method Comparison on **cobas** 8000 modular analyzer series demonstrated close agreement to **MODULAR** *ANALYTICS* system using routine reagents.

Test, Unit, Method	cobas Repeatability %CV				Method Comparison				
	mean	1 pipettor	mean	all**	n	range	slope	intercept	r
AST, U/L, IFCC	49.10	1.0	48.61	1.6	269	9-1077	1.00	-0.3	0.999
GGT, U/L, Szasz	41.59	0.9	40.63	1.6	270	4-1260	0.995	1.45	0.999
CHOL, mg/dL, AK	97.80	0.8	95.42	1.1	270	42-432	0.996	4.217	0.997
CREA, mg/dL, enzy	1.06	0.8	1.02	1.4	270	0.2-15.7	1.103	0.008	0.999
GLUC, mg/dL, HK	98.9	0.4	95.9	0.8	270	20-704	1.034	1.683	0.998
BUN, mg/dL, urease	19.79	0.7	18.87	1.3	230	2-164	1.05	-0.725	0.997
CA, mg/dL, o-cresol.	8.43	0.5	8.59	0.9	270	2.6-11.0	1.056	-0.239	0.924
Na, mmol/L, ISE	123.0	0.2	122.5	0.4	269	107-171	1.04	-3.48	0.974
K, mmol/L, ISE	3.38	0.3	3.37	0.5	268	1.5-6.9	0.988	0.166	0.992
CL, mmol/L, ISE	83.74	0.3	82.85	0.6	269	82-133	1.046	-5.346	0.976
CRP, mg/dL, latex	1.25	0.8	1.25	2.6	197	0.1-35.6	1.026	0.005	0.998
** ISE 2 modules, others 4 pipetting units									

On-board QC stability was evaluated on two control materials stored on-board and analyzed on one pipetting unit in singlicate at baseline and every hour for eight hours. Reagent and calibration drift was evaluated on three materials analyzed on one pipetting unit at baseline and every half hour using fresh aliquots for eight hours. During both experiments, no systematic deviation >3% from the initial value was observed after eight hours. Daily QC with two control levels was performed prior to every chemistry performance experiment on all pipetting units. All selected assays demonstrated control results within ±2SD of the mean.

Conclusion: Analytical performance on the **cobas** 8000 modular analyzer series met acceptance criteria for repeatability, correlation, on-board QC stability, calibration and reagent drift.

*This analyzer is currently under development and has not been cleared for use in the US by FDA.

A-33

Patient Misidentifications Caused By Errors in Standard Barcode Technology

M. L. Snyder[1], A. Carter[2], C. R. Fantz[2]. *[1]Brigham and Woman's Hospital, Boston, MA, [2]Emory University, Atlanta, GA,*

Barcode technology is utilized in nearly every facet of healthcare, from pharmacy and point of care testing to inventory and nutrition. Although linear barcode technology has improved the incidence of identification errors in many healthcare applications, barcode-related misidentification can still occur.

Objective: The objective of this study was to investigate sources of barcode decoding errors and suggest solutions to prevent their occurrence.

Methods: At an academic medical center, using hospitalized inpatient wristbands, defective and control barcodes were visually analyzed for damage and printing errors. Barcodes were then scanned by two or more operators using five different scanner models. Barcode substitution and rejection rates were determined for each barcode and scanner.

Data integrity algorithms were manually calculated for each defective barcode. **Results:** Eleven misprinted armband barcodes were shown to generate as many as three incorrect patient identifiers when scanned multiple times using several scanner models. Of the five scanner models tested, the Roche ACCU-CHEK® (RACG) glucometer had the highest substitution error rate (as high as 96.7%). Barcode scanner misreads were not unique to the RACG glucometers. Laser, CCD/LED and omni-directional barcode scanners were all shown to generate errors. Interestingly, the two laser-based scanners generated vastly different substitution and rejection error rates. While the RACG produced the highest substitution rates of all scanners tested, the Metrologic scanner was more likely to reject the defective barcodes, yielding a 0% substitution rate, but significantly higher rejection rate. The remaining scanners had similar substitution and rejection rates, falling between relative extremes observed for the two laser scanners. Barcode character substitution errors were always found to correspond to misprinted areas of the barcode. The incorrectly accepted patient identifiers produced check characters identical to those printed in the barcodes, by-passing the internal data integrity check safeguard.
Conclusions: Minor barcode imperfections caused by malfunctioning printheads, failure to control for barcode scanner resolution requirements, and less than optimal barcode orientation and width were determined to be sources of error. This report demonstrates that linear barcode identification technology is not fail-safe. To our knowledge, we are the first to describe how misread wristband barcodes generated incorrect patient identifiers, preventing filing of point-of-care testing glucose results. In the worst case, these misidentified results could have been transmitted to the incorrect patient medical record. Careful control of barcode scanning and printing equipment specifications will minimize this threat to patient safety. Ultimately, healthcare device manufacturers should adopt more robust and higher fidelity alternatives to linear barcode symbology.

A-34

An evaluation of the analytical performance of the AU5800® Clinical Chemistry System using a panel of AU reagents*

C. Neville, C. O'Brien, L. Jennings, H. Dineen, J. Reynolds, E. Twomey, M. Cavalleri, M. D. McCusker. *Beckman Coulter Inc., Co. Clare, Ireland,*

Background: The Beckman Coulter AU5800 Clinical Chemistry System is the newest addition to the AU series of Clinical Chemistry Systems and is designed for ultra high-throughput laboratories. A single-photometric unit of the AU5800 has a throughput of 2000 tests/hour with four connected units completing 8000 tests/hour. The reactant volume of the AU5800 is 90µl to 287µl with sample volumes as low as 1µl.
Methods: All currently available AU reagents were evaluated as part of this study. The data below was gathered using a representative panel of 12 reagents which were used to evaluate Precision, Linearity, and Method Comparison. Precision studies were carried out over a period of 20 days using a protocol based on the CLSI guideline EP5-A2. Linearity was confirmed as per the claimed dynamic range (based on CLSI guideline EP6-A). Method Comparison data was generated using a minimum of 100 serum samples spanning the dynamic range based on CLSI guideline EP9-A2. Results are calculated using Deming regression analysis.
Results: Results for this study are summarized in the table.
Conclusions: The study demonstrated the performance characteristics of the Beckman Coulter AU5800 Clinical Chemistry System.

Reagent	Dynamic Range	Total Precision (CV%)		Method Comparison AU5800 v AU2700				
		Low Concentration	High Concentration	N	Slope	Intercept	R	Sy,x
Glucose	10-800 mg/dL	1.0	0.7	173	0.993	-1.62	0.9998	3.207
Creatinine	0.2-25 mg/dL	1.6	2.0	103	0.986	0.02	0.9997	0.092
Total Protein	3-12 g/dL	2.3	1.7	107	1.013	-0.10	0.9990	0.065
Albumin	1.5-6.0 g/dL	0.4	0.9	105	0.996	-0.05	0.9978	0.054
Urea	5-278 mg/dL	1.7	1.5	113	0.996	0.33	0.9997	0.682
Total Bilirubin	0-30 mg/dL	2.3	0.8	135	1.016	-0.01	0.9999	0.113
Calcium Arsenazo	4-18 mg/dL	0.6	0.8	138	0.974	0.15	0.9994	0.090
AST	3-1000 U/L	2.7	1.4	127	0.950	-0.55	0.9996	4.742
Cholesterol	25-700 mg/dL	0.7	0.7	128	1.019	-0.23	0.9990	5.216
CRP	1-480 mg/L	1.4	0.9	122	0.988	0.73	0.9997	3.550
α-Amylase	10-2000 U/L	2.4	2.0	105	1.024	-2.04	0.9999	8.392
Urinary/CSF Protein	4-200 mg/dL	3.2	1.5	169	1.034	-0.15	0.9996	1.134

* System under evaluation and currently not available for clinical use

A-35

The New respons®920 System - Adaption of a Panel of Kidney Disease Markers

H. Baethies, B. Wehle, A. Nadem, S. Caspari, S. Hoffmann, R. Schenk, E. Metzmann, T. Hektor. *DiaSys Diagnostic Systems GmbH, Holzheim, Germany,*

Introduction: The respons®920 system is a fully automated, random access analyzer designed for small to medium size workloads. The system has a throughput of about 240 tests/h with ISE and 200 tests/h without. Key features are the one-grip loading of a complete reagent (twin-container concept), up to 30 refrigerated and barcoded methods on board and an optional 4 channel ISE. A panel of more than 60 clinical chemistry and immunoturbidimetric methods is available.
Because of the growing relevance and the steadily increasing number of cases of metabolic syndrome and diabetes in many countries, the adaption of a panel of kidney disease markers has been chosen to demonstrate the performance of the system.
This kidney disease package includes the following assays:
- creatinine
- cystatin C
- total protein
- urea
- urinary albumin

Materials & Methods: The assay adaption and performance verification have been carried out on 3 respons®920 systems in parallel. All reagents, calibrators and controls were from DiaSys Diagnostic Systems GmbH. Method comparisons have been performed on respons®920 and Hitachi 917 as a reference system. The data have been evaluated by using regression analysis according to Passing and Bablok (*J. Clin. Chem. Clin. Biochem.*, 1983). Inter- and intra-assays imprecision were performed according to a DiaSys internal protocol in serial 20-fold repetition and a 4-fold day-to-day repetition over 5 days, respectively. The analytical sensitivity has been determined by adding at least 3 times the SD to the mean of the signals of a 20-fold repeated blank measurement. The assay lipemia interference has been assessed by mixing serial dilutions of a high concentrated triglycerides matrix with an analyte containing human plasma or serum sample.
Results:

		measuring range			method comparison slope	intercept	r	n
urea	mg/dL	3	-	300	1.01	1.12	0.999	110
urinary albumin	mg/L	2.0	-	310	0.935	1.4	0.9985	92
total protein	g/dL	0.05	-	15	1.02	0.017	0.955	110
creatinine	mg/dL	0.03	-	30	1.00	-0.04	0.999	101
cystatin C	mg/L	0.1	-	7.89	0.955	-0.032	0.9978	100

		intra-assy-imprecision sample 1 mean	CV %	sample 2 mean	CV %	sample 3 mean	CV %
urea	mg/dL	39.2	2.54	77.8	2.90	152	2.34
urinary albumin	mg/L	20.3	3.01	34.1	1.55	106	0.56
total protein	g/dL	5.09	1.02	6.20	0.93	10.9	0.90
creatinine	mg/dL	1.02	2.68	1.21	3.01	7.57	0.88
cystatin C	mg/L	0.70	2.33	0.95	2.26	3.08	1.88

		inter-assay-imprecision sample 1 mean	CV %	sample 2 mean	CV %	sample 3 mean	CV %
urea	mg/dL	39.8	2.22	66.9	3.68	150	2.24
urinary albumin	mg/L	20.8	3.46	35.0	2.91	110	1.94
total protein	g/dL	4.91	2.11	5.96	1.62	11.0	2.25
creatinine	mg/dL	1.00	3.21	1.11	2.59	7.53	2.63
cystatin C	mg/L	0.91	3.71	1.12	3.08	3.44	3.53

Conclusion: The respons®920 system demonstrated good precision and accuracy and is fully compliant with the demands of a state of the art clinical laboratory.

A-36

Analysis of metanephrines in plasma by automated solid phase extraction with ACQUITY® Amide hydrophilic interaction liquid chromatography tandem mass spectrometry

H. A. Brown[1], L. J. Calton[1], K. Graham[2], M. Eastwood[1], B. Molloy[1], R. T. Peaston[3], D. Cooper[1]. *[1]Waters Corporation, Manchester, United Kingdom, [2]Waters Corporation, Milford, MA, [3]Department of Clinical Biochemistry, Newcastle Hospitals NHS Trust, United Kingdom,*

Background: Plasma metanephrines are a highly sensitive test for pheochromocytoma. Our aim was to develop an automated method for the solid phase extraction (SPE) of metanephrines from plasma for analysis by liquid chromatography tandem mass spectrometry.
Methods: Extraction of metanephrine (M), normetanephrine (NM) and 3-methoxytyramine (3MT) from plasma was achieved using Waters® Oasis® weak cationic exchange 96-well µElution plates with liquid transfer and SPE automated onboard the Tecan® Freedom EVO® 100. Plasma diluted with deuterated internal standards (D3-M and -NM, D4-3MT) in water was transferred to the µElution plate and washed with water, methanol and acidified acetonitrile (0.2%v/v formic acid) prior

to elution in 2%v/v formic acid in acetontrile. Extracted samples were eluted from an Waters ACQUITY UPLC® 2.1 x 50mm amide column at 0.2mL/min in a gradient of 100mm ammonium formate, pH 3 and acetonitrile at 3.1, 3.3 and 3.0min for M, NM and 3MT, respectively, with an injection-to-injection time of 5mins. Analytes were detected as multiple-reaction monitoring experiments by the ACQUITY TQ Detector operating in electrospray positive ionisation mode using *m/z* ion transitions *180>148*, *166>134*, *151>91* for M, NM and 3MT, respectively. Validation was conducted using in-house calibrators (0.17 to 24.55nmol/L) and quality control material (M: 0.43, 1.07 and 4.28; NM: 0.46, 1.14 and 4.55; 3MT: 0.49, 1.23 and 4.91nmol/L) prepared from independent 0.1M HCl stocks of metanephrines diluted into 0.1M phosphate buffered saline, 0.1%w/v bovine serum albumin (PBS/BSA). PBS/BSA matrix was considered a suitable matrix following demonstration of parallel recovery compared with plasma (n=6) supplemented to QC concetrations with mixed metanephrines, with mean recoveries of 85, 92 and 91% for M, NM and 3MT, respectively.

Results: Analysis of replicate calibration curves (n=10) showed linearity of detector response across the calibration range, demonstrated as r^2>0.996 and <10% deviation from the nominal concentration, with the exception of the lowest calibrator, where <20% deviation was accepted. The limit of detection was 0.01, 0.02 and 0.07nmol/L for M, NM and 3MT, respectively, based on the average signal-to-noise (SN) ratio of patient samples (n=10) and assuming a SN>3. Similarly, the lower limit of quantification was estimated as 0.06, 0.08 and 0.24nmol/L, for M, NM and 3MT, respectively, assuming a SN>10. The between- (n=25) and within-batch (n=15) imprecision, evaluated by analysis of low, mid and high level QC material was acceptable with CVs <6.7, 9.0 and 5.3%, for M, NM and 3MT, respectively. Method comparison by analysis of patient samples (n=30) previously analysed by an EQA-enrolled plasma M and MN LCMSMS service at Freeman Hospital, Newcastle, UK, was described by the Passing-Bablok equation Waters=0.01+1.01Freeman for M and Waters=0.08+0.95Freeman for NM, with no systematic or proportional biases across the range of the tested material (0.09 to 7.70nmol/L). A comparator method for 3MT is sought.

Conclusion: We have developed a robust, sensitive and precise method for the analysis of M, NM and 3MT in plasma. Combining the Tecan with Oasis SPE technology, this automated solution enables rapid, uninterrupted processing of patient samples and is suitable for routine use in the busy clinical laboratory.

A-37

Urinalysis: comparison between microscopic analysis and a new automated microscopy image-based urine sediment instrument

P. V. Bottini[1], C. R. Garlipp[1], L. F. Franco-Fernandes[2]. *[1]University of Campinas, Campinas, SP, Brazil, [2]Medlab Group, São Paulo, SP, Brazil,*

Urinalysis is a high demand procedure, with large amount of manual labor and poorly standardized. Recently a new walk-away automated urine microscopy analyzer has been introduced. It is based on the capture and recognition of images of monolayered urine sediment in a cuvette with a digital camera. In order to evaluate the performance of this new sediment analyzer compared to microscopic analysis we analyzed 400 urine samples originating from patients with several clinical conditions.

All samples were analyzed by light field microscopy using the KOVA® method with the results expressed in terms of quantitative elements (erythrocytes-RBC and leukocytes-WBC; mean number of 10 fields of 400x) and qualitative elements (casts, crystals and bacteria, considering all fields) and by fully automated sediment analyzer based on the KOVA® method with a on-screen review of the images (UriSed - 77 Elektronika Kft, Budapest, Hungary).

Statistical analysis included evaluation of agreement, sensitivity, specificity, positive and negative predictive values (table 2x2, chi-square method).

All the elements showed a good agreement between both methods, as seen in the table below

	RBC	WBC	Casts	Crystals	Bacteria
Agreement	95.0	91.2	86.6	100.0	80.1
Sensitivity	74.6	72.0	25.8	100.0	69.4
Specificity	98.8	97.6	98.8	100.0	85.0
Positive Predictive value	94.0	93.5	81.0	100.0	68.8
Negative Predictive value	95.9	92.0	87.0	100.0	86.2
* results expressed in percentage basis					

The low sensitivity observed for casts is due to the microscopic examination of all the fields in the KOVA® system versus the15 standard image of the UriSed system.

UriSed eliminates the need of a microscopic review of the samples. It has a strong correlation with the results obtained by microscopic analysis and allows a better workflow and significantly improves turnaround time. The use of a system that photographs and stores sample images provides an invaluable training tool for technologists and medical students.

Tuesday AM, July 27

Poster Session: 10:00 am – 12:30 pm
Factors Affecting Test Results

A-38

Fully Automated Sample Preparation, PCR Amplification, and Melt Curve Analysis Integrated Into a Cassette and Instrument for Molecular Diagnostic Applications

Y. E. Lin[1], D. Birch[1], K. Estis[1], A. Tran[1], A. Benn[1], B. Baker[1], M. Fairchild[1], B. Richardson[2], J. Ching[1]. *[1]Progentech, Emeryville, CA, [2]Accel Biotech Inc., Campbell, CA,*

An instrument was designed to integrate sample preparation (i.e., pathogen lysis and nucleic acid purification), nucleic acid quantitation through real-time polymerase chain reaction (PCR), and melt curve analysis of PCR product. The instrument accommodates multiple target-specific cassettes and fully automates the sample preparation, amplification, and analysis. The combination of a closed cassette and instrument function allowed processing of clinical specimens with full automation, potentially free of external- and cross-contamination.

Pre-loaded cassettes contained all of the reagents and components needed for pathogen (i.e., bacterial and viral) lysis, nucleic acid purification with magnetic beads, amplification, and analysis. After the specimen was directly added to the cassette and the cassette loaded into the instrument, stepwise reagent addition, sonication for lysis, heating for enzyme activation, fluidic mixing, and a magnetic transfer system provided a state-of-the-art bead-based nucleic acid purification for target nucleic acid in clinical specimens such as plasma and nasal swabs. Within the cassette, isolated nucleic acid proceeded to thermocycling with precise thermal control allowing rapid PCR amplification and high-resolution (0.1-0.2ºC/sec) PCR product melting. The fluorescent detection optics of the instrument provided 6 channels for multiplexed real-time quantitative PCR and qualitative PCR product melting curve analysis. The detection chemistries were non-destructive probe detection with fluorescent tag and quencher. Each detection probe contained a minor groove binder (MGB) and modified nucleotides that increase binding specificity and protect them from digestion with polymerase. The broad range of melting curve detection (Tm at 50-80ºC) plus 6 channel filters for emission detection allow for an unprecedented level of quantitative PCR multiplexing.

The performance metrics of the cassette were divided into sample preparation, real-time PCR, and melt analysis. Sample preparation efficiency on the cassette was demonstrated with genomic and viral DNA at a DNA recovery of 91.98% ±10.10 (N=21). Detection of Cytomegalovirus (CMV) and Epstein Bar virus (EBV) plasmid isolated from 200 µL plasma yielded real-time thermocyling plots with melt analysis (i.e., PCR product and MGB hybridizing probe) as follows: EBV Tm= 80ºC and CMV Tm= 74ºC. We have also demonstrated a multiplex detection of influenza (flu) A, flu B, respiratory syncytial virus (RSV), and RNA internal control (RNA bacteria phage, MS2). The melt analysis for flu used an intercalating dye (Sybr Green) for identification and differentiation of target organisms in the multiplexed assays. With these results, the cassette and instrument demonstrated full automation that included sample preparation, real-time PCR amplification and detection, and melt analysis of PCR product for potential use in molecular diagnostic applications.

A-39

Linearity Study on Testing Creatinine with Various Assay Instruments

Q. Zhou, X. Li, J. Xu, W. Xie, S. Li. *Beijing Hospital, National Center for Clinical Laboratories, Beijing, China,*

Background The EP6-A protocol and Dr. Kroll's EP6-A extended methods are newly approved schemes for assessing linearity. The purposes of surveying linearity are to evaluate the performances of clinical laboratories and improve the quality of tests.

Methods Sample data were taken from an EQA program of linearity survey and calibration verification. Serum samples with five different concentrations of creatinine were measured four times in each sample. The data were grouped according to the analytical instruments used: Beckman LX (28 laboratories), Beckman CX (14 laboratories), Hitachi (62 laboratories) and Olympus (72 laboratories). Statistically significant linearity and nonlinearity were judged using the polynomial regression technique recommended by the NCCLS EP6-A guideline. The *P* values for the nonlinear coefficients (b_2 and b_3) were investigated using t-test, the data set was considered linear if none of the nonlinear coefficients were significant ($p>0.05$) and nonlinear if any of the nonlinear coefficients were significant ($p<0.05$). The imprecision of analytical data were analyzed and the extents of nonlinearity were determined using Dr. Kroll's EP6-A extended methods.

Results This study found that in samples analyzed using Beckman LX, Beckman CX, Hitachi and Olympus instruments, the degrees of imprecision ranged from 0.30% to 3.01%, 0.09% to 3.46%, 0.14% to 4.91% and 0.17% to 16.44%, respectively; the average deviations from linearity ranged from 0% to 2.38%, 0% to 2.51%, 0% to 5.46% and 0% to 4.66%, respectively; the percentages of tests showing statistical linearity were 21.4%, 35.7%, 11.3% and 18.1%, respectively; and the percentages showing clinical linearity were 78.6%, 64.3%, 88.7% and 80.6%, respectively; the percentages of laboratories whose analyses met the linearity criteria were 100%, 100%, 100% and 98.6%, respectively.

Conclusions The level of imprecision in one laboratory in the group of Olympus exceeded the acceptable limit, while the remaining laboratories were considered to be suitable for testing creatinine.

The Results of Linearity Assessment on Testing Creatinine

Group	First-order Polynomial		Second-order Polynomial		Third-order Polynomial		Nonlinearity	Imprecision	Pass the Survey
	range of imprecision (%)	*ADL* (%)	range of imprecision (%)	*ADL* (%)	range of imprecision (%)	*ADL* (%)	number (percent) of labs	number (percent) of labs	number (percent) of labs
Beckman LX	0.58-3.01	0-0	0.50-1.59	0.48-1.09	0.30-1.58	0.41-2.38	0(0%)	0(0%)	28(100%)
Beckman CX	0.93-2.00	0-0	1.29-3.46	0.73-2.51	0.09-1.00	0.29-1.50	0(0%)	0(0%)	14(100%)
Hitachi	0.58-2.18	0-0	0.14-4.35	0.08-4.27	0.15-4.91	0.18-5.46	0(0%)	0(0%)	62(100%)
Olympus	0.62-16.44	0-0	0.25-2.47	0.42-3.35	0.17-4.20	0.17-4.66	0(0%)	1(1.4%)	71(98.6%)

A-40

Estimation of plasma Total Iron Binding Capacity and Transferrin Saturation using the Siemens Dimension® Vista® 500 Intelligent Lab System and Siemens Flex® Iron, TIBC and Transferrin reagents

R. R. Wiedmann[1], A. L. Liddle[2], B. R. Morgan[3]. *[1]University of Wisconsin Stevens Point, Stevens Point, WI, [2]Sacred Heart-St. Mary's Hospitals, Ministry Health Care, Rhinelander, WI, [3]Sacred Heart-St. Mary's Hospitals, Ministry Health Care/ Ministry Medical Group, Rhinelander, WI,*

The Siemens Dimension® Vista® 500 Intelligent Lab System Transferrin Flex® assay, using Becton-Dickinson Vacutainer®PST™Gel and Lithium Heparin Blood Collection Tubes, provides an acceptable estimate of total iron binding capacity (TIBC) when compared to the Siemens TIBC Flex® assay (determined TIBC). The % Transferrin Saturation (%TS) calculated using Vista Flex iron reagent, TIBC estimated from plasma transferrin concentration in umol/L (calculated TIBC) and from determined TIBC in umol/L correlate acceptably.

Fifty plasma specimens were collected by standard phlebotomy technique, centrifuged 10min at 1800g, and all iron/Flex TIBC testing in duplicate completed within 1.5 hours of collection. Aliquots of the plasma specimens for duplicate transferrin determination were frozen at -20°C within 4 hours of collection and determinations performed within 2 weeks of collection. Statistical analysis using Microsoft® Excel® Program consisted of ordinary linear regression analysis, sign test, difference plots, and assessment of clinical significance of differences at medical decision levels. Siemens calibrators for the tests were traceable to these reference materials: TIBC and iron, NIST SRM937, transferrin, ERM®-DA470 (CRM 470) and ferritin, 3rdIS 94/572.

Plasma determined TIBC ranged from 27 to 81umol/L and transferrin concentration from 14 to 40umol/L (calibrated in g/L, converted to umol/L using transferrin molecular weight 79,540g/mol). The average within-run CV for the paired determined was 1.1% for TIBC and 1.8% for transferrin. Linear regression analysis on a plot of determined TIBC umol/L vs. calculated TIBC (assuming each transferrin molecule binding 2 iron molecules), yielded a line with equation Y = 0.870X + 5.05umol/L, R^2=0.939, SEE 2.72, slope 95% CI 0.806 - 0.935, y-int. 95% CI 1.24 - 8.86, maximum differences -13% and +9%. Comparison of %TS calculated using iron, determined TIBC vs. %TS using the calculated TIBC, with linear regression analysis, yielded a line with equation Y = 0.983X + 1.28%, R^2=0.991, SEE 1.26, maximum absolute differences -4% and +3%, slope 95% CI 0.956 - 1.01, y-int. 95% CI 0.545 - 2.01. Sign test on the TIBC and %TS paired data sets were significant at P0.05. Plots of ferritin concentration vs. %TS, transferrin concentration and plasma iron concentration showed no correlation at R^2=0.139, R^2=0.142, and R^2=0.044, respectively.

Interestingly, TIBC calculated from the transferrin concentration by this system and calibrators referenced to CRM 470 overestimated the total iron binding capacity in 39 of the 50 specimens compared to the actual determined TIBC with calibration referenced to SRM 937, and was unrelated to the degree of % transferrin saturation. This is contrary to what one might expect given that non-transferrin proteins can also bind iron as saturation becomes high. The observed differences in %Transferrin Saturation appeared clinically insignificant whether calculated from transferrin or by determined TIBC.
The Siemens Dimension® Vista® 500 Intelligent Lab System Transferrin Flex® assay, using Becton-Dickinson Vacutainer®PST™Gel and Lithium Heparin Blood Collection Tubes, provides an acceptable estimate for calculating total iron binding capacity that could be used in-lieu of the determined TIBC to calculate a clinically relevant %Transferrin Saturation.

A-41

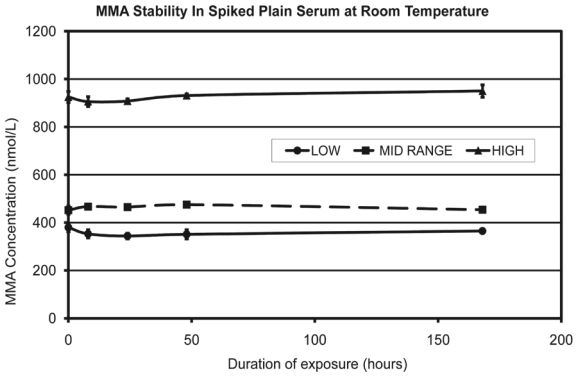
Comparison Of Five Different Methods For Serum Albumin Assay
S. Dumanlı, T. Turhan, Y. Koca, S. Sezer. *healty ministry, Ankara, Turkey,*

There are various methods used for albumin assay. Within these methods, there are differences in cost, assay results and requirement for special analyzer. Bromcresol purple (BCP) and Bromcresol green (BCG) methods are the most preferred assays that have low cost and do not require a special analyzer. But studies show that, Nephelometry (NM) that has a relative high cost and requires a special analyzer, gives more reliable results so is accepted as a gold standard method. In this study, we looked for the relation between BCP, BCG and Turbidimetry (TM) methods of low cost and Capillary electrophoresis (CE) of high cost, by comparing them with respect to Nm method, which is taken as reference method. Firstly, to evaluate the precision of all methods, serum pools of low and high levels were used. For method comparison, 40 blood samples from patients who applied to Numune Hospital and had normal liver/kidney functions were included in the study.
By the statistical analysis, it was seen that except TM method, all of the other four methods satisfied the %CV≤5 target advised by CLIA'88. With low-level serum pool, wider %CV values were obtained. Except TM method, random errors of within and between days were found to be 4SD<Ea and 3SD<Ea respectively, random errors of the other four methods were accepted while random error of TM method was rejected owing to dilutions done for the study.
Before the comparison of methods, Kolmogorov-Smirnow Normality test was applied and assay results of all methods were found to fit to the Normal distribution. So, comparison results were studied with t-test, correlation and regression analysis, which are parametric tests. By t-test all methods except CE displayed significant differences with respect to NM for averages (p<0,01) and by correlation analysis paired correlations for all methods except TM were found above 0,970. Due to high correlations, paired regression analyses were performed between all methods. For each regression with NM as independent variable and one of other four methods as dependent variable, F statistics came out to be significant (p<0,01).
Except for TM-NM equation (r^2=0,775), r^2 values of equations are found to be over 0,970 corresponding to high determination power. Except TM slopes of equations were very close to 1 and intercepts were below limits of ±2 g/L.
Regression results proved that except TN, other three methods had consistent results with NM method. Bland-Altmann graphs displayed that differences between the other methods and NM were independent from assay levels. Thus, for acceptance of method performances, total errors were checked. For each method, total error (TE) was calculated by adding the random error form precision and systematic error from regression equations with Xc=35g/L as independent variable. Except TM, for other three methods, TE<Ea was satisfied so BCP, BCG and CE were accepted as consistent with NM.
As a result, BCP and BCG methods give consistent results with NM. So, low cost methods BCP and BCG with a high cost method CE are accepted as reliable methods for albumin assay.

A-42

Effect of storage temperature or collection tube type on blood methylmalonic acid levels measured by gas chromatography-mass spectrometry
A. Hughes, E. Reineks, S. Wang. *Cleveland Clinic, Cleveland, OH,*

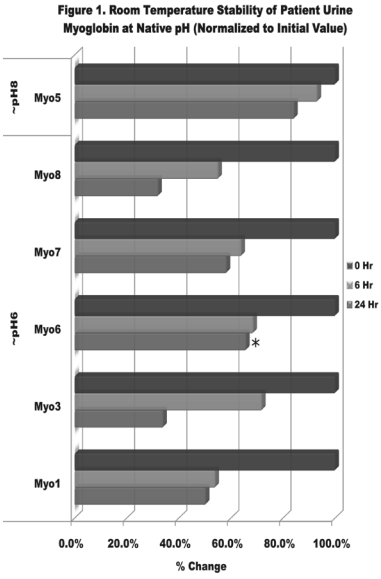
Background Methylmalonic acid (MMA) is used clinically for evaluating the cause of macrocytic anemia. However, the impact of storage temperature and tube types on MMA stability in serum/plasma has not been thoroughly studied.
Objective To evaluate the effects of storage temperature and tube types on blood MMA measured by a gas chromatography-mass spectrometry assay.
Methods Pools of de-identified leftover serum in plain red-top tubes were spiked at three different concentration levels at or above the upper reference range. Aliquots were kept at room temperature at set time points for up to one week. At each time point 3 aliquots from each pool were frozen at -70 °C until analysis. In a different experiment, EDTA additive plasma or serum in gel separator tubes were analyzed along with their corresponding plain serum with the same collection time. Due to the lack of heparin tubes with corresponding plain serum tubes, half of the serum was poured into an empty heparin tube and analyzed in pair.
Results The spiked serum MMA samples showed <10% change at room temperature for up to a week. The mean difference between serum in gel separator tubes and plain serum (n=5) was 8.8% (p=0.433). Two EDTA specimens failed minimum internal standard response requirements, suggesting that the EDTA additive may interfere with the assay. The mean difference between the 3 remaining EDTA specimens and the plain serum specimens was 5.5% (p>0.999). The heparin specimens (n=5) compared nicely with the plain serum with a mean difference of 3.7% (p=0.778).
Conclusion The MMA concentration in plain serum samples remains stable for up to one week at room temperature. Serum gel separator and heparin tubes are suitable for this assay, but plasma EDTA is not an ideal additive.



A-43

pH dependence of urine myoglobin stability as measured on Roche E170 immunoassay analyzer in a clinically relevant patient population
D. R. Bunch, S. Gebreselassie, S. Wang. *Cleveland Clinic Foundation, Cleveland, OH,*

BACKGROUND Rhabdomyolysis may result in myoglobinuria which can cause pigment induced acute renal failure. Urine myoglobin (myo) measurement is used clinically for both early detection and response to treatment to avoid acute renal failure. Prior studies showed that myo is more stable in alkaline urine. OBJECTIVE To study the stability of urine myo at different pH in specimens from a clinically relevant population to formulate a reliable sample handling process for real patient specimens.
METHOD Leftover patient specimens were de-identified and frozen at -70ºC until tested. Samples were allowed to reach room temperature then each sample's pH was measured using pHydrion™ papers. Each sample was split and adjusted to either pH 6 or pH 8 using either potassium hydroxide or hydrochloric acid solution (1.0 M). Aliquots were kept at room temperature for 0, 6, 18, or 24 hr then frozen at -70ºC. The urine myo was measured in batch on Roche E170 using an electrochemiluminescent immunoassay. A decrease >20% was considered significant for this study.
RESULTS Native patient urine samples (n=6) varied in pH from 6-8. Specimen 5's native pH was 8 and its myo level decreased <20% over 24 hrs, while the remaining patient specimens had pH 6 (n=5) and myo level decreased 34-68% (Figure 1, * below the analytical measurement range). Furthermore, the pH 6 specimens were not stable during the 6 hr time period with an average decrease of 35%. Additionally, the pair-wise adjusted patient samples (n=7) show a marked increase in stability at pH 8 while specimen 5 stability decreased at pH 6 (data not shown).
CONCLUSION Urine myo with pH ≥8 was stable at room temperature for up to 24 hr. Urine specimens need adjustment to pH ≥8 immediately after collection to stabilize myo.



A-44

Comparison of the results for Electrochemical Luminescence Assay and ELISA in determining Hepatitis B virus serum markers

W. Xu, Y. Li, M. Wang. *Renmin Hospital of Wuhan University, Wuhan, China,*

Background: To evaluate the differences of the test results between electrochemical luminescence assay and ELISA in determining serum markers of hepatitis B virus (HBV).
Methods: 180 specimens were collected and determined by electrochemical luminescence system and ELISA simultaneously, the sample can be divided into two types-type 1 and type 2 (coexistence of HBsAg and HBsAb or HBeAg and HBeAb), 90 specimens were collected for each type.
Results: Concurrence between electrochemical luminescence system and ELISA was 98.9% for HBsAg, 90% for HBsAb,100% for HBeAg, 84.4% for HBeAb, and 61.1% for HBcAb while determining type 1 specimens; the paired chi-square test showed there were significant statistically differences between the two different methods in determining HBeAg and HBcAb (P<0.05). Concurrence between the two methods was 93.3% for HBsAg, 46.7% for HBsAb, 85.5% for HBeAg, 83.3% for HBeAb, and 95.6% for HBcAb determining in type 2 specimens; the paired chi-square test showed there were significant statistically differences between the two different methods in determining HBsAb, HBeAg and HBeAb (P<0.05). Positive rate using electrochemical luminescence assay was higher than ELISA.
Conclusion: Significant discrepancies were observed between results for the two immunoassay methods, especially for HBeAb and HBcAb results in type 1 specimens and HBsAb and HBeAb results in type 2 specimens. That mostly relies on the high sensitivity of electrochemical luminescence system.

A-45

Effects of Hemolysis, Icterus, and Lipemia on Serum Osmolality Results using the Advanced® Model 3250 Single-Sample Osmometer

J. Rosenman, E. Garry, M. Pesta, J. Sacks, A. Curria. *Advanced Instruments, Inc., Norwood, MA,*

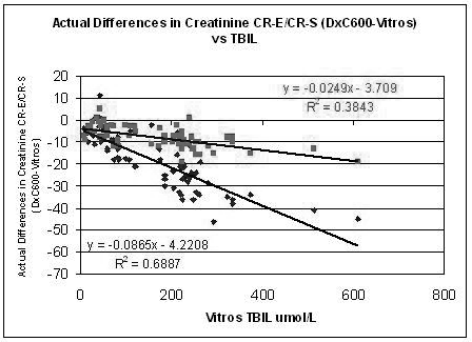
Background: Hemolysis, icterus, and lipemia may affect the results of routine chemistry tests and thereby lead to erroneous clinical interpretations and inappropriate actions regarding patient care. The accurate determination of serum osmolality in the clinical laboratory is of critical importance to the differential diagnosis of many disorders involving water balance. This study was conducted to quantify the influence of hemolysis, icterus, and lipemia on serum osmolality results using the Advanced® Model 3250 Single-Sample Osmometer.
Methods: Normal human serum was spiked with varying concentrations of hemolysate, bilirubin, and Intralipid following NCCLS EP7-A dose-response guidelines in order to simulate hemolysed, icteric, and lipemic specimens, respectively. Hemolysis, icterus, and lipemia were studied up to concentrations of 500 mg/dL hemoglobin, 36 mg/dL bilirubin, and 3030 mg/dL triglycerides. Samples were tested for osmolality in quintuplicate.
Results: The data were analyzed using Microsoft® Office Excel 2007. Using a significance criterion of 3 SD from the mean osmolality of the neat serum, hemolyzed, icteric, and lipemic serum samples were reliably tested up to 31.25 mg/dL hemoglobin (Δ_{mOsm} = -1.2), 25.1 mg/dL bilirubin (Δ_{mOsm} = -1.2), and 1557 mg/dL triglycerides (Δ_{mOsm} = 2.0), respectively.
Conclusion: The results of this study demonstrate that serum can be reliably tested for osmolality with considerable levels of hemoglobin, bilirubin, and triglycerides.

A-46

Bilirubin Interference on DxC800 & DxC600 Creatinine: Improvement with new Enzymatic Creatinine

W. Skoropadyk[1], G. Blakney[2], G. Cembrowski[1], D. Levesque[1]. [1]*University of Alberta Hospital, Edmonton, AB, Canada,* [2]*Royal Alexander Alberta Hospital, Edmonton, AB, Canada,*

Background: Bilirubin is known to interfere negatively with the Jaffe creatinine assay. Until last fall, Beckman only provided the Jaffe' method on its Synchron® DxC systems. In its product literature, Beckman stated that the minimum bilirubin concentrations to cause this interference were 256 umol/L and 342 umol/L for the DxC 600 (modified Jaffe' method) and the DxC 800 system (Jaffe' method), respectively. Because of recurring interference flags associated with elevated bilirubin, we assessed the lowest bilirubins which were associated with invalid DxC 600 and DxC 800 creatinine measurements. Subsequent to our fall evaluation, Beckman released an enzymatic creatinine assay, which we tested on the DxC 600.
Methods: The Vitros® 350 (Enzymatic Method) was the comparative 'reference' method for both bilirubin and creatinine measurements. In the fall study, a total of 74 previously analyzed plasma samples (patient population 5 days to 77 years) were analyzed for total bilirubin and creatinine by the DXC 800 and DXC 600. In the winter study, 72 previously analyzed plasma samples (patient population 2 months to 67 years) were analyzed for total bilirubin and creatinine by the DXC 600 (Enzymatic Creatinine) .
Results: The graph compares the new Enzymatic Creatinine to the modified Jaffe' method on the DxC 600. The minimum bilirubin concentration associated with a -20 umol/L bias are 600 umol/L and 180 umol/L for the enzymatic and Jaffe method, respectively. For the DxC 800, graph not shown; the minimum interfering bilirubin is 350 umol/L.
Conclusion: The DxC 600 modified Jaffe' method has serious limitations especially in the pediatric population where bias interferences of even <20 umol/L may be clinically significant. We recommend that the enzymatic Creatinine be used by DxC 600 systems, while it would remain optional for the DxC 800 systems.



A-47

Study of High Dose Hook Effect on Centaur total HCG Assay

D. Chen. *NYU Med. School/Bellevue Hospital, New York, NY,*

Objective: High dose hook effect is a well known phenomenon in one-step wash heterogeneous immunoassays. The analytical impact of hook effect is a falsely low result of the affected test. In this study, a patient sample with total HCG concentration around two million mIU/ml was found affected by hook effect. Subsequently, the tolerance limit for hook effect in total HCG Centaur assay was investigated.
Material and Methods: The HCG sample was from a patient with trophoblastic disease. The total HCG of the patient sample was measured by Centaur total HCG assay. The sample was measured directly without dilution, and with several different ratio dilutions by HCG diluent. The Centaur assay's linearity range is up to 1000 mIU/ml, any sample with HCG level higher than 1000 mIU/ml will be resulted as " >1000 mIU/ml", and reflexed to further dilution. As a comparison to Centaur method, the same sample was also measured by the Ortho VITROS ECI total HCG assay in a different laboratory.
Results: The original patient HCG concentration of this sample was resulted as 616 mIU/

ml, which was significantly lower than the previous result (400,000 mIU/ml) of same patient done three months ago. Physician questioned this test result, since it is unlikely to have patient HCG dropped so much while the patient's trophoblastic disease was still uncured. Potential hook effect was investigated: patient sample was manually diluted 1:10 by HCG diluent and then tested on Centaur again. This time, the diluted sample was resulted as >1000 mIU/ml and the sample's HCG concentration was eventually resulted as 2,177,320 mIU/ml with further dilution. The HCG concentration of this sample was also confirmed by ECI method from a different laboratory, which resulted it as 2,273,800 mIU/ml.

The concentration of HCG level to trigger hook effect was investigated using different dilutions. When the sample was diluted to 60% of the original HCG concentration the Centaur assay was still able to result it as >1000; while a sample with 75% of the original concentration HCG was affected by hook effect. Further dilution and repeats confirmed that when sample total HCG was at 1,280,000 mIU/ml, it was still unaffected by hook effect when assayed by Centaur. It was affected by hook effect when HCG sample concentration was at 1,500,000 mIU/ml.

Conclusion and Discussion: This study indicated that Centaur total HCG assay has very high tolerance to high dose hook effect: it can still function normally even when serum total HCG concentration reaches 1,280,000 mIU/ml. This concentration is significantly higher than the hook effect claim in Centaur total HCG assay insert, which states that the assay is still unaffected by high dose hook effect when total HCG concentration is up to 400,000 mIU/ml. Based on this study, the total HCG concentration to trigger hook effect on Centaur HCG assay is between 1,280,000 to 1,500,000 mIU/ml.

A-48

Effects of Whole Blood Storage on HbA1c Measurements with Five Current Assay Methods.

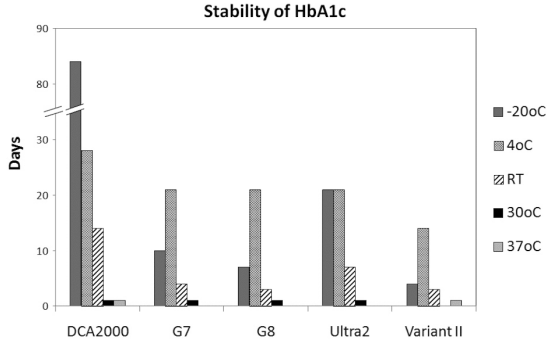
C. Rohlfing, S. Hanson, A. Tennill, R. Little. *University of Missouri, Columbia, MO,*

Background: Hemoglobin A1c (HbA1c) is an important index of average glycemia in patients with diabetes mellitus, and is also utilized in clinical trials and large-scale epidemiological studies. For these applications specimens are often stored for a time then shipped to a central laboratory. Previous studies have shown that exposure to improper temperature conditions can cause erroneous HbA1c results, and that in general, ion-exchange assay methods tend to be more sensitive to storage issues than boronate affinity or immunoassay methods. We examined the effect of storage at different temperatures with five current HbA1c methods: Tosoh G7 and G8, and Bio-Rad Variant II (ion-exchange HPLC); Bayer DCA2000 (immunoassay) and Trinity Biotech Ultra2 (boronate affinity HPLC).

Methods: Whole blood specimens with five different HbA1c levels were analyzed by each assay method on Day 0, then divided into aliquots that were stored at six different temperatures (-70ºC, -20ºC, 4ºC, room temperature, 30ºC and 37ºC) for analyses on subsequent days out to Day 84. Acceptance limits were calculated based on -70ºC; for the remaining temperatures, results outside +/-3sd of all -70ºC results for each sample were considered unacceptable. Stability was considered acceptable for a given temperature only if results for all 5 specimens were acceptable on that day.

Results: Results are shown in the figure. Stability was best for the DCA2000, and stability for the G7 and G8 methods was comparable to that of the Ultra2 at temperatures above -20ºC. No methods demonstrated stability at 30ºC or 37ºC for more than one day.

Conclusion: Exposure of specimens to high temperatures should be avoided regardless of assay methodology, and for the ion-exchange methods tested 4ºC storage is preferable to -20ºC (stability 14-21 days vs. 4-10 days). For studies where long-term stability is required, samples should be stored at -70ºC or colder.



A-49

Stability of immunosuppressants monitored for organ transplant drug therapy - whole blood kept at room temperature, 4ºC and -20ºC.

W. Y. Ng, S. C. Chong, E. Jacob. *Singapore General Hospital, Singapore, Singapore,*

Background: The stability of immunosuppressant medications (assayed with immunoassays) at various storage conditions have been shown. With change to the liquid chromatography tandem mass spectrometry (LC-MS/MS) technique for measurements, we re-evaluated immunosuppressants' stability in blood specimens at storage conditions typically adopted in clinical laboratories.

Methods: Whole blood specimens (up to 3 levels) of immunosuppressants (tacrolimus TAC, sirolimus SRL, everolimus ERL and cyclosporine CSA), were collected, pooled and measured by LC-MS/MS before separate aliquots prepared for the study. Specimen aliquots (200-500uL) were then kept at room temperature (in the dark), in a 4ºC refrigerator and -20ºC freezer for 2, 3, 7 and 14 days before further testing for the immunosuppressants. Stability was evaluated against measured levels of fresh specimens.

Results: Levels of the immunosuppressants were: TAC 3.1, 5.1, 9.2 µg/L; SRL 2.7, 9.7, 16.6 µg/L, ERL 4.8, 6.5 µg/L and CSA 207, 1449 and 2005 µg/L. Acceptance criteria for stability were based on absolute differences for tacrolimus, sirolimus and everolimus (1-5 µg/L: +/-1.0 µg/L; 6-10 µg/L: +/-2.0 µg/L; 11-50 µg/L: +/-5.0 µg/L) and percent difference for cyclosporine (100-2000 µg/L: +/-25.0%). The drug with least drift observed was tacrolimus. Tacrolimus, sirolimus and everolimus stability were maintained to the 14th day when kept at 4ºC. At -20ºC, frozen specimens (with lysis observed during the thawing process) generally showed consistent but lower results over the various test dates to the 14th day. Cyclosporine stability was also demonstrated at room temperature and 4ºC. Similar to the three other drugs, there was a greater negative drift for frozen samples' cyclosporine results from the initial test values with fresh specimens. Frozen aliquots would allow a good estimate of the initial level but due to lysis during thawing, the change in erythrocyte/plasma distribution contributed to the lower measured levels.**Conclusions:** A common practice of storing blood specimens at 4ºC for 3 days maximum in the clinical laboratory should give similar test (repeat) levels compared to initial testing of fresh samples. Amongst the immunosuppressants tested, tacrolimus showed the least drift from initial testing. Blood specimens should be kept cool (4ºC) with ice-packs for short periods of transportation to the laboratory. However, grossly hemolysed blood specimens are not acceptable for immunosuppressant testing.

A-50

Abbott Architect Clinical Chemistry and Immunoassay Systems Digoxin Assays are Free of Interferences from Spironolactone, Potassium Canrenoate and Their Common Metabolite Canrenone

A. DeFrance[1], D. Armbruster[1], D. Petty[1], K. Cooper[1], A. Dasgupta[2]. *[1]Abbott Laboratories, Irving, TX, [2]University of Texas Health Science Center, Houston, Houston, TX,*

***Objective*:** Samples with varying amounts of aldosterone antagonists that may interfere with digoxin assays were tested on three ARCHITECT clinical chemistry platforms using the Clinical Chemistry Digoxin (cDig, particle enahanced turbidimetric immunoassay) and on an ARCHITECT i2000sr using the chemiluminescent microparticle Digoxin (iDig, CMIA) to evaluate interference.

Background: Spironolactone is a potassium sparing diuretic commonly used to correct volume expansion or hypertension. Spironolactone is also commonly prescribed for congestive heart failure patients who also often take digoxin. Due to structural similarities, spironolactone interference with digoxin immunoassays has been widely reported. Potassium canrenoate is a similar drug, not approved for use in the U.S but prescribed in Europe and other parts of the world. Both potassium canrenoate and spironolactone are metabolized to canrenone which also interferes with various digoxin immunoassays. Steimer et al. reported significant negative interference of spironolactone, potassium canrenoate and canrenone in the MEIA II Digoxin assay (Steimer et al Clin Chem 2002; 48: 507-508) causing potential digoxin toxicity if digoxin dosage is increased based on falsely low digoxin result.

Methodology: Aliquots of drug free serum pool were supplemented with various amounts of spironolactone, potassium canrenoate and canrenone representing therapeutic concentrations as well as concentrations expected in overdose and digoxin was measured with cDig on Architect c4000, c8000 and c16000 and with iDig on i2000sr.

Results: No significant difference in results was observed between baseline drug free serum pool and spiked samples. No significant differences in results were observed between a digoxin serum pool and the same serum pool spiked with increasing amounts of drugs. See table below for summary.

	Drug Free Serum Pool				Serum Digoxin Pool			
	c8000	c16000	c4000	i2000sr	c8000	c16000	c4000	i2000sr
Baseline	0.0	0.0	0.0	<0.3	1.1	1.1	1.1	0.9
Spironolactone (ng/mL)								
250	0.0	0.1	0.1	<0.3	1.1	1.1	1.1	0.9
500	0.0	0.1	0.1	<0.3	1.1	1.1	1.1	0.9
1000	0.0	0.1	0.1	<0.3	1.1	1.1	1.1	0.9
Potassium Canrenoate (ng/mL)								
500	0.0	0.1	0.1	<0.3	1.1	1.1	1.1	0.9
1000	0.0	0.1	0.0	<0.3	1.1	1.1	1.1	0.9
2000	0.0	0.1	0.0	<0.3	1.1	1.1	1.1	0.9
Canrenone (ng/mL)								
500	0.1	0.1	0.1	<0.3	1.1	1.1	1.1	0.9
1000	0.1	0.1	0.1	<0.3	1.1	1.1	1.1	0.9
2000	0.1	0.1	0.1	<0.3	1.2	1.1	1.1	0.9

Conclusion: The ARCHITECT cDig and iDig assays showed no interference from the specified drugs. The cDig and iDig assays are free from interference due to spironolactone, potassium canrenoate, and their common metabolite canrenone.

A-51

Distribution of areas of filter paper bloodspots derived from fixed volume (50 µL) application of EDTA-whole blood for a normal adult population, and correlation of areas to hemoglobin and hematocrit

S. D. Schutte, J. D. Landmark, D. F. Stickle. *University of Nebraska Medical Center, Omaha, NE,*

Background: Use of bloodspot samples for quantitative whole blood analysis has been described for numerous analytes. Depending on the details of calibration, for some methods there may be an inherent degree of uncertainty in quantitation using this sample type due to the fact that there is interpatient variability in the relationship between volume applied and the area of the bloodspot, with concomitant variability in the volume of blood contained within a fixed-area bloodspot punch used for measurement. It is worthwhile to have an assessment of the degree of variation in blood volume/area that exists among a given target population of subjects. Given advancement of potential use of bloodspot samples in applications such as testing for vitamin D deficiency among otherwise healthy adults, our objective in this study was to examine the distribution of bloodspot areas for fixed-volume applications of EDTA-whole blood from a population of apparently normal (ambulatory, non-clinic) adults. Because hematocrit is known to influence spreading properties of blood on filter paper, we also examined correlation of bloodspot area with hematocrit and hemoglobin.

Methods: EDTA-whole blood specimens were de-identified, to-be-disposed refrigerated specimens from adult "health fair" subjects. These were obtained from the laboratory along with age, sex, and test results for hemoglobin and hematocrit. 48 specimens (25 male; 23 female; age range 24-71 years) were obtained in a single day for use in the experiment. Specimens were mixed gently at room temperature for 30 min by rocking. Bloodspot samples were prepared in duplicate by vertical pipetting of 50 µL onto standard "903" filter paper cards suspended horizontally. Samples were allowed to dry at room temperature for 24 h. Relative areas of bloodspots were determined by color image analysis of bitmap images of the filter paper cards obtained by scanner. It was previously established that bloodspot areas obtained for fresh EDTA-whole blood were indistinguishable from those obtained from fresh non-anticoagulated whole blood.

Results: The distribution of bloodspot areas across the population was well characterized (r^2 = 0.994) by a parametric normal distribution based on the arithmetic mean and standard deviation of the raw area data (# of pixels): bloodspot area = 6910 ± 243 pixels (coefficient of variation (CV) = 3.5%; range = 6430-7390 pixels; calibration area = 6590 pixels/cm^2). Hematocrits of the original samples were normally distributed (hematocrit = 14.4 ± 1.3%) and ranged from 12-18%. Correlations of areas with hematocrit (r^2 = 0.291) and total hemoglobin (r^2 = 0.345) were relatively poor.

Conclusions: Areas of constant volume bloodspots derived from normal adult whole blood samples were normally distributed, with CV = 3.5%. Areas were not highly correlated with hematocrit or hemoglobin. The results indicate that, due solely to interpatient variation in blood volume per bloodspot area, an inherent variation of ±7% (±2 standard deviation range) could in principle be observed across bloodspot results from among adult samples having identical whole blood concentrations of analyte.

A-52

Paraprotein Interference in Clinical Chemistry Assays

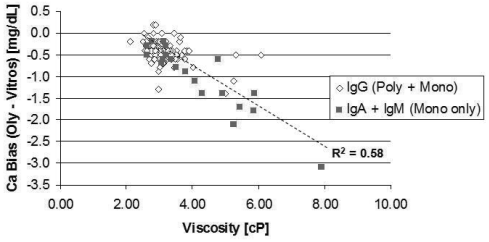
S. E. Melanson, P. Jarolim. *Brigham and Womens Hospital, Boston, MA,*

Background Previous reports indicate that paraproteins, particularly IgM paraproteins, introduce negative or positive bias into automated chemistry assays. However, a systematic study examining the effects of both polyclonal and monoclonal proteins and of specimen viscosity on the performance of automated chemistry analyzers has not been performed.

Methods We compared results for 11 analytes in patient samples in each category: 1) total protein between 6.0-8.0 g/dL (n=69), 2) total protein >8.0 g/dL (n= 65) and 3) total protein >8.0 g/dL and the presence of monoclonal gammopathy (n=51). Specimens were analyzed for electrolytes, glucose, creatinine, calcium, phosphate, total bilirubin, uric acid, iron and HDL on the Ortho VITROS 5,1 FS, Olympus AU2700 and Siemens Dimension RxL analyzers. The Ortho VITROS 5,1 FS measured viscosity. The bias between methods was calculated and the relationship of total protein and viscosity was determined.

Results Groups 1, 2 and 3 had average total protein concentrations of 7.11 ± 0.49 g/dL, 8.36 ± 0.31 g/dL and 9.21 ± 1.15 g/dL; respectively. Viscosity was 2.53 ± 0.25 cP, 3.09 ± 0.48 cP, 3.51 ± 1.09 cP in groups 1, 2 and 3 respectively. Clinically significant biases were seen when comparing calcium, glucose, HDL and iron results. An excellent correlation between bias and viscosity was seen (Figure). The bias was significant in 77% of specimens with a viscosity > 3.0 cP.

Conclusions Similar to other studies we found that the mechanism of interference is dependent on a variety of factors including methodology, stabilizing reagents, pH, and type of protein. We verified that for certain analytes a bias between analyzers is present that correlates well with viscosity. Because not all factors contributing to protein interferences can be readily measured, we recommend using viscosity to predict the likelihood of interference with certain analytes.



R2 = 0.16 (IgG)
R2 = 0.86 (IgA and IgM)

A-53

Impact of Pre-analytical Processes on the Measurement of Protein Biomarkers of Atherothrombotic Risk

S. Esayanur Sujatha Sarma, A. Vahedi, R. Nghima-Tahiri, Y. Cheung, L. Millar, S. Vijayakumar, B. Wang, N. Nolan, E. Hytopoulos, B. Phelps, M. Beggs. *Aviir Inc, Palo Alto, CA,*

The determination of protein biomarker levels in blood is emerging as an important means to assess the level of coronary heart disease (CHD) in an individual and predict a person's risk for atherothrombosis. How a blood sample is collected, handled, and stored prior to analysis has been reported to greatly impact the levels of biomarkers measured. Yet the stabilities of many of the proteins associated with the biological pathways that may be activated in CHD have not been systematically evaluated.

Objective: We studied the impact of blood collection tube-type (glass or plastic, with and without separator gel), centrifugation speed, cell contamination, and storage temperature including post-thawed conditions (with multiple freeze-thaw cycles) on the measurement of serum protein biomarkers by xMAP™ assays.

Methods: Three panels (a total of 47 individual protein assays) were tested. They included the Bio-Rad Human Cytokine 21-plex assays, RDS Custom "High Abundance" 12-plex assays, and RDS Custom "Low Abundance" 14-plex assays. These assays are sandwich bead-based immunoassays carried out in a 96-well plate format and read using the Bio-Plex™ xMAP instrument system. Serum samples (up to n=54 depending on the condition tested) were collected from patients who had a cardiac event ("case") and from normal blood donors with no evidence of CHD ("control"). P-values by ANOVA were calculated for all 47 biomarkers to determine the significance of the effects caused by tube type and temperatures.

Results: The assay results were analyzed in terms of fluorescence signal and concentration (pg/mL). Six markers (CTACK, MIF, SCGF-b, RANTES, sCD40L, and VEGF) were significantly impacted by sample handling and storage conditions ($p < 0.05$). CTACK showed an increased concentration trend when the serum samples were stored at 4C and 25C ($p<0.0001$), whereas sCD40L levels were decreased in samples stored at 25C($p<0.0001$). Both of these biomarkers were also strongly impacted by blood collection tube type. MIF showed a surprising peak in concentration levels when samples were stored at -20C compared to -80C or 4C ($p<0.0001$), although there was a concentration dependent decrease in samples stored at 25C and 4C.

Conclusions: These observations provide important information that must be considered when serum samples are tested for protein biomarker levels. We have defined a sample

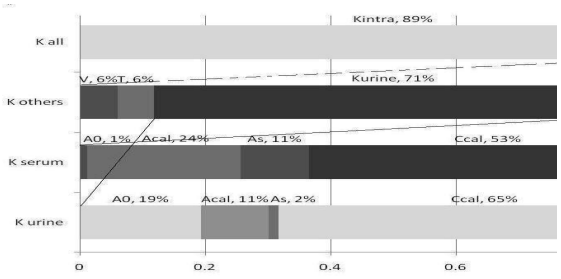
handling process that minimizes the exposure of proteins of interest to conditions that we have shown could alter measured levels significantly. Knowing that a particular protein is sensitive to certain pre-analytical handling conditions, we are able to choose the more reliable and robust protein biomarkers to include in the final clinical test panel of protein assays. By minimizing pre-analytical variation, changes in measured protein concentrations can be used with greater confidence to predict a person's risk for atherothrombosis. Our findings are also important for investigators to consider when designing a multi-site blood sample study protocol. If all sites adhere rigorously to a pre-defined pre-analytical sample handling protocol, the validity of the study results can be viewed with greater confidence.

A-54

Estimation of the Measurement Uncertainty of Creatinine Clearance Rate

J. Li, X. B. Zhao, S. K. Yan. *Dept.of Lab. Med.,China-Japan Friendship Hospital,Ministry of Health, Beijing, China,*

Background It is well known that incomplete urine collection or the relative bias of creatinine concentrations had direct effects on the creatinine clearance rate (CCr) test. Laboratories should set routine performance goals for measurement uncertainty (MU) based on the clinical use of the CCr results.
Objective To explore the effects of different parameters on the CCr results in the clinical laboratory, and estimate the MU of CCr.
Methods According to the rules of the ISO Guide to the Expression of Uncertainty in Measurement (GUM) and other guidelines, the MU of serum and urine creatinine, and CCr were estimated in our laboratory by using standard procedures with Hitachi 7600 automatic biochemical analyzer.
Results Base on the uncertainty components and their values of serum and urine creatinine, contributions of different parameters to the MU of CCr was estimated (Fig. 1).
$\mathbf{K}_{all}$: all contributions to CCr combined uncertainty; $\mathbf{K}_{others}$: other contributions to CCr combined uncertainty exclude the individual biological variation; $\mathbf{K}_{serum}$: contributions come from serum sample; $\mathbf{K}_{urine}$: contributions come from urine sample.
Fig. 1 Contributions of different parameters to the MU of CCr
When the CCr results of patient 1 and 2 were 66.88ml/min and 30.93ml/min, the expanded uncertainty were 16.98ml/min and 7.84ml/min, and the relative standard uncertainty were 12.70% and 12.67%; The factors of biological variation excluded, the expanded uncertainty is 5.48 ml/min and 2.50ml/min, and the relative standard uncertainty were 4.09% and 4.04%.
Conclusion The CCr concentrations had no effects on the MU estimation. The impacts of biological variability excluded, pre-analytical variation is the major factor for estimating the MU of CCr.
Kay word: creatinine clearance rate, measurement uncertainty



A-55

Evaluation of the BD Vacutainer Rapid Serum Tube with BD Hemogard Closure for Select Analytes at Initial Time and 24 Hours and with Alternate Centrifugation Conditions

D. Szamosi, T. Finocchio, A. Stankovic, E. Croneberger, J. Berube. *BD Diagnostics, Franklin Lakes, NJ,*

Objectives: The clinical performance of the BD Vacutainer® Rapid Serum Tube Blood Collection Tube with BD Hemogard™ Closure (BD RST) was evaluated in comparison with the BD Vacutainer® SST™ Blood Collection Tube (BD SST™) for 40 analytes at initial time and at 24 hours and for specimen quality in the BD RST. In a second study, using four centrifugation conditions, the BD RST was also compared with the BD SST for four analytes; clot time for the BD RST was recorded in a third study.
Background: The BD Vacutainer Rapid Serum Tube is a new blood collection tube that contains a thrombin-based clot activator and a gel barrier with a BD Hemogard closure that minimizes splatter during cap removal. The thrombin promotes rapid clotting of the blood, allowing centrifugation five minutes after collection to provide a serum specimen that can be sampled directly from the primary tube. This reduced clot time can enable faster laboratory turnaround time, which is particularly vital for hospital units in which test results drive important medical decisions on patient treatment.
Methods: Fresh blood specimens were collected for the studies by routine venipuncture from apparently healthy adult subjects. After a time interval to allow clotting, the tubes were centrifuged to provide separated serum specimens. Serum was then tested for 40 analytes at initial time (t=0) and following storage at 24 hours (t=24h) at room temperature on a Roche Integra® 800 and Siemens ADVIA® Centaur. Using four centrifugation conditions (2000g/10 min, 2000g/4 min, 4000g/3 min, 1500g/10 min), four analytes (AST, LD, K, Phos) were assessed in the BD RST vs the BD SST on the Roche Integra 800.
Results: Clinical equivalence on average was demonstrated in the BD RST and BD SST at initial time and 24 hours for all analytes. For each centrifugation condition, the BD RST met the bias requirement for equivalence with the BD SST for the select analytes. Visual observations recorded no significant tube/time interval difference for RBC on barrier, fibrin strand, fibrin mass and hemolysis in the BD RST at initial time and 24 hours. Clot time was observed for the BD RST in under 5 minutes. Overall, results obtained with the BD RST demonstrated comparable performance with the BD SST for all analytes evaluated.
The 510(k) clearance of the BD RST with BD Hemogard Closure in the US is pending.

A-56

Interferences from hemolysis, bilirubin and turbidity on analytes using two different integrated analytical systems

A. Baeuerle-Bubeck[1], I. Bauer[1], O. Sonntag[2], H. Baum[1]. *[1]Regionale Kliniken Holding RKH GmbH, Ludwigsburg, Germany, [2]Ortho Clinical Diagnostics GmbH, Neckargemuend, Germany,*

Introduction: Hemolysis, bilirubinemia or turbidity (lipemia) lead to many problems in the correct determination of analytes measurable in serum or plasma specimen. Therefore, detection and removal of possible interfering substances are of great importance in daily routine. In this study, we investigated the possible interference of hemolysis, bilirubin and turbidity (lipemia) on several routine parameters compared to unspiked sera on a VITROS® 5600 integrated system and a cobas® 6000 analyzer. An additional study was undertaken to compare coloured samples and samples with no intensive colour with the two analytical systems.
Materials and Methods: Serum samples from 4 healthy individuals were collected. The sera were divided in 4 portions and spiked with different amounts of hemoglobin, bilirubin or Intralipid®. In all samples several analytes were measured covering the assay spectrum of both the VITROS 5600 integrated system and the cobas 6000. To better quantify the degree of colour the serum index was determined in each sample. Additional we tested 150 coloured and 100 uncoloured native serum samples on both systems and did comparative statistical analysis.
Results: Compared to unspiked sera, in several parameters there was a significant interference with the spiked samples. On the VITROS 5600 the interference could be correlated to the semiquantitative measured amount of the interfering substance. Compared to the cobas analyzer there were lesser interferences especially in those parameters measured with the MicroSlide technology and spiked with bilirubin or lipids. The method comparison with uncoloured and coloured samples delivered much broader differences as found in the in-vitro testing.
Conclusion: The VITROS 5600 showed lesser interferences with classical interfering substances compared to another widely used integrated system but is limited to those parameters which are determined with the MicroSlide technology. One has to take into account that in-vitro studies do not reflect the real situation in native patient material.

A-57

The use of indices for the determination of cut off levels for the influence of hemolysis, icteria and lipemia on 35 parameters measured on the Architect C8000 analyser

E. C. Michielsen, M. Bour, B. H. E. Hendrickx, M. E. P. van Drunen, J. C. J. M. Swaanenburg. *Department of Clinical Chemistry and Hematology, VieCuri Medical Center for Noord-Limburg, Venlo, Netherlands,*

Objective: When measuring on a routine clinical chemistry analyzer, one should always be aware of the influence of hemolysis, icteria and lipemia (HIL). The aim of this study was to investigate the influence of HIL for 35 routine clinical chemistry parameters on the Architect C8000 analyzer (Abbott Diagnostics) by the HIL-indices that are automatically determined for each sample.
Methods: Basic solutions were prepared with lysed erytrocytes, bilirubin and intra-lipid® for calculating the influence of hemolysis, icteria and lipemia, respectively. Also, a

serum pool was prepared with concentrations within the appropriate reference ranges for each parameter. From this serum pool 10 working solutions were prepared by adding 10 increasing amounts of the appropriate basic solution and 10 decreasing amounts of 0.9% saline solution in order to maintain equal total end volumes. The highest concentrations hemoglobin, bilirubin and intra-lipid (triglycerides) were 0,62 mmol/l, 820 µmol/l and 48 mmol/l, respectively. From these data we determined cut off values for HIL indices above which a parameter result was measured that deviated more than 10% and 20% from the native pool serum result.

Results: In the evaluated ranges, hemolysis was of significant influence on LDH, ASAT, ALAT, gamma-GT, uric acid, ammonia, total protein, iron, potassium, conjugated bilirubin and magnesium. Icteria was of significant influence on creatinin (Jaffé), ammonia, cholesterol, gamma-GT, triglycerides, lithium and lipase. Lipemia was of significant influence on albumin (BCP), bilirubin total, conjugated bilirubin, calcium, iron, lipase, lithium, magnesium, total protein and uric acid.

Conclusion: Most of the evaluated parameters were not influenced by hemolysis, icteria or lipemia in the investigated ranges. For the aforementioned parameters that were influenced we installed cut off values for HIL-indices above which a result was generated with a deviation of more than 10% from the native pool serum result (remark on patient report: minor deviation due to interference). For HIL-indices resulting in a deviation of more than 20% from the native pool serum we installed cut off values that blocked reporting of the result (remark on patient report: result blocked due to interference). For potassium, these cut off values were installed at deviations of 5% and 10%, respectively.

A-58

Spurious results of vancomycin and other chemistry analytes in a patient with undiagnosed paraproteinemia

M. R. Al-Turkmani, J. Barron, L. V. Rao. *UMass Memorial Medical Center, Worcester, MA,*

OBJECTIVE: To highlight the significance of paraprotein interference with vancomycin and other routine laboratory assays.

BACKGROUND AND CASE DESCRIPTION: Monoclonal immunoglobulins, also known as paraproteins, have been reported to interfere with a number of routine chemistry assays. Paraprotein interference with an automated vancomycin assay has been recently reported in two lymphoma patients (1). The patient described in this report is an 87-year-old male with a history of chronic obstructive pulmonary disease (COPD), chronic renal insufficiency, and anemia. He was admitted to the medical floor with septicemia and subsequently managed with supportive care and antibiotic therapy that included intravenous administration of vancomycin (1g every 24 h).

METHODS: Serum vancomycin levels were determined using a turbidimetric immunoassay (LX, Beckman Coulter). The patient's serum samples were then pooled and vancomycin levels were measured using three different assays: the Beckman assay, a similar turbidimetric immunoassay on the Architect analyzer (Abbott), and a fluorescence polarization immunoassay (FPIA) on the Cobas Integra analyzer (Roche). In a follow-up study, six samples with different M-protein components (IGgκ, IgGλ, IgMκ, IgMλ, IgAκ, IgAλ) were spiked with vancomycin to achieve a final concentration of 30 µg/mL and later tested on all three platforms.

RESULTS: Serum vancomycin measurements on the LX analyzer gave a < 2.0 µg/mL result, which was incompatible with the ongoing vancomycin therapy the patient was receiving. This falsely low result indicated potential interference by paraproteins. Subsequent serum protein electrophoresis and immunofixation revealed an IgMκ monoclonal component of 24 g/L in the patient's serum that the medical staff were not aware of. Testing the patient's serum pool on the three platforms demonstrated that vancomycin levels were assay-dependent (15.3, 29.6, 46.9 µg/mL by LX, Architect, and Integra, respectively). In addition to vancomycin, the pooled serum was tested for commonly measured analytes and no significant differences were found among the three platforms except for inorganic phosphate (suppressed by LX, 3.5 and 4.2 mg/dL by Architect and Cobas, respectively), and HDL-C levels (suppressed by LX and spuriously low by Architect and Cobas). Measuring vancomycin levels by LX, Architect, and Integra in the spiked samples that contained different M-proteins gave comparable results with no evidence of interference. The inorganic phosphate and HDL-C results were either suppressed or spuriously low only in the sample containing IgMλ, indicating that the interference might be dependent in certain cases on the class of paraprotein.

CONCLUSION: Spurious or suppressed test results of vancomycin or other selected chemistry analytes should prompt suspicion of paraprotein interference, especially when these results do not match the clinical picture of a patient. Additionally, test results of these analytes should be interpreted carefully in patients with known paraproteinemia.

(1) Simons SA et al. Two cases with unusual vancomycin measurements. Clin Chem.2009 Mar;55(3):578-80.

A-59

Stability of Vitamin C in Whole Blood and Serum

M. Zhang[1], R. L. Schleicher[1], M. Sternberg[1], M. Xu[1], H. Chen[1], C. M. Pfeiffer[1], L. E. Mechanic[2]. *[1]Centers for Disease Control & Prevention, Atlanta, GA, [2]Westat, Rockville, MD,*

Objective: Multiple studies have shown that vitamin C (VIC) rapidly degrades in serum unless it is promptly acidified. We re-evaluated VIC stability before addition of meta-phosphoric acid (MPA) to provide a more practical procedure for sample collection and processing in future studies.

Relevance: VIC is essential for good health. It is a cofactor in many important enzymatic reactions and a highly effective water-soluble antioxidant. Total ascorbic acid in serum is a biomarker of VIC status. Deficiency is indicated by values <0.2 mg/dL.

Methodology: Whole blood (WB) samples were drawn into 5 red-top Vacutainers™ from 33 healthy donors (mean±SD: 1.24±0.44 mg/dL). After 30 min, an individual's serum was separated from tube 1 and 6% MPA was added (1:4); this acidified reference serum was stored at -70°C. Non-acidified serum from tube 1, aliquots of WB from tube 2, and WB in unopened Vacutainers™ (tubes 3-5) were stored at 4°C for 1, 4 and 6 days. At the end of each storage period, serum was prepared and/or VIC was stabilized with 6% MPA, and aliquots were transferred to -70°C until analysis (experiment 1; n=10). We also tested VIC stability when non-acidified serum samples were stored at -70°C for up to 14 days before addition of MPA (experiment 2; n=23) and the effects of different amounts/age of MPA on serum VIC (experiment 3; n=10). Total VIC (oxidized and reduced) was measured using HPLC separation with electrochemical detection (analytical CV 5.3%). Paired t-tests without correction for multiple comparisons were used to test for significance against reference serum ($p<0.05$).

Results: VIC concentrations decreased substantially in non-acidified serum stored at 4°C ($p<0.0001$): -14% (1 d), -41% (4 d) and -61% (6 d). Little change was observed when whole blood was kept at 4°C either in opened or unopened tubes: +2.3% ($p=0.17$) or -3.9% ($p=0.04$) at 1 d, +1.4% ($p=0.58$) or -4.5% ($p=0.05$) at 4 d and -4.2% ($p=0.10$) or -7.5% ($p=0.03$) at 6 d. When non-acidified serum was frozen at -70°C and stored for up to 14 days, VIC decreased only a little: -5.0% ($p=0.0009$) at 1 d, -5.5% ($p<0.0001$) at 7 d and -3.8% ($p=0.0002$) at 14 d. Compared with freshly prepared 6% MPA (routine procedure), 8% MPA or 6% MPA stored frozen at -70°C for more than 3 years were equally effective in stabilizing VIC: -2.7% ($p=0.03$) and 0.1% ($p=0.94$), respectively. Substantial loss of VIC was observed when 3% MPA was used: -75% ($p<0.0001$).

Conclusions: While VIC was not stable in non-acidified serum stored at 4 °C, it showed reasonable stability in refrigerated WB for 1-6 days (<7% change) or in serum frozen at -70°C for up to 14 days (<6% change) without need for acid stabilization. Individual aliquots of 6% MPA stored frozen may be used for laboratory convenience. Simplified handling of blood and shipment to a laboratory for acidification would facilitate field studies of VIC status. Further studies of VIC stability in blood and serum should be undertaken in persons with poor VIC status.

A-60

Evaluation of the Clinical Performance of the New BD Microtainer® MAP Microtube for Automated Process with K_2EDTA for Routine Hematology Testing on the Sysmex® XE-2100 and Beckman Coulter® LH 750

N. Niwinski, A. Stankovic. *BD Diagnostics, Franklin Lakes, NJ,*

Objectives: The clinical performance of the new BD Microtainer® MAP Microtube for Automated Process was evaluated in comparison with the current BD Microtainer® Tube with Microgard™ Closure for routine hematology testing at initial time on two instrument platforms_the Sysmex® XE-2100 and Beckman Coulter® LH 750 and over time on the Beckman Coulter LH 750.

Background: The BD Microtainer MAP Microtube for Automated Process (BD MAP) is a blood collection tube that is intended for collecting low volumes of capillary blood. The BD MAP, which is spray coated with K_2EDTA anticoagulant, enables the collection, anticoagulation, transport and storage of skin puncture blood specimens for routine hematology testing on automated hematology systems. The tube is especially beneficial for blood collection from geriatric, oncology, pediatric, neonatal and intensive care unit patients, as well as the general population. The standard 13x75 mm size of the BD MAP enables full-size labeling to eliminate potential specimen labeling errors, which could result in possible life-threatening consequences to the patient. Since the design fits standard instrument racks and is compatible with the most commonly used hematology analyzers, the BD MAP can assist in improving workflow efficiency.

Methods: Blood specimens were collected for the studies by two separate finger punctures from a total of 200 adult subjects from both in-patient and out-patient populations. Specimens were allowed to sit for a minimum of 30 minutes from time of collection at room temperature prior to testing. Testing was then performed

on specimens from both tubes for a complete blood count, including platelets and reticulocytes, on the Sysmex XE-2100 and Beckman Coulter LH 750 at initial time (within 4 hours from time of collection) and after 12 hours on the Beckman Coulter LH 750. Specimens were processed in the BD MAP using automated mode and in the BD Microtainer Tube using traditional manual mode.
Results: Clinically acceptable performance was demonstrated for the BD MAP for all hematology parameters measured on the Sysmex XE-2100 and Beckman Coulter LH 750 as compared to the BD Microtainer Tube with Microgard Closure at initial time. Stability over time for up to 12 hours when stored at room temperature was observed in the BD MAP on the Beckman Coulter LH 750. Overall, clinically acceptable performance was demonstrated for the BD MAP in comparison with the BD Microtainer Tube with Microgard Closure for all hematology parameters evaluated.

A-61

Influence of a regular, standardized meal on Clinical Chemistry Analytes.
G. Lippi[1], G. Salvagno[2], G. S. Lima-Oliveira[3], M. Gelati[2], M. Montagnana[2], E. Danese[2], G. Picheth[4], A. Duarte[3], G. Guidi[2]. *[1]U.O. di Diagnostica Ematochimica, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy, [2]Sez. Chimica Clinica, Dip. Scienze Morfologico-Biomediche, Verona, Italy, [3]University of São Paulo, São Paulo, Brazil, [4]Federal University of Parana, Curitiba, Brazil,*

Background: Although it is widely acknowledged that the lipaemia influences the concentration and/or the activity of several analytes in blood, the time of fasting is often disregarded as a potential source of preanalytical variability.
Objective: The purpose of this study was to evaluate the effects of time of fasting on clinical chemistry analytes.
Methods: Venous blood specimens were drawn in the morning from 17 fasting volunteers who gave informed consent for participating to the study. The subjects consumed a regular, standardized meal, contain carbohydrates, protein and lipids (563 Kcal) in 10 min. Sequential venipunctures were performed before the meal and 1, 2 and 4 hours later on four different veins of the upper arms. Blood was collected by a single, expert phlebotomist by venipuncture with 20 G straight needles, directly into 3.5-mL vacuum tubes containing gel and lithium heparin (Terumo Europe, Haasrode, Belgium). The concentrations of several clinical chemistry analytes were assayed on Roche/Hitachi Modular System P (Roche Diagnostics GmbH) (See Table 1). Significance of differences between samples was assessed by paired Student's t-test. The level of statistical significance was set at $p<0.05$. The biases following 1, 2 and 4 hours after the meal were compared with the current desirable quality specifications for bias, derived from biologic variation.
Results: Statistically significant differences exceeded the analytical quality specifications for desirable bias for phosphate (P), glucose (GLU), triglycerides (TG), Iron (Fe), total proteins (TP), sodium (Na), calcium (Ca), magnesium (Mg) and albumin (Alb) (Table 1). No significant biases could be recorded for the others analytes.**Conclusion:** The significant variation of several clinical chemistry parameters after a light meal demonstrates that the fasting time needs to be carefully considered when performing testing, in order to prevent spurious results especially in the emergency setting.

Table 1 Statistical analysis of clinical chemistry testing in plasma specimens collected before and after a standardized meal (0, 2 and 4 hours).

	Desirable Bias (%)	Baseline specimen Mean value (SEM)	1 h after meal Mean value (SEM)	Mean % Difference	P	2 h after meal Mean value (SEM)	Mean % Difference	P	4 h after meal Mean value (SEM)	Mean % Difference	P
P	3,2	3,84(0,11	3,58 (0,10)	-6,5	0,001	3,65 (0,08)	-4,7	0,005	4,1 (0,10)	7,7	0,003
BT	11,4	0,52 (0,07)	0,50 (0,07)	-4,8	0,049	0,46 (0,07)	-11,2	0,0001	0,45(0,06)	-13,5	0,001
BD	14,2	0,17 (0,07)	0,17 (0,02)	-2,3	0,01	0,15 (0,02)	-12,2	0,002	0,15(0,02)	-11,6	0,013
ALP	6,4	58,97 (4,40)	60,59 (4,49)	2,8	0,01	60,20 (4,44)	2,1	0,029	59,54(4,56)	1	0,057
GLU	2,2	94,46 (10,53)	95,41 (17,60)	4,5	0,174	92,31 (16,82)	-1,2	0,355	90,84 (14,70)	-3,2	0,484
AU	4,9	4,01 (0,32)	4,10 (0,31)	2,1	0,007	3,95 (0,30)	-1,6	0,007	3,78(0,30)	-5,7	0,0001
BUN	7	15,19 (0,84)	14,70 (0,75)	-3,1	0,001	14,62 (0,78)	-3,7	0,001	14,79(0,30)	-2,4	0,05
AMYL		60,71 (4,30)	58,76 (4,32)	-2,8	0,143	57,80 (4,09)	-4,4	0,41	59,20(4,30)	-2,1	0,177
AMY-P		24,47 (4,30)	23,99 (2,15)	-1,8	0,144	23,44 (2,08)	-4	0,023	24,50(2,14)	0,4	0,391
COL	4	188,118(6,66)	187,56 (7,76)	-0,2	0,469	184,71(6,99)	-1,8	0,005	187,24(7,46)	-0,4	0,388
CK	11,5	90,84 (9,00)	92,80 (10,17)	2,2	0,201	93,02 (10,52)	2,5	0,087	96,97(9,62)	6,8	0,001
LDH	4,3	279,46 (10,38)	283,04 (10,22)	1,4	0,195	284,42 (10,44)	1,9	0,106	290,55(11,02)	4,1	0,009
AST	5,4	21,94(2,50)	21,71 (2,60)	-0,9	0,378	21,99 (2,52)	0,3	0,395	23,69(2,55)	8,3	0,0001
LIP	10,1	33,66 (4,90)	34,41 (4,63)	2,6	0,32	33,73 (4,94)	0,7	0,361	33,59(4,28)	0,2	0,322
CRE	3,8	0,83 (0,04)	0,84 (0,04)	1,8	0,11	0,82 (0,04)	-1,2	0,058	0,81(0,04)	-2,6	0,024
TG	10,7	96,01 (13,95)	103,79 (13,67)	9,7	0,152	106,70 (13,14)	14,8	0,158	104,46 (13,14)	18,9	0,201
ALT	12	17,34 (2,76)	17,95 (2,76)	3,9	0,077	18,24(0,02)	5,4	0,168	19,91 (3,12)	15,2	0,0001
HDL	5,2	55,46 (2,97)	55,95 (3)	0,9	0,113	55,21 (2,86)	-0,3	0,318	55,72(2,70)	0,6	0,471
FE	8,8	73,70 (5,93)	76,35 (6,92)	6,5	0,0001	80,44(6,96)	9,3	0,0001	77,81 (6,67)	6	0,013
GGT	10,8	12,25 (1,41)	12,79 (1,41)	4,6	0,017	12,64(1,40)	3,5	0,041	11,69 (1,49)	-4,7	0,089
TP	1,2	73,77 (1,03)	76,28 (1,75)	3,8	0,066	74,51 (1,09)	0,7	0,068	75,55(1,09)	2,4	0,001
NA	0,3	138,81 (0,46)	140,40 (0,48)	1,2	0,001	139,69 (0,51)	0,6	0,001	139,40(0,52)	0,4	0,082
K	1,8	4,20(0,06)	4,15 (0,08)	-0,9	0,271	4,24(0,06)	1,1	0,257	4,45(0,09)	6,2	0,004
CL	0,5	103,46 (0,54)	103,69 (0,51)	0,2	0,205	103,75(0,47)	0,3	0,103	103,40(0,43)	0	0,438
CA	0,8	9,12(0,06)	9,33(0,06)	2,3	0,0001	9,37(0,05)	2,8	0,0001	9,46(0,06)	3,6	0,0001
MG	1,8	2,13(0,03)	2,07 (0,04)	-2,8	0,0001	2,11(0,04)	-1,2	0,055	2,22(0,03)	4,3	0,0001
ALB	1,3	46,89 (0,56)	46,51 (0,70)	1,4	0,016	46,57(0,57)	1,5	0,002	47,43(0,49)	3,4	0,0001
CRP	13,9	0,41 (0,47)	0,45 (0,46)	11,3	0,417	0,41(0,49)	7,1	0,2	0,48(0,51)	27,1	0,04

alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), amylase, amylase pancreatic, aspartate aminotransferase (AST), direct bilirubin (BD), total bilirubin (ST), blood urea nitrogen (BUN), calcium, chloride, cholesterol, C-reactive protein (CRP), creatinine, creatine kinase (CK), g-glutamyltransferase (GGT), glucose (GLU), highdensity lipoprotein-cholesterol (HDL-C), iron (Fe), lactate dehydrogenase (LDH), magnesium (Mg), phosphate (P), potassium (K), total protein (TP), sodium (Na), triglycerides (TG) and uric acid (UA).

A-62

Heterophilic antibody interference in a chemiluminescent immunometric erythropoietin assay
L. Spruill, M. A. Constantin, D. M. Andrews, Y. Zhu. *Medical University of South Carolina, Charleston, SC,*

Background: Erythropoietin (EPO) is a glycoprotein hormone responsible for the tight regulation of erythropoiesis. EPO is typically measured in clinical laboratories using two-site sandwich immunoassays. It is well documented that sandwich immunoassays can be limited by heterophilic antibodies within patient serum, due to the fact that the capture antibody and detection antibody can be joined by heterophilic antibodies in the absence of antigen (measured analyte). Our **objective** is to report the first published case in which EPO measurement has been confounded by heterophilic antibodies.
Methods: EPO was measured with a solid-phase, chemiluminescent immunometric assay on the IMMULITE 2000 analyzer (Siemens Medical Solutions Diagnostics), which consists of a ligand-labeled monoclonal anti-EPO capture antibody, an alkaline phosphate-labeled polyclonal anti-EPO conjugate antibody, and solid-phase anti-ligand-coated polystyrene beads. The assay has an analytical measurement range of 1.5 - 200 mIU/mL, where the lowest reportable result is 1.5 mIU/mL as established by our laboratory. If the sample concentration is > 200 mIU/mL, the sample will be diluted to endpoint using the on-board dilution protocol. In a normal population, the median EPO level is 10.2 mIU/mL with a 95th percentile level of 19.6 mIU/mL, and the absolute range is 3.7 to 31.5 mIU/mL.
Results: In a 73 years old patient with anemia of unknown etiology, initial EPO level was greater than 200 mIU/mL, which is above the assay measurable range (1.5-200 mIU/mL). Dilution of the sample resulted in a non-linear decrease in the EPO level of 68.2 mIU/mL (1:10 dilution), which was inconsistent with a truly elevated level. Use of heterophilic antibody blocking tubes (Scantibodies Laboratories) yielded a result of 123 mIU/mL, a decrease consistent with the presence of heterophilic antibodies. The specimen was sent to a reference laboratory (ARUP) that employs a different immunochemiluminescent assay using the DxI 800 analyzer (Beckman Coulter). The reference laboratory returned a result of 9 mU/mL, which is within the range of normal (4-27 mU/mL).
Conclusion: The current report is the first documented case of interference from heterophilic antibodies using the chemiluminescent immunometric EPO assay on the IMMULITE 2000 analyzer. Clinical awareness of this possibility is necessary in order to prevent misdiagnosis of a patient due to inaccurate interpretation of results.

A-63

Incidence of Catecholamine Interference in the Roche Enzymatic Creatinine Assay
A. Wockenfus, C. Koch, M. Onsum, A. Saenger, A. Jaffe, B. Karon. *Mayo Clinic, Rochester, MN,*

Background: We have reported that some patients receiving intravenous catecholamines can have markedly low creatinine values due to analytical interference on the Roche (Roche Diagnostics, Indianapolis IN) enzymatic creatinine assay. This effect was not observed when the Roche Jaffe creatinine method was used**.** To understand the frequency of this effect and the factors associated with it, we compared creatinine results obtained by the enzymatic and Jaffe methods on the Roche Cobas Integra 400 analyzer (Roche Diagnostics, Indianapolis, IN) using samples from patients known to be receiving intravenous catecholamines.
Method: In our practice, patients on intravenous epinephrine, norepinephrine, dopamine, dobutamine, or vasopressin automatically have creatinine analyzed by a Jaffe method to avoid possible interference. We measured the frequency of this interference by running enzymatic and Jaffe creatinines in parallel on 491 specimens received in the laboratory from patients with a Jaffe creatinine ordered due to catecholamine administration. We defined interference to be a Jaffe creatinine value at least 0.3 mg/dL greater than the enzymatic creatinine value. We recorded the draw type and any sample comments (i.e. hemolysis, lipema, etc.) attached to those results in the Laboratory Information System (LIS).
Results: Of 491 creatinine Jaffe specimens, 360 (73.3%) were collected from arterial lines, 73 (14.9%) from venous lines, and 58 (11.8%) by venipuncture. Mean bias between enzymatic and Jaffe methods (n=491) was 0.0212 mg/dL. Catecholamine interference was observed in 7 of 491 (1.4%) samples. Of those specimens demonstrating interference 4 were collected from arterial lines, 2 from venous lines, and 1 by venipuncture. No sample comments (hemolysis, lipemia, etc) were noted in the LIS for any of the specimens demonstrating catecholamine interference.
Conclusions: Catecholamine interference with enzymatic creatinine measurements occurs infrequently in patients receiving intravenous catecholamines. Samples drawn from venous lines were over-represented among the samples with interference (2 of 6 vs. only 15% of overall samples); however interference was not limited to venous line

draws. Because interference is infrequent and not limited to any one draw method; avoiding catecholamine interference in the enzymatic creatinine assay will pose significant process problems for labs using that assay.

A-64

Lipid Emulsion Solution: A novel cause of hemolysis in serum and plasma samples

E. A. Jaben, C. D. Koch, B. S. Karon. *Mayo Clinic, Rochester, MN,*

Background: While investigating the cause of hemolyzed blood samples, we found that several pediatric patients who had 3 or more hemolyzed samples were on a combination of total parenteral nutrition (TPN) and lipid emulsion solution. In these patients TPN and lipid emulsion were administered together in varying ratios for nutritional support. In one of these patients, whole blood specimens collected in blood gas syringes were not visibly hemolyzed (after plasma separation), while multiple serum and plasma specimens collected in vacutainer tubes were grossly hemolyzed. We investigated the potential for TPN and lipid emulsion to cause hemolysis in lithium heparin plasma collected in vacutainer tubes and blood gas syringes.

Methods: Blood collected from normal healthy volunteer donors was spiked with a mixture of 20% lipid emulsion (Intralipid, Fresenius Kabi, Homburg Germany) and TPN at varying proportions (1:1, 1:4 and 1:8) to a final concentration of 10% (1 part TPN/lipid emulsion to 9 parts whole blood). The blood and TPN/lipid emulsion mixture was transferred into blood gas syringes and plasma separator tubes (vacutainer tubes) each containing lithium heparin, and the tubes and syringes were mixed on a rocker for 10-60 minutes at room temperature. Specimens were then centrifuged for 3 minutes at 4000 x g, and the H-index of the plasma was determined on a Roche Modular Analytics system (Roche Diagnostics, Indianapolis IN).

Results: Hemolysis in both vacutainer tubes and blood gas syringe samples was proportional to the amount of lipid emulsion (relative to TPN) in the sample. At the highest ratio of lipid emulsion to TPN tested (1:1), spiking blood with 10% TPN/lipid emulsion caused gross hemolysis in vacutainer tube samples (H index >300) but only moderate hemolysis in blood gas samples (H index 100-150). Increasing incubation time from 10-60 minutes resulted in increased hemolysis in vacutainer tubes (H index > 600 after 30 minutes) but did not significantly influence the amount of hemolysis in blood gas syringes. At lower ratios of lipid emulsion to TPN (1:4 and 1:8) only slight hemolysis was noted in either sample type (H index of 20-40 in vacutainer tubes and 10-20 in blood gas samples).

Conclusion: Blood specimens containing significant amounts of lipid emulsion solution are prone to gross hemolysis. If blood specimens must be collected while lipid emulsion solution is being administered to pediatric patients, collection in blood gas syringes may prevent hemolysis of samples.

A-65

The Influence of Centrifugation, Freeze-Thaw, and Hemolysis on the Measurement of Plasma TARC (CCL17) by ELISA

M. Peach, J. Bigler, D. Tran, Y. Pan, R. Xu. *Amgen Inc., Seattle, WA,*

Introduction (Relevance): Pre-analytical variation in sample collection, preparation, and handling can have a significant impact on analyte measurement. Analytes associated with platelets may be especially susceptible to pre-analytical variation due to the contribution of platelet-derived analyte in the sample. TARC (CCL17) is a chemokine that has been associated with platelets and has been measured from purified platelets after lysis or thrombin activation (Fujisawa et al. 2002). Because of the potential for platelet-derived TARC to alter plasma concentrations, the influence of pre-analytical sample handling procedures needs to be understood. Factors that can affect platelet contribution to plasma TARC levels include centrifugation speed at plasma collection and number of plasma freeze thaw cycles. Additionally, factors that lead to hemolysis during sample acquisition can also result in platelet lysis and higher TARC concentrations.

Objective: To determine the effect of variation in centrifugation, plasma freeze-thaw, and sample hemolysis on plasma TARC concentrations measured by ELISA.

Materials and Methods: TARC concentrations were determined using the Human CCL17/TARC Quantikine ELISA from R&D Systems (Minneapolis, MN). Plasma was purchased from Bioreclamation Inc. (Hicksville, NY) or prepared from in-house donors. Purified red blood cells were isolated from blood by ficoll. Platelets in plasma were counted on the Siemens Advia 120 Hematology System.

Results (Validation): Centrifugation conditions were varied to prepare matched plasma samples containing variable numbers of platelets. Samples prepared at lower centrifugation speeds contained significantly more TARC than samples centrifuged at higher speeds and plasma TARC concentrations were strongly correlated with platelet count. Additionally, TARC concentrations in "platelet-free" plasma, prepared specifically to remove platelets, were significantly reduced compared to matched plasma samples prepared using conventional methods. TARC concentrations were also shown to increase with additional freeze-thaw cycles. Plasma TARC from samples prepared by centrifuging at 2000g was, on average, 69% higher after three cycles than samples that did not undergo any freeze-thaw. The magnitude of the freeze-thaw effect was also affected by centrifugation speed. Samples centrifuged at higher speeds were less affected by freeze-thaw than samples centrifuged at lower speeds. TARC concentrations from hemolysed plasma samples were nearly five times higher than non-hemolysed samples. Increased TARC in hemolysed samples was independent of red blood cell lysis as plasma spiked with purified red blood cell lysates failed to shown significant increases in TARC.

Conclusions: Centrifugation, sample freeze-thaw, and sample hemolysis can influence the measurement of TARC from plasma and are likely a result of platelet-derived TARC. Lower centrifugation speeds, greater number of platelets, and higher overall TARC levels were correlated. Higher TARC levels in samples that had undergone several freeze-thaw cycles are consistent with platelet lysis during freeze-thaws. Finally, hemolysed samples may indicate "sample trauma" that may cause platelet lysis or activation. As a result of these findings, strict compliance with sample collection protocols is essential to reduce artefactual variation in TARC plasma levels and sample freeze-thaw need to be limited. Results from hemolysed samples should be interpreted with caution.

A-66

The Interference of Insulin Antibodies and Cross-reactivities with Two Human Recombinant Insulin Analogues in a Insulin Immunoassay<!--StartFragment-->

Y. Yun, S. Kim, M. Hur, H. Moon. *Konkuk Univserity Medical Center and School of Medicine, Seoul, Korea, Republic of,*

Background: Insulin measurement is used for the diagnosis of hypoglycemia, and may be useful in determining the pathogenesis of type 1 and 2 diabetes. However each commercially available insulin assay has known to have some limitations: these include various degrees of cross-reactivities with recombinant insulin analogues and the interferences of anti-insulin antibodies. We evaluated the usefulness of insulin immunoassay (Immuno-eletrochemiluminescent-metric Assay, IECMA) by assessing the interference of insulin antibodies and cross-reactivities with two insulin analogues, and by comparing with other two insulin assays

Methods: Sera were obtainted from 73 type 2 DM patients under the continuous subcutaneous insulin infusion therapy(CSII) and 20 healthy controls attending the diabetic Out-Patient Clinics and medical health examination center of the Konkuk University Hospital, Seoul. Insulin concentrations were determined using MODULAR analyzer E170 (Roche Diagnostics, Mannheim, German), Advia Centaur Immunoassay System (Simens, Erlangen, Germany), and manual INS-IRMA assay (BioSource, Nivelles, Belgium). To investigate the interference of anti-insulin antibodies, we performed anti-insulin antibody tests(anti-insulin IRMA, CIS Bio International, Areva S.A., France), and analysed the differences between direct insulin and two PEG-treated insulin levels. We obtained vials of two insulin analogues (insulin aspart and glulisine) with nominal concentration of 100 IU/mL and suitable for injection, and diluted them with 7% BSA to final concentrations of 30, 100, 300, and 1000 mIU/L. The insulin levels of all dilutions were measured by E170 analyzer, and cross-reactivities (%) were calculated from the ratio of the measeured and nominal concentrations.

Results: The patients were grouped by the anti-insulin antibody levels (≤ 5.4%, 5.4~20%, 20~40%, > 40%), the mean values of direct, total, and free insulin obtained from E170 increased in higher level group. The differences of direct, total, and free insulin values in the anti-insulin antibody negative group(< 5.4%) were not significant (p=0.619), while those values in the antibody positive group(≥ 5.4%) were significantly different each other(p=0.031). Anti-insulin antibody and E170 total, free insulin levels were slightly correlated with fasting glucose level. Insulin values obtained from E170 analyzer were strongly correlated with those from Advia Centaur than those from insulin IRMA assay (R2= 0.421, p< .001 vs. R2= 0.767, p< .001). The average difference between E170 and IRMA values in B-A difference plot was 26.14%, the E170 insulin values had a higher tendency and showed a significant proportional bias (y = 2.0899x - 0.5491). The E170 insulin assay showed low cross-reactivity to both insulin analog aspart, and glulisine (< 0.7%).

Conclusions: The comparison between E170 and Advia Centaur insulin results showed good correlation, but poor between E170 and IRMA assay. The E170 insulin assay was more sensitive to interference from anti-insulin antibodies than others. We demonstrated that E170 insulin assay have no cross-reactivities with both insulin aspart and glulisine, this is the E170 measures only human endogeneous insulin. E170 insulin assay could be available to diabetes patients undergoing insulin therapy for the assessment of β-cell function and insulin resistance, and especially to anti-insulin antibody-positive patients, a PEG precipitation could be required of this assay.

A-67

25-OH vitamin D is stable in the clinical setting under many different collection and storage conditions

M. Burson[1], J. Puhl[2], G. S. Bodor[2]. *[1]Denver Office of the Medical Examiner, Denver, CO, [2]VA Medical Center, Denver, CO,*

Objective: There is contradictory information regarding vitamin D (VitD) stability in human blood. Our lab used to require collection and transport of samples in dark tubes and storage below -20C until testing. To eliminate the need for these error prone practices we evaluated VitD stability at ambient, refrigerated and frozen temperatures, using no additive (red top, RTT) and gel separator (SST) collection tubes. We also investigated the effect of hemolysis and exposure to fluorescent light on VitD concentration during storage.

Methods: Our in-house LC-MSMS VitD assay uses a Shimadzu binary HPLC system and AB 3200 Q-Trap triple-quadrupole mass spectrometer. 25-OH vitamin D2 (OHD2), 25-OH vitamin D3 (OHD3), parent vitamins D2 and D3 and their respective hexa-deuterated internal standards are separated on a C18 column following protein precipitation. MS analysis is performed using APCI positive ionization. Run time is 9.7 minutes. Assay measuring range is 5 to 150 ng/mL for each analyte. Total imprecision is 7 % to 11% throughout the AMR range. We used left over serum, or serum from volunteers for our study. Fresh serum was kept at ambient temperature, under fluorescent light on the bench for up to 24 hours to assess short term stability. Aliquots were taken at 3, 6, and 24 hours and frozen until assayed. Long term stability of VitD was assessed by comparing results of fresh (T0) samples from RTT to that of the SST serum. Centrifuged samples were left in the SST tubes at 4C to for up to 19 days to determine potential absorption of VitD by the gel. Frozen stability was assessed by re-assaying left over samples, kept below -20C for as long as sufficient amount of serum was available for testing. We added increasing amounts of hemolyzed RBC to pooled sera to assess interference from hemolysis. Each sample was assayed twice. Samples from the short term stability and hemolysis interference studies were analyzed within the same run. Samples from the long term stability study were extracted and assayed in separate runs, on days when VitD testing was performed.

Results: OHD3 concentrations in samples of the short term study ranged from 5 to 60 ng/mL. All results from T0 to T24 hours were within the 2SD range of our method. No statistical difference between time points and no trend could be detected within 24 hours of storage. No significant difference between OHD3 result of RTT and SST serum could be detected at T0. All results from later time were within assay imprecision. No difference between RTT and SST was found for up to 19 days but a slight upward trend in concentration could be observed. Hemolysis (up to 1.2 g/dl hemoglobin) had no effect on VitD concentration. Frozen samples were stable for at least six months. We could ascertain OHD3 stability only because none of the samples contained OHD2.

Conclusion: OHD3 is surprisingly stable at ambient, refrigerated and frozen temperatures, or when exposed to fluorescent light. Storage in RTT or SST does not affect stability.

A-68

Alternate Method for Determination of Long Term Frozen Storage Stability of Biomarkers

C. Hill, P. E. Oefinger, S. McLaughlin. *Covance Central Laboratories, Indianapolis, IN,*

Background: A preferred method of determining long term frozen stability of analytes is to prepare multiple aliquots of six patient samples and test one set of aliquots at 1, 3, 6, 9, and 12 months. Occasionally, an alternate method to assess long term stability is needed. For esoteric enzyme-linked immunosorbent assays (ELISA), two levels of quality control (QC) material were prepared in-house by pooling patient samples, aliquotting, and freezing at -70 degrees C. No preservative or stabilizer was added to the QC material. Target means and % coefficient of variation (CV) were established for each lot of QC material using 20 replicate aliquots. The two levels of QC material were then tested with each run of patient samples for up to one year. Achieved means and achieved %CV were evaluated against target means and target %CV as well as observation of trend analysis to determine long term frozen stability for each analyte.

Objective: This study purpose was to evaluate the use of in-house prepared patient sample based QC material to determine long term frozen stability of analytes.

Method: Thirteen analytes with quantitative ELISA were evaluated with the alternative method. The 13 quantitative ELISA included Adiponectin, C1CP, IL-1 receptor antagonist, IL-13, SHBG, D-Dimer, Anti ds DNA, IGF-II, Diphtheria IgG, Amyloid A, Anti CCP, Cotinine, and MCP-1. In addition, two analytes with qualitative ELISA were also evaluated with the alternate method. For both the qualitative ELISA for detection of Cotinine and Giardia Antigen, aliquots of one positive and one negative sample were prepared for use in parallel testing of new lots of reagents.

Result: Data were analyzed using the BioRad QC on Call software. The acceptable %CV level was established over time, ranging from 8.6 to 17.3 for various quantitative assays. The QC frozen stability for each assay was confirmed for a range of 5 to 11 months for the 13 quantitative ELISA, using the %CV as a target for accuracy. For qualitative ELISA, the frozen stability has been proven for at least 2 years. The stability studies continue to be on-going.

Conclusion: The alternate method of using in-house prepared patient sample based QC material was successful in establishing the storage stability of samples. Unlike the method of testing aliquots of six patient samples at specified time points, the alternate method incorporates more data sets that allow for trend analysis, thereby reducing individual run variation leading to erroneously assigned shortened stabilities. The alternate method also allows for establishment of stability time points over a continuum, instead of at defined intervals.

A-69

Improved Buprenorphine Immunoassay Performance after Pretreatment of Urine with Beta-Glucuronidase

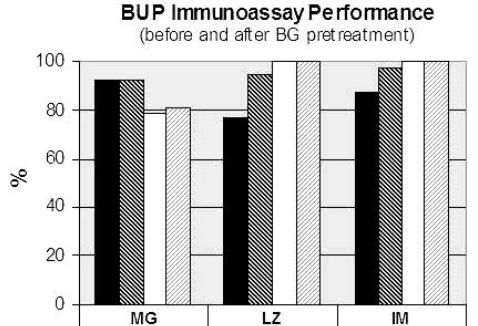
M. L. Snyder[1], S. E. Melanson[1], J. G. Flood[2], P. Jarolim[1]. *[1]Brigham and Women's Hospital, Boston, MA, [2]Massachusetts General Hospital, Boston, MA,*

Background: Immunoassay screening methods are increasingly being used by clinical labs to monitor buprenorphine compliance and abuse. However, these assays suffer from either limited sensitivity due to minimal cross-reactivity with highly excreted buprenorphine metabolites and/or glucuronide conjugates or limited specificity due to cross-reactivity with other commonly prescribed analgesics. A urine screening method with higher combined sensitivity and specificity is preferred. Objective: To evaluate beta-glucuronidase (BG) enzymatic sample pretreatment to improve performance of buprenorphine screening assays.

Method: Urine samples sent to our laboratories for buprenorphine testing (39 positive, 138 negative by LC/MS and medical record review) were analyzed using Microgenics CEDIA (MG), Lin Zhi International, Inc., EIA (LZ), and Immunalysis EIA (IM) semi-quantitative immunoassays on the Olympus AU480 analyzer (Beckman Coulter, Inc.), using a 5-ng/mL cutoff. BG pretreatment was performed by incubating urine with BG in acetate buffer for 1 hr at 60°C.

Results: Urine BG pretreatment significantly improved LZ and IM buprenorphine sensitivities, while assay specificities and MG sensitivity were unchanged (see figure). MG was the least specific for buprenorphine, yielding a false positive rate of 43%. The LZ and IM methods were 100% specific, even with high levels of other potentially interfering substances, such as opioids or other analgesics. Lowering the IM assay cutoff from 5 to 1 ng/mL would have increased sensitivity to 100%, with no decrease in specificity.

Conclusions: Rapid and simple BG urine pretreatment increases accurate detection of buprenorphine use with the LZ and IM methods to 95% and 97%, respectively, and should facilitate accurate BUP testing in clinical laboratories that do not perform confirmatory testing. Continuing investigations to further shorten the BG pretreatment and to fully automate this method are underway.



	MG	LZ	IM
Sensitivity	92.3	76.9	87.2
Sensitivity-post BG	92.3	94.9	97.4
Specificity	79	100	100
Specificity-post BG	81.2	100	100

A-70

Effects of Hemoglobin S Variants on the Measurement of Glycosylated Hemoglobin A1c by Four Analytical Methods.

W. Tamimi[1], S. Aljaser[1], R. Dafterdar[2], N. Aldegaither[1], M. Alrawaili[1], A. Elfatih[1], W. Alsaeed[1], R. Hasanato[3]. *[1]King Fahad National Guard Hospital, King Saud Bin Abdulaziz Univresity for Health Sciences, Riyadh, Saudi Arabia, [2]Riyadh Military Hospital, Riyadh, Saudi Arabia, [3]King Khalid Hospital, King Saud University, Riyadh, Saudi Arabia,*

Objectives: Glycosylated hemoglobin (HbA1c) is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. HbA1c is typically measured by either HPLC or immunoassay. However, measurement of HbA1c test can lead to false outcomes in patients with inherited hemoglobin variants such as hemoglobin S which is a prevalent variant in the Middle East population. Although it is expected that the homozygous variant of hemoglobin S may interferes with many HbA1c methods, the heterozygous form is not well reported among our population. Therefore, the purpose of this study was to evaluate four HbA1c different methods in patients with sickle cell anemia (SCA) and sickle cell trait. To the best of knowledge this was the first study to evaluate HbA1c among this ethnic population in the Middle East.

Method: Blood samples were collected in EDTA tubes from 38 patients 18 males and 20 females with either SCA 23 (60 %) or SCT 15 (40 %). Samples were splitted and performed at three different hospitals using 3 different HbA1c methods, Cobas Intergra (Roche, SZ), Dimension (Siemens, USA) and DCA2000 (Siemens, USA). All methods were compared to HPLC Variant II (Bio-Rad, USA).

Results: There was an excellent correlation (r=0.987, slope= 1.1, intercept= -0.6) between Dimension and Cobas Intergra followed by Cobas Intergra and DCA 2000 (r=0.725, slope= 0.710, intercept= 1.6) and then Dimension and DCA 2000 (r=0.701, slope= 0.596, intercept= 2.1). All three Dimension, Cobas Intergra and DCA 2000 were poorly correlated with Variant II method (r=0.0103, r=0.085, and r=0.0644) respectively. All four methods were interfered with hemoglobin S. We found that 9 samples (7 SCA & 2 SCT) were interfered with Variant method, 3 (1 SCA & 2 SCT) with COBAS INTEGRA, 10 (5 SCA & 5 SCT) with DCA2000, and 10 (7 SCA & 3 SCT) with Dimension.

Conclusion: HbA1c measurement interfered with both forms of Hemoglobin S homozygous and heterozygous in all studied methods for this ethnic population. While HbA1c levels were undetected in some method, other methods were perfectly measured HbA1c. This was due to differences in the design of each method. Therefore, we recommend that any interfered samples for HbA1c should be measured by another alternative method.

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Extended Stability of Free and Total PSA in Frozen Male Serum

J. E. Loebel[1], H. A. Fritsche[2], R. P. Thiel[3], T. F. Soriano[1]. *[1]DOCRO, Inc., Seymour, CT, [2]University of Texas, MD Anderson Cancer Center, Houston, TX, [3]Thiel Statistical Consulting, Oxford, CT,*

Objective: To verify long term stability of free and total PSA in male serum frozen at -70ºC and to demonstrate long-term serum storage does not affect PSA studies, initial PSA results were compared to those using new frozen aliquots from the same subjects 8.5 years later.

Background: Subjects aged 50 - 75 years, referred for prostate cancer workups, including sextant biopsy, donated blood August 1999 through July 2001 prior to DRE/biopsy. Serum was separated from cells, kept at 4 - 8 ºC until frozen at < -70 ºC within 36 hours of collection for long term storage in 0.5 mL aliquots in cryovials (Fisher Scientific 02-681-343). Serum remained frozen until just prior to testing.

Methodology: In August 2001, free and total PSA assays were calibrated on the Dade (Siemens) Dimension RxL. Free and total PSA were measured and f/t PSA ratio% calculated on a frozen serum specimen aliquot from urologically-referred subjects as described above. In January 2010, the same Dimension RxL was again calibrated and used to measure free and total PSA on a freshly defrosted aliquot from 160 of the subjects previously tested. The time period between the initial and final measurements of free and total PSA was 8.5 years with many of the specimens having been drawn more than ten years prior to the latest testing date.

Validation: y = tPSA 2010 and x = tPSA 2001, Y = 0.2127 + 1.0057x R^2 = 0.9945 y = fPSA 2010 and x = fPSA 2001, y = -0.02673 + 1.0549x R^2 = 0.9931

Statistic	tPSA July 2001 ng/mL	tPSA Jan 2010 ng/mL	Ratio tPSA 2010 2001	fPSA July 2001 ng/mL	fPSA Jan 2010 ng/mL	Ratio fPSA 2010 2001	f/t PSA% July 2001	f/t PSA% Jan 2010	Ratio of f/t PSA%2010 2001
Mean	7.48	7.73	1.03	0.95	0.97	1.03	0.15	0.14	0.96
Std Dev	8.29	8.36	1.01	0.95	1.00	1.06	0.09	0.09	1.00
Minimum	0.07	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.00
Q1	3.96	3.91	0.99	0.39	0.41	1.04	0.09	0.08	0.96
Q2 (Med)	5.71	6.07	1.06	0.69	0.68	0.99	0.13	0.12	0.97
Q3	8.01	8.43	1.05	1.12	1.13	1.01	0.18	0.18	0.96
Maximum	69.94	70.25	1.00	5.72	6.11	1.07	0.47	0.44	0.92
Range	69.87	70.25	1.01	5.70	6.11	1.07	0.45	0.44	0.98
Interquartile Range	4.06	4.52	1.11	0.73	0.72	0.99	0.10	0.09	0.96

Conclusion: Free and total PSA concentrations in serum are not affected by storage at <-70 ºC for a period of at least 8.5 years. Results using specimens stored in this way are equivalent to those obtained from freshly drawn serum immediately tested for free and total PSA.

A-72

Assessment of the Effect of Hemolysis on PTH STAT Assay Using Roche e601 Cobas Analyzer

L. Chen, N. Adams, K. Ryan, J. Drader, B. Meiklejohn, T. Kwong. *University of Rochester, Rochester, NY,*

Background and Objective: A negative interference by slight hemolysis on the 9-minute Roche PTH STAT assay has been reported recently, which may affect the clinical interpretation when used for interoperative PTH measurement during parathyroidectomy. We performed the hemolysis analysis using spiked pooled serum and compared our results with the documented hemolysis analysis provided by the manufacturer to determine the cutoffs for sample rejection.

Methodology: To test if a PTH concentration-dependent effect on interference by hemolysis using the Roche PTH STAT assay exists, pooled serum with PTH levels at 15, 20, 40, 63, 107 and 198 pg/ml were spiked with hemolysate to produce serial hemolyzed specimens with hemolysis indexes of 95, 186, 837 and 1678 (corresponding to 0.168, 0.336, 1.68 and 3.36 g/dL Hb respectively); each specimen was measured five times serially with Elecsys PTH STAT assay on e601.

Results and the Conclusion: The results of twenty two immunoassays in our test menu are relatively unaffected by hemolysis, which agreed with manufacturer's specifications. However, Roche PTH STAT assay was negatively affected by hemolysis and the interference caused 16% and 26% reduction for the specimen with hemolysis index 95 and 186 (moderate hemolysis), respectively when PTH level was at 22 pg/ml. In a further investigation using spiked serum with various PTH levels and hemolysis index (95, 189, 837 and 1678), we found that when serum PTH levels were 40, 63, 107 and 200 pg/ml the differences were all within 10% range of the non-hemolyzed samples across the moderate and grossly hemolyzed specimens, as shown in Table 1. We concluded that negative interference by hemolysis on Elecsys PTH STAT assay only exists when PTH values are at low normal range (<40 pg/ml), which has minimal impact on result interpretations with our current sample rejection criteria set at hemolysis index >200.

Percentage Bias Caused by Hemolysis on Roche PTH STAT Assay Vary with Serum PTH Levels

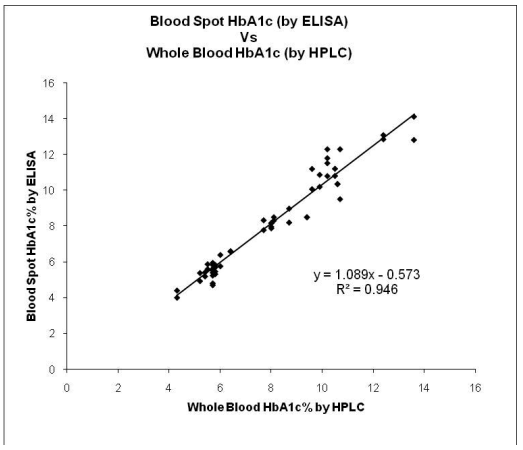
	Hemolysis Index			
PTH Levels	95	186	837	1678
16 pg/ml	-7.50%	-26.17%	-41.13%	-43.82%
22 pg/ml	-16.15%	-26.83%	-	-
40 pg/ml	6.64%	8.98%	8.98%	8.98%
63 pg/ml	-3.95%	-2.41%	-7.27%	-3.55%
107 pg/ml	10.09%	3.87%	0.07%	-0.75%
199 pg/ml	8.98%	6.43%	6.23%	3.06%

A-73

Measurement of HbA_{1C} in eluted blood spot samples by a novel ELISA - a method for assessing the diabetic status of patients in resource limited settings

S. Packer[1], F. Ghafoor[2], S. Naz[2], R. Edwards[3], P. Timms[4], S. Ajodha[3].
[1]Scipac Ltd, Sittingbourne, United Kingdom, [2]NHRC, Shaikh Zayed Medical Complex, Lahore, Pakistan, [3]Netria Ltd, Sittingbourne, United Kingdom, [4]Homerton Hospital, London, United Kingdom,

With the increase in standards of living in the developing countries the incidence of diabetes is increasing dramatically. Monitoring HbA1c is well recognized as an important way of monitoring long term glycemic control for diabetic patients. A sensitive ELISA for HbA_{1C} has been developed using monoclonal antibody detection for use with filter paper blood spot samples. In developing countries the logistics systems for transportation of specimens are not well developed, equipment for testing is located only in major industrial centers and there is a shortage of trained phlebotomists. Blood spot samples were chosen for ease of collection and distribution from widespread rural populations. Samples can be collected in the field and sent by post to central laboratories for elution and testing. The high sensitivity ELISA was developed with optimal binding of hemoglobin (glycated and non-glycated). This was achieved by coating microtitre plate wells with purified haptoglobin and using a specific monoclonal antibody to the glycated region of the HbA1c molecule conjugated to HRP to produce a signal. The assay was calibrated using stabilized HbA1c solutions and the assay was validated using external control materials. Samples of whole blood were collected in EDTA tubes and spotted on specially prepared filter paper and allowed to dry. After several days samples of blood spots were eluted with a stabilizing buffer solution, samples were then lysed and tested in the ELISA. The ELISA result was compared with results from whole blood samples measured by HPLC (Bio-Rad Variant II). Results from 54 patients within a normal laboratory population of diabetic patient requests were assessed using the two methods. The results for the blood spots produced a highly significant correlation with HPLC results ($R^2 = 0.946$).



A-74

Effect of repeated centrifugation of PST tubes on common chemistry analytes

A. L. Pyle, S. Dawling, A. Woodworth. *Vanderbilt University, Nashville, TN,*

Introduction: Since studies have shown that several analytes are not stable in un-separated whole blood, many out-reach sites centrifuge samples to separate plasma from cell components prior to sending to the reference laboratory. Use of gel-separator tubes enables fast and reliable sample separation, but once in the laboratory the primary tubes may be re-spun by automated processing systems to expedite sample flow.
Objectives: To test the stability of common analytes in Lithium-Heparin plasma separator tubes (PST) after prolonged storage refrigerated and at room temperature and after recentrifugation.
Methods: Blood was drawn in BD Vacutainer PSTs and subjected to multiple storage and treatment conditions. Four tubes were collected from each of 16 healthy volunteers and divided into 4 groups of 4 subjects each as follows: (1) Spun prior to storage at room temperature (RT), or (2) at 4°C; and (3) un-spun and stored at RT, or (4) 4°C, with centrifugation after the storage period. All groups were stored in their respective conditions for 0, 4, 8, and 24hrs. 22 common chemistry and immunochemistry assays were performed on Beckman Coulter UniCel DxC 800 and DxI 600 analyzers (first round analysis). Once analysis was completed, all tubes from the spun storage group were immediately re-centrifuged and re-analyzed (second-round analysis). Statistical change limit (SCL) was calculated for each analyte.
Results: At first round analysis of spun samples stored at RT (Group 1), most of the analytes remained within the SCL except glucose and total bilirubin (TBili) which fell below the SCL at 24h, and all time points, respectively. Aspartate aminotransferase (AST) was elevated above the SCL at 24 hr RT storage. TBili and AST both dropped below the SCL at all time points in samples spun and stored at 4°C (Group 2). Most samples stored un-spun for 4, 8, and 24 hr were stable at RT (Group 3), with some exceptions: glucose decreased at 4, 8, and 24 hr, TBili decreased at 24 hr, and AST decreased at 8 hr and increased at 24 hr. Consistent with published reports, a significant increase in potassium (K) was observed at all time points in samples stored un-spun at 4°C (Group 4). Both glucose and TBili were decreased at 24hr.
In second round analysis, several analytes changed significantly. Glucose concentrations dropped further in samples stored for 4, 8, and 24 hrs (Group 3). TBili rose back to within SCL with repeated centrifugation. Ferritin and AST rose above the SCL at 24hr, and all time points, respectively. Samples stored spun at 4°C and then subjected to repeat centrifugation showed elevated K and TBili after 24 hr storage, a drop in glucose after 8 and 24 hr, and a significant decrease in TBili and AST after 4 and 8 hr storage (Group 4).
Conclusions: This study demonstrates the unique finding that various analyte concentrations change significantly following repeat centrifugation in PSTs. Most notable were K, glucose, TBili, and AST, which all exceeded their SCL. These results should be considered when processing PST tubes from out-reach sites.

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Evaluation of different tube types and reducing interferences for the measurement of 25-hydroxy vitamin D2 and D3 by tandem mass spectrometry

A. Munar, C. C. Frazee, A. M. Ferguson, U. Garg. *Children's Mercy Hospital, Kansas City, MO,*

Background: Vitamin D plays a vital role in health and disease including bone metabolism, immune system, cardiovascular diseases, diabetes and cancer. Vitamin D metabolites, 25-OH vitamin D2 and D3 are used as markers of vitamin D nutritional deficiency. One of the commonly used methods for the measurement of 25-OH-vitamin D2 and D3 is tandem mass spectrometry (MS-MS). Although MS-MS is generally considered specific and free from interferences, they do occur. While evaluating MS-MS method for the assay of 25-OH vitamin D2 and D3, we found interfering peaks with a commonly used multiple reaction monitoring (MRM) ion pair of m/z 401.2/ 383.2. A recent article (Clinical Laboratory News December 2009) reported a positive interference at m/z 383 for 25-OH-D3 with a certain brand of polypropylene containers. We investigated if other tube types also show interference with m/z 401.2 to 383.2 MRM. We also investigated other MRMs to see if this interference could be eliminated.
Methods: Blood from 18 volunteers was collected in serum clot activator, no gel, red top tubes (Vacuette, Greiner bio-one, Inc), and EDTA purple, sodium heparin and lithium heparin tubes (BD Vacutainer, Inc). To 200 µL serum or plasma, 200 µL of acetonitrile containing 100 ng/mL estriol and 50 ng/mL deuterated internal standard 25-OH-vitamin-D3 (26, 26, 26, 27, 27, 27-d6) was added. The samples were vortexed and left at room temperature for 10 minutes. After centrifuging at 10,000 g for 10 minutes, the supernatants were transferred to autosampler vials. The analysis involved injection of 30 µL supernatants, 5 cm x 46 mm 5 µm C18 column, Shimadzu UFLC and Applied Biosystems 4000 QTrap tandem mass spectrometer. MS-MS was used with an atmospheric pressure chemical ionization (APCI) and multiple reactions monitoring (MRM) mode. Calibrators were prepared in 7% BSA in phosphate buffered saline and ranged from 4.6 to 74 ng/mL for 25-OH-vitamin D3 and 3.9 to 62 ng/mL for 25-OH-vitamin D2. MRM transitions were: 25-OH vitamin D2 (395.5/377.5 and 395.5/269.5), 25-OH vitamin D3 (401.2/383.2, 383.4/257.3 and 383.4/365.5) and 25-OH vitamin D3-d6 (389.3/211.5).
Results and Conclusion: When 25-OH-vitamin D3 was measured using 401.2/383.2 MRM, serum clot activator tubes showed interfering peaks. The interfering peaks were also seen when calibrators prepared in 7% BSA/PBS were added to these tubes. The interference peaks were not observed with the other tubes. Other MRMs for 25-OH-vitamin D3 were optimized, and by using 383.4/257.3 MRM, the interference peaks could be eliminated. With this MRM transition, the average 25-OH-vitamin D3 concentrations in different tube types, serum clot activator- no gel, EDTA, sodium heparin and lithium heparin tubes were 32, 33, 32, 31 ng/mL. None of the MRMs listed above for 25-OH-vitamin D2 were affected by serum clot activator. In conclusion a commonly used 401.2 to 383.2 MRM shows interference in certain tube types. This interference can be eliminated by using 383.4 to 257.3 MRM. Using this method, several other tube types are also suitable for blood collection for the measurement of 25-OH vitamin D2 and D3.

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Robust features of an assay for measurement of serum vitamin C using high performance liquid chromatography with electrochemical detection (HPLC-ED)

H. Chen[1], M. Xu[2], R. L. Schleicher[1]. *[1]Centers for Disease Control & Prevention, Atlanta, GA, [2]Battelle, Atlanta, GA,*

Background: CDC routinely quantitates serum vitamin C (ascorbic acid) using HPLC-ED. In the field, one part serum is mixed with four parts 6% MPA to acidify the serum and stabilize ascorbic acid, then the specimens are frozen and stored at -70ºC. To begin the analysis, specimens are thawed and centrifuged to remove particulate; supernatants are decanted and gently hand-mixed with trisodium phosphate and dithiothreitol to reduce dehydroascorbate. An internal standard (1-methyl uric acid) is included to monitor recovery. Samples are re-acidified with 40% MPA, filtered and promptly injected onto a C-18 column eluted with an acidic mobile phase. Analytical reagents are prepared using 18 megohm water. ED voltage is maintained at +650 mV and current is measured.
Objective: As vitamin C is readily oxidized, assay robustness (small variations in method parameters) is an ongoing concern. Steps in the analysis process in which small changes might lead to additional oxidation were investigated including, more vigorous mixing, delayed chromatographic separation (samples held at 4ºC), or lower water quality. Independent experiments were performed for each variable using either QC pools (duplicates per pool) or patient samples. Significance was tested using paired t-tests.
Results:

Serum Vitamin C Mean±SD mg/dL	**Mixing**		**Delayed HPLC (Hr)**			**Deionized water (MOhm)**	
	hand	**vortex**	**0**	**24**	**48**	**18**	**5**
Low QC	0.22±0.02	0.22±0.02	0.24±0.02	0.24±0.01	0.25±0.02		
Med QC	1.06±0.06	1.09±0.05	1.12±0.04	1.12±0.04	1.06±0.04		
High QC	2.14±0.09	2.16±0.04	2.19±0.08	2.19±0.09	2.14±0.03		
Patients (n=51; 1 run)						1.05±0.48	1.12±0.58
Patients (n=143; 6 runs)			1.16±0.63	1.16±0.64			
Patients (n=8; 3 runs)			2.61±0.66		2.69±0.68		

Reference conditions: hand, 0 or 18; all p>0.05
Conclusions: None of the changes in protocol significantly affected the results. The HPLC-ED method that we use in our lab is robust under the non-standard conditions described.

A-77

Are specimen collections for vancomycin trough concentrations appropriately timed?

A. P. Morrison, M. J. Tanasijevic, M. G. Carty, P. Szumita, S. E. Melanson. *Brigham and Womens Hospital, Boston, MA,*

Background: Therapeutic drug monitoring of vancomycin is common in clinical practice. For clinical scenarios which require monitoring, serum trough concentrations are recommended as a surrogate marker of pharmacodynamic target attainment, a predictor of vancomycin efficacy. Peak levels to monitor toxicity are not recommended. Trough levels must be drawn at the correct time to have clinical value. Interpreting a vancomycin level, that is not a true trough, as a true trough may lead to improper dosing and treatment failure. The extent to which levels are drawn at incorrect times is unknown.
Objective: To determine the frequency with which phlebotomy and nursing collections for vancomycin trough levels are inappropriately timed and to identify targets for improvement in the ordering and collection process.
Methods: We analyzed one month of vancomycin levels measured at our hospital (n=827, April 2009). Both nursing and phlebotomy collections were examined. We excluded 244 levels (30%) with uncertain collection or vancomycin administration times. We defined a level as appropriately timed if it met any of three appropriateness criteria: 1) collected within 2 hours of next given vancomycin dose, 2) collected within 2 hours of next scheduled dose if next dose not given, 3) collected at the correct interval after the last given dose. We first determined the percentage of all included levels (n=583) that met the first criteria, then reviewed a sample of the remaining levels using the additional criteria. We excluded all tests found upon chart review to be drawn in the following clinical scenarios: rapidly changing renal function, checking after a high level, checking shortly after admission, and checking before discharge- as these represented non-trough draws. We then estimated the percentage appropriately timed versus inappropriately timed.
Results: 25% of levels (146/583) were collected within two hours of the next dose. From review of a sample (n=150) of the remaining levels, we estimate an additional 14% were appropriate by further criteria, 43% met exclusion criteria, and 19% of levels were inappropriately timed. After removing the exclusions, an estimated 68% of troughs were correctly timed and 32% were incorrectly timed. A similar percentage of correctly and incorrectly timed levels (72% vs. 68%) had instructions within the order to collect the specimen as a trough. As opposed to correctly timed levels, incorrectly timed levels were more often collected during hours when routine morning labs were drawn at our hospital (54% for incorrectly timed vs. 23% for correctly timed). Phlebotomy-collected and nursing-collected levels were analyzed separately but did not differ significantly.
Conclusion: An estimated one third of vancomycin levels intended to be drawn as troughs at our hospital are drawn at incorrect times and thus may be potentially clinically misleading. We plan to implement targeted solutions to reduce the number of incorrectly timed draws through direct education of nurses and ordering clinicians, decision support at the time of order entry, and electronic solutions to both enhance multidisciplinary communication and allow collections to be coordinated with electronic medication administration data.

A-78

Effects of Hemolysis on High-Sensitivity Troponin T Values During Serial Sampling

L. J. Ouverson, S. C. Bryant, B. S. Karon, A. S. Jaffe, A. K. Saenger. *Mayo Clinic, Rochester, MN,*

Background: Cardiac troponin is the biomarker of choice for diagnosis of acute myocardial infarction when a changing pattern of values is present. Our current cardiac biomarker panel measures troponin T (cTnT) upon suspicion of AMI (baseline, 0 h) and subsequently at 3 and 6 hours. An elevated cTnT value (i.e above the 99th% of the reference range) is defined as ≥ 0.01 ng/mL. We further report a "delta", calculated from the difference in cTnT at the 0-3 and 0-6 hour time points based on analytic precision. Significant deltas are defined based on the initial cTnT concentration. Hemolysis is a commonly encountered interference which causes falsely low cTnT results. Combined with analytic imprecision, there is the potential for these conjoint effects to confound our change criteria. Accordingly, we evaluated the effect of hemolysis on the current 4th generation cTnT assay and the new high-sensitivity cTnT (hscTnT) assay and modeled results to determine what level of hemolysis will clinically impact interpretation of serial troponin results.
Methods: Erythrocytes were lysed and preparation of hemolysates yielded hemoglobin concentrations ranging from 0.5 to 8.0 g/dL (corresponding to an H-index of 50 to 800). Manufacturer recommendations suggest utilizing an H-index cutoff of 100. Eight serum and two lithium heparin plasma pools with varying troponin concentrations were spiked with hemolysate solutions, resulting in 110 unique samples with varying cTnT and H-index measurements. cTnT (4th generation and hscTnT) and H-indices were measured on the Roche Modular E170 or Cobas e601 (Roche Diagnostics, Indianapolis, IN). Generalized estimating equations were used to assess the relationship between H-Index and the amount of negative interference, while adjusting for dilution effects. Subsequent general linear models (GLM) were used to assess the equivalence of pools within each cTnT assay. All analyses were performed in SAS 9.1.3.
Results: A proportional negative relationship between the initial cTnT concentration and H-index was observed with both the 4th generation cTnT and hscTnT assays. The slope (% bias vs H-Index) of individual pools spiked with hemolysate was significantly different from one another and different between the standard and hscTnT assays ($p < 0.004$ for cTnT; $p < 0.01$ for hscTnT). There was a -5.1% bias per 100 units of H-Index with the cTnT assay, and a -4.4% bias per 100 units of H-Index with the hscTnT assay (both $p < 0.002$). The effect of hemolysis was similar regardless of sample type. Interassay precision of serum pools was 10.5% at 0.01 ng/mL and 13.4% at 0.0069 pg/mL for TnT and hsTnT, respectively.
Conclusions: Even mild-moderate hemolysis produces a small negative bias (<5%) in cTnT and hscTnT results. This effect could be of significance for patients whose values are near the cut off value of the 99th%. The combination of hemolysis and analytical imprecision has the potential to even further skew the clinical interpretation of serial troponin results. These data suggest that conservative cutoffs for hemolysis should be used for all cTnT assays but especially the hscTnT assay to minimize the likelihood of false negative results.

A-79

Preanalytical Variables Affecting the Detection of microRNA in Serum and Plasma Specimens

J. S. Hackbarth, D. Milosevic, S. K. Grebe, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN,*

Background: MicroRNAs (miRNAs) are short, non-coding RNA sequences that regulate gene expression by inducing mRNA degradation or inhibiting translation. The recent isolation of miRNAs from plasma and serum samples and the detection of unique miRNA fingerprints in blood samples of patients with cancer and other disease states has generated great interest in the use of circulating miRNAs as blood-based markers in clinical diagnosis. However, in order for these markers to be successfully implemented in the clinical laboratories a number of parameters need to be thoroughly characterized including pre-analytical factors of sample collection, extraction of small amounts of miRNA from serum and plasma and data normalization. The purpose of this study was to perform a detailed evaluation of the pre-analytical factors that affect miRNA analysis, including comparing serum versus plasma specimens and determining the inherent assay variability, inter-individual variation, and miRNA stability.
Method: Three miRNAs (miR-15b, miR-16 and miR-24) were extracted from serum and plasma samples obtained from healthy volunteers using the mirVana PARIS isolation kit (Ambion) and amplified using Taqman-based qRT-PCR (Applied Biosystems). miRNA quantitation is represented by the cycle threshold (Cq). Synthesized *C. elegans* miRNAs were spiked into samples before extraction to serve as internal controls. A nested design experiment was used to determine the amount of variability introduced during the extraction, RT, and qPCR steps. Stability of miRNAs in serum was tested at ambient temperature, 4ºC, and -20ºC. Statistical analysis was performed using JMP statistical software (SAS)
Results: miRNA concentrations were significantly higher in plasma samples compared to serum. Additional processing of the plasma to remove possible cellular contaminants significantly decreased the amount of miRNA to the concentrations detected in serum. Analysis of the contribution of each step to the overall noise using a nested experiment approach showed a mean Cq variance of 0.27 (0.20-0.41), which correspond to less than a 2 fold variation in sample recovery. Breakdown of the source of variation showed most of the variability was due to the miRNA extraction process and inter-individual variability. Analysis of miRNA stability showed that miRNA are stable for 24 hours at ambient temperature, and 3 days refrigerated or frozen.
Conclusion: Detailed validation of the pre-analytical steps affecting miRNA detection and quantitation is critical when considering the use of these markers in the clinical laboratory. All study parameters should be standardized when establishing the clinical significance of miRNAs to avoid introducing additional variability from factors including the extraction method or cellular contamination of serum or plasma samples. Finally, ideal miRNA candidates for identifying a disease state should display a difference between the control and disease group that greatly exceeds the range of variability inherent in the assay. This variability should be established for each miRNA marker used in the clinical setting.

A-80

Specialty Hemostasis Test Compatibility with Capped Tubes

K. Finnegan[1], G. Cutsforth[2]. *[1]SUNY Stony Brook, Stony Brook, NY, [2]Diagnostica Stago, Inc., Parsippany, NJ,*

Several manufacturers have introduced cap-piercing technology on their automated blood coagulation analyzers. The study was initiated to determine the substantial equivalence of hemostasis testing (AT and vWf) on capped versus uncapped evacuated blood collection tubes from two manufacturers. The use of cap-piercing technology reduces technologist exposure to blood borne pathogens. However, cap-piercing technology must be validated to ensure that a bias or additional variability is not introduced. Seventy one volunteers donated two hemostasis collection tubes (one each of two manufacturers) and the AT and vWf assays performed first on the capped tube, then on the same tube, uncapped. Demographic data (age and sex) and coumadin/coumarin/warfarin therapy were collected. Samples were drawn by routine venipuncture and processed for platelet poor plasma within 4 hours of collection adhering to CLSI H21- A5 Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline. The AT and vWf were performed on a Stago STA®-Compact® with STA® System Controls N&P, STA® Liatest Controls N&P, STA® Liatest VWF (Immuno-turbidimetric) and STA® Stachrom ATIII (Functional-Chromogenic) reagents. Assays were performed in singlicate and the values were compared between capped and uncapped by linear regression. Linear regression analysis demonstrated a correlation between capped and uncapped values ($R^2 > 0.80$) for both assays. Student's t-test analysis was not performed on the data sets because the differences in values is dependent on the level of the value.

	Linear Regression	Linear Regression	Linear Regression
	Slope	Intercept	R / R2
AT	0.867	21.0	0.918 / 0.843
vWF	0.907	19.4	0.936 / 0.876

Given the inherent biological variation of AT and vWF, our conclusion is that there is no significant difference between AT and vWF analysis on the capped or uncapped blood collection tubes tested in this study.

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Comparison of ViveST™, a Novel Dried Plasma Transportation Matrix, to Frozen Plasma Using a Quantitative HIV-1 Viral Load Assay

R. M. Lloyd[1], C. Loveday[2], R. S. Diaz[3], K. Presley[1], R. L. Mathis[1], R. Kantor[4], Z. Grossman[5], M. Holodniy[6]. *[1]Research Think Tank, Inc., Buford, GA, [2]ICVC Charitable Trust HQ, Buckinghamshire, United Kingdom, [3]Federal University of São Paulo, São Paulo, Brazil, [4]Brown University Medical School, Providence, RI, [5]Sheba Medical Center, Ramat Gan, Israel, [6]VA Palo Alto Healthcare System, Palo Alto, CA,*

Background: There are currently many methods for the transportation of human biological specimens: 1) frozen plasma or frozen serum on dry ice; 2) dry blood spots (DBS) or 3) whole blood, serum, or plasma at ambient or refrigerated temperatures. The ViveST™ device is a high volume (1 mL) dried specimen transport and storage system that does not require refrigeration. The biological specimen is loaded onto the absorbent matrix and dried. Specimens improperly dried during the drying process will arrive at the testing site, laboratory, or storage facility with a visible color change in the Color Indicating Capsule used for laboratory quality assurance. The dried plasma specimen is reconstituted with the supplied buffer and released from the absorbent matrix using a supplied disposable recovery apparatus. The recovered specimen is then suitable for testing.
Objective: This study presents an evaluation of k2EDTA frozen plasma compared to ViveST using HIV viral load (VL). The study also compares the utility of the method in relation to long-term shipping or storage at three temperature ranges.
Materials and Methods: Five 1 mL replicates were created from pooling remnant patient specimen representing approximately 350,000 copies/mL and 3,500 copies/mL replicate panel members and VL control reference standards. One mL of the experimental pooled plasma was directly applied to 5 separate ViveST devices to determine short-term stability at: room temperature for 22°C, 37°C, and -80°C. Each experimental temperature had the following end points at Day 2, 7, 14, 21, 28, and 56. ViveST units were then placed in an externally venting biological safety cabinet running overnight with a relative humidity range of 32-35%. HIV-1 VL was determined using the Roche COBAS Amplicor HIV-1 Monitor standard assay.
Results: Overall intra-assay mean variance among 5 dried replicates across all time points and temperatures was < 0.20 log10 copies/mL (P = NS). Compared to baseline frozen plasma, there was a mean reduction in viral load of 0.38-0.45 and 0.23-0.53 log10 copies/mL at the high and low copy samples respectively at day 2 depending on the temperature of storage (all comparisons, $P < 0.05$). However, when day 2 ST was compared to day 56 ST, there was no significant difference in HIV log copy number out to 56 days of storage at 23°C or -80°C, and out to 28 days at 37°C, but there was a further significant decline in VL in samples held at 37°C for 56 days ($P < .002$).
Conclusions: Viral load results from dried samples using ViveST were highly reproducible. Although quantitation of HIV-1 VL using dried plasma always yielded slightly lower values compared to frozen plasma, results were generally within the accepted assay variation for replicate samples and demonstrated marked stability in conditions that simulate weather conditions. The data on stability and reproducibility suggest that ViveST has promise for use in the research and diagnostic laboratories.

A-82

Solution Densities and Estimated Total Protein Concentrations Associated with Inappropriate Floatation of Separator Gel in Different Types of Blood Collection Tubes

R. C. Faught, R. Calloway, R. Norquist, J. Marshall, J. Bornhorst. *University of Arkansas for Medical Sciences, Little Rock, AR,*

Background: Inappropriate flotation of separator gel to the top of clinical laboratory specimens following centrifugation has the potential to be highly disruptive to laboratory instruments upon probe aspiration. This problem is especially apparent in automated processing systems where it can lead to analytical errors. Unusually high density of the aqueous portion in clinical samples containing high total protein content has been associated with this phenomenon. While gel floatation has been previously reported in Plasma BD Vacutainer® PST™ (Becton, Dickinson

and Company, Franklin Lakes, NJ) sample tubes, we observed a clinical sample where inappropriate gel formation occurred in Greiner Bio-One Vacuette® serum tubes (Greiner Bio-One, Frickenhausen Germany), but not in BD Serum Vacutainer® SST™ tubes. The tubes (13 x 100mm) were centrifuged at 3000 rpm (1912 rcf) for 6min, and repeated centrifugation yielded no change in gel placement. This led us to initiate a comparative study of gel floatation in different specimen container types.
Methods: Different 13x100mm BD and Greiner specimen tubes with two different lot numbers of each type were purchased. The specimen tube types examined were BD Serum Vacutainer® SST™, BD Li-heparin Plasma Vacutainer® PST™, Greiner Serum Separator Vacuette® and Greiner Li-Heparin plasma separator Vacuette® tubes. Different dextran solutions (500 mL) were generated by dissolving high fraction dextran (Acros Organics, New Jersey, USA) in normal saline solution. The density of the dextran solutions were measured by means of Fisherbrand (Fisher Scientific, Pittsburg, PA) floating traceable NIST certified hydrometer (specific gravity 1.000-1.070). The dextran solutions (4.5mL) were added to the different specimen tubes and were then centrifuged as described above. The tubes were then visually inspected for separator gel rising to the top of the dextran solution.
Results: The dextran solution specific gravity at which inappropriate gel flotation was observed is described as follows (highest density for which no flotation was observed: lowest density for which gel flotation was observed). While some differences between tube lots were observed, only data for the lots for which the lowest density caused gel floatation is presented here. For serum tubes the results were (1.054: 1.057) for BD and (1.044:1.047) for Greiner. For Li-heparin plasma tubes the results were (1.054:1.057) for BD and (1.047:1.048) for Greiner. Using an equation obtained from Moore and Van Slyke (*Journal of Clinical Investigation*,(1925)), total plasma protein content can be calculated from the sample specific gravity (SG), where total protein(g/dL)=343(SG-1.007). The calculated corresponding estimated total protein content (g/dL) from the lowest measured dextrose solution SG in which gel floatation was observed was 13.72 g/dL (serum tube) and 14.06 g/dL (plasma tube) in Greiner tubes as compared to 17.15 g/dL (serum and plasma) in BD tubes. The total protein content of the original clinical sample described above was 14.4 g/dL, and the observed flotation pattern is predicted by the data obtained from the dextran SG experiments.
Conclusion: Differences were observed in the solution density between gel specimen types and manufacturer lots. Laboratories wishing to avoid problems with inappropriate gel floatation should consider these observations.

A-83

Cross-reactivity of Sertraline, Buprenorphine, Buspirone, and Bupropion with the Vitros Drugs of Abuse Assays

D. D. Baker[1], R. E. Winecker[2], C. A. Hammett-Stabler[1]. *[1]University of North Carolina, Chapel Hill, NC, [2]Office of the Chief Medical Examiner, UNC, Chapel Hill, NC,*

Background: Widely used to provide rapid turnaround times in clinical settings, the immunoassays designed to detect the presence of drugs belonging to large classes, such as the amphetamines, benzodiazepines, and opiates, are subject to cross-reactivity with other, often unrelated drugs. In response to an increased number of urine drug screens for these classes that did not confirm using GC/MS, we investigated the potential cross-reactivity of several medications found to be common to many of these samples. The compounds tested included sertraline, buprenorphine, buspirone, bupropion, and two metabolites (desmethylsertraline and hydroxybupropion). Of these, sertraline was reported by the manufacturer (Ortho Clinical Diagnostics, Inc.) to cross-react with the methadone assay (0.05% at 300,000 ng/mL) and the opiate assay (at 10 mg/dL), and bupropion with the amphetamine assay (0.05% at >100,000 ng/mL).
Method: Individual samples were prepared by adding increasing concentrations of each compound to drug free urine. Each sample was tested for amphetamines (cut-off = 500 ng/mL), barbiturates (200 ng/mL), benzodiazepines (200 ng/mL), cocaine metabolite (150 ng/mL), methadone (300 ng/mL), opiates (300 ng/mL) and THC (20 ng/mL) (Vitros 5600 Ortho Clinical Diagnostics) and propoxyphene (300 ng/mL Siemens Healthcare Diagnostics EMIT II reagent modified for the Vitros 5600). The response obtained for each compound was compared to the response of a drug free sample for each assay.
Results: Sertraline cross-reacted with the propoxyphene, benzodiazepine, methadone, and opiate assays when the sertraline concentration exceeded 50 ng/mL, 100 ng/mL, 500 ng/mL, and 500 ng/mL, respectively. The desmethylsertraline metabolite cross-reacted with the benzodiazepine and propoxyphene assays at concentrations exceeding 100 ng/mL, and the amphetamine and methadone assays at concentrations exceeding 500 ng/mL. Bupropion was found to cross-react with the amphetamine assay at a concentrations exceeding 16,000 ng/mL while the metabolite cross-reacted when concentrations exceeded 8000 ng/mL. No cross-reactivity was observed between buprenorphone or buspirone and any of the assays. Conclusions: We report potential cross-reactivities which may be important in the interpretation of immunoassay screening methods for drugs of abuse.

A-84

Evaluation of ViveST™ vs Frozen Plasma for HIV-1 Viral Load Testing in Brazil

M. Zanoni[1], R. S. Diaz[1], M. C. Sucupira[1], R. Cortes[1], C. Loveday[2], M. Holodniy[3], R. M. Lloyd[4]. *[1]Federal University of São Paulo, São Paulo, Brazil, [2]ICVC Charitable Trust HQ, Buckinghamshire, United Kingdom, [3]VA Palo Alto Healthcare System, Palo Alto, CA, [4]Research Think Tank, Inc., Buford, GA,*

Background: ViveST™ (ST) is an inexpensive transportation system for HIV-1 virologic assays of dried plasma. Herein we describe the precision, and reproducibility of using ViveST as a transportation method for shipping specimens and subsequent HIV-1 viral load (VL) testing.
Method: Ten-fold dilutions from clinical plasma samples with HIV VL > 5 $\log_{10}$/mL were created to generate samples with values of 4 $\log_{10}$, 3 $\log_{10}$ and 2 $\log_{10}$ copies/mL. Thirty singlicate samples at each dilution from ST were compared to frozen (F) (180 samples). Ten samples in triplicate were compared F to ST (60 samples). Finally, 299 samples with HIV VL <50 copies/mL (99); 1.7 $\log_{10}$ to 3.99 $\log_{10}$ (100); and 4 $\log_{10}$ to 5.99 $\log_{10}$ (100) were applied to ST, dried via driDOC for 12 hours, stored at room temperature for up to 4 days, and reconstituted using 1.175mL of buffer and compared to F using the Siemens VERSANT® HIV-1 RNA 3.0 Assay (bDNA). Significance was analyzed using Student t-test and Pearson correlation.
Results: F vs. ST dried plasma intra-assay mean variance among 3 dried replicates was < 0.15 $\log_{10}$ copies/mL (P = NS). Compared to F plasma, there was a mean reduction in viral load of 0.3, 0.27, and 0.35 $\log_{10}$ copies/mL at the 4, 3, 2 $\log_{10}$ copy/mL samples respectively (all comparisons, P < 0.01). 12/99 undetectable F VL were positive with ST, whereas 5/100 F detectable VL were undetectable with ST (mean VL 2.1, 2.3 $\log_{10}$/mL respectively). Overall correlation between F and ST was r =0.97.
Conclusion: Viral load results using ViveST were highly reproducible. Quantitation of HIV-1 viral load assays using dried plasma yielded minimally lower values compared to frozen plasma, and were within accepted assay variation for replicate samples. Our comparative data suggests that ViveST has promise for use in HIV clinical practice.

A-85

A Simple Solid Matrix Transport Device (ViveST) for Economic Collection, Storage, and Transport of Patient Plasma Between Laboratories for HIV-1 Resistance Testing

C. Loveday[1], R. M. Lloyd[2], E. MaCrae[1], Z. Grossman[3], R. Mathis[2], D. Burns[2], R. Diaz[4], M. Holodniy[5]. *[1]ICVC Charitable Trust HQ, Buckinghamshire, United Kingdom, [2]Research Think Tank, Inc., Buford, GA, [3]Sheba Medical Center, Tel-Hashomer, Israel, [4]Federal University of São Paulo, São Paulo, Brazil, [5]VA Palo Alto Healthcare System, Palo Alto, CA,*

Background: Worldwide, HIV-1 molecular and clinical laboratory testing requires transport of frozen plasma samples with which has become prohibitively expensive. ViveST (VST) is a biomatrix for transport of dried plasma at room temperature that can be used to move patients' samples from resource-limited to resource rich-settings for HIV-1, HBV and HCV genotypic analysis to support real-time clinical care.
Objective: This study evaluated the utility of VST versus frozen plasma ('gold standard') for accurate determination of HIV-1 genotypic resistance in paired clinical samples sent from UK to USA.
Methods: Paired, consecutive 1ml clinical samples of frozen plasma from our HIV/AIDS cohort (VL >1000c/ml) were compared with VST preparations. The VST was loaded with 1ml of plasma, air-dried overnight in a hood, sealed and stored. Each 1 ml of plasma was frozen at -70C until dispatch. The paired samples were analysed for genotypic resistance using the TRUGENE HIV-1 Genotyping Kit and HIV-1 GeneTanker PR/RT Select Assay. Analyses were performed to compare overall nucleotide sequence similarity and codon (including resistance associated amino acid mutations (IASUSA 2008). In 2 sample pairs (VL 1,000c/ml) sequencing was repeated on multiple occasions to assess base agreement rates. **Results**: 137 of 172 plasma/VST-paired sequences were successfully obtained for comparison. The mean base agreement between plasma and VST for 1137 paired sequences was >98% (range: 98.5% - 100 %). The mean codon agreement between the VST sequences and frozen plasma sequence was 98.6% for all codons in the sequence (range: 92.81% to 100%). There were no discordant pairs at the codon level associated with ART resistance.
Conclusions: This study showed highly accurate and reproducible HIV-1 resistance genotyping using VST comparable with the 'gold standard' frozen plasma single pass sequence, with >98% base agreement, 98.6% codon agreement and >98% reproducibility. This tool provides access to sophisticated laboratory technologies from distant and resource-limited settings for real-time patient clinical care in adults and children.

A-86

Between Lot Variation of the BGM Galectin-3™ assay evaluated at a CLIA Laboratory.

C. Adiletto[1], S. Wang[1], N. Gordon[1], R. H. Christenson[2]. *[1]BG Medicine, Inc., Waltham, MA, [2]University of Maryland, Baltimore, MD,*

Objective: To assess the between lot variation of the BGM Galectin-3™ assay using three individual kit lots that were manufactured according to Good Manufacturing Practices (GMPs). This study was designed to test actual clinical specimens by clinical laboratorians (intended users), in the intended use setting (CLIA laboratory).

Relevance: Reproducible measurement of clinical analytes is critical to ensuring quality in laboratory medicine. Furthermore, consistency between lots of reagents is a critical element in the total quality management program of a clinical laboratory, plus a key element in complying with the CLIA requirements for calibration verification upon introduction of new reagent lots (Ref. CLIA Sec. 493.1255). Manufacturers of *in vitro* diagnostic devices are challenged with developing innovative technologies while ensuring consistent and high volume manufacturing of quality devices over an extended period of time. BG Medicine recently developed a novel microtiter plate-based ELISA assay for the quantitative measurement of galectin-3 in serum and plasma. BGM Galectin-3 is intended to be used in conjunction with clinical evaluation as aid in the stratification of patients diagnosed with heart failure. Having recently achieved CE Mark status, the assay is available for clinical use in Europe and is pending clearance by the FDA.

Methodology: We present the results of a clinical laboratory evaluation of the between lot consistency of three individual lots of the galectin-3 product that were all manufactured according to Good Manufacturing Practices (GMPs). The lots are referred to as Lots 1, 2 & 3. The study was performed at the University of Maryland CLIA-Certified Clinical Laboratory. One hundred fourteen (114) EDTA plasma samples were analyzed using the BGM Galectin-3 assay according to the instructions for use. Absorbance at 450nm was measured with the BioTek ELx-800 plate reader using the recommended seven point standard curve. Testing was performed over three sequential days by a single operator. On each day, 38 samples were analyzed in duplicate on a single plate, using each of the three different lots. Results of the 114 samples were analyzed in a pair-wise comparison using the Bland-Altman method. Detailed statistics from the Bland-Altman analysis include the mean bias and 95% confidence interval.

Results: The pair-wise lot comparison of Lot 2 vs. Lot 1 resulted in a bias of 3.1% (95% CI 2.0 to 4.2); comparison of Lot 3 vs. Lot 1 resulted in a bias of -1.6% (95% CI -2.9 to -0.3) and comparison of Lot 2 vs. Lot 3 resulted in a bias of -1.5% (95% CI -2.9 to -0.1).

Conclusion: The results demonstrate excellent agreement between three individual lots of BGM Galectin-3 kits as shown by thewith biases of 3.1%, -1.6% and -1.5% as assessed with 114 plasma samples. Between lot consistency will enable clinical laboratories to readily introduce new lots of BG Galectin-3 kits while not significantly shifting control values or patient test results.

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High Density, Room Temperature Storage of Clinical Laboratory Samples for Future Genomic Analysis

H. M. Martinez[1], K. A. B. Goddard[2], S. Smith[2], C. Woffendin[3], M. Hogan[1]. *[1]GenVault, Carlsbad, CA, [2]Center for Health Research Kaiser Permanente Northwest, Portland, OR, [3]Oregon Health & Sciences University, Portland, OR,*

Objectives: Whatman FTA paper cards are an effective way to preserve DNA in whole blood and crude biosamples in the dry state at room temperature. However, such cards are not ideal for high density storage of clinical samples. We hypothesized that storage of buffy coat in a 384-well FTA format might be a very efficient way to store up to 1µg of DNA per well. Given that 250ng of DNA is now sufficient to perform whole genome SNP analysis on a microarray platform, and that 1-2µg of DNA is becoming sufficient to perform targeted sequencing on next generation sequencing platforms, we decided to explore the use of high density FTA technology, coupled to the storage of crude buffy coat samples, as the basis for storage of clinical laboratory samples for future genomic analysis.

Methods: Buffy coat from four anonymous, healthy donors was prepared from two 4 ml EDTA vacutainers simultaneously and centrifuged to generate a lymphocyte-enriched buffy coat pellet, which was re-suspended in 200µL of PBS. The buffy coat from one tube was immediately stored at -70°C. Ten µL aliquots of the freshly prepared buffy coat sample from the remaining tube were applied directly to at least 7 replicate wells of a GenPlate. GenPlates (GenVault) contain 6mm hemispherical FTA disks, arranged in a 384-well microplate. Samples were air-dried for 48 hours in the presence of desiccants, sealed with plastic, and stored at lab ambient temperature (25°C+/-3°C) and >40% relative humidity for 4 weeks prior to analysis. DNA was recovered from the FTA paper elements using GenVault's GenSolve kit according to the manufacturer's protocol. After purification, the yield of recovered duplex DNA was quantified by PicoGreen fluorimetry. The length of the recovered DNA was assessed by native 1% agarose gel electrophoresis. The chemical integrity of the purified DNA was also assessed by quantitative real-time PCR. Genotyping was performed using the Illumina Infinium assay on the HumanCytoSNP-12 Beadchip including 299,931 SNPs. The genotyping module of the Illumina BeadStudio software was used to call genotypes.

Results: We show that 0.6µg to 0.9µg of high molecular weight, native duplex DNA can be recovered, per well, from crude blood lymphocytes stored in the dry state at room temperature in the 384-well FTA format. Performance in qRT-PCR was not influenced by FTA paper storage of lymphocytes prior to DNA recovery, and no detectable PCR inhibition was observed. High quality and accurate genotypes were obtained when compared with paired samples processed in parallel, but stored frozen.

Conclusions: We examined the use of standard chemically-treated FTA paper technology, arranged in a novel 384 well format, for use in storage of a crude, enriched buffy coat fraction, rather than whole blood. We demonstrate that it is possible to store an average of 0.75µg of high molecular weight duplex DNA in one well, which is roughly three times the amount required to support whole genome scanning technologies. This methodology could serve as the basis for high density, low cost, energy-free storage of clinical laboratory samples for future genomic analysis.

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Homocysteine Measurement in Samples from End-Stage Renal Disease Patients - Comparison of Three Methods

M. Sheehan[1], R. J. Liedtke[2], L. Liedtke[2]. *[1]Kaiser Regional Reference Laboratory, Denver, CO, [2]Catch Inc., Bothell, WA,*

Background: Nearly all patients with end-stage renal disease (ESRD) have markedly elevated levels of homocysteine. The mean serum homocysteine concentration for this population is approximately 30 µmol/L versus 10 µmol/L for a normal population. In ESRD, sulfur containing amino acid metabolism is severely altered. The concentrations of cystathionine, S-adenosylhomocysteine and S-adenosylmethionine are increased. These amino acids have the potential to interfere with commonly used methods for homocysteine measurement. The Catch homocysteine method cycles homocysteine and cystathionine. The Diazyme method cycles homocysteine and S-adenosylhomocysteine. Other automated homocysteine methods currently available involve conversion of homocysteine to S-adenosylhomocysteine with subsequent immunological assay of S-adenosylhomocysteine. The Advia® Centaur assay is an example of this approach.

Objective: Our goal for this study was to determine if the concentration increases of these sulfur containing amino acids would result in any significant errors in measurement of homocysteine in samples from ESRD patients by the methods described above.

Methods: We measured homocysteine in serum samples from139 patients undergoing renal dialysis using the Catch and Diazyme 2-part liquid stable homocysteine assays and a Cobas MIRA Plus® analyzer. The Catch assay is calibrated using one non-zero aqueous calibrator traceable to SRM 1955. The Diazyme assay uses five serum-based calibrators. Assays were run simultaneously. Catch and Diazyme controls were run to verify proper assay performance. Four runs were done over a period of several weeks. The Advia Centaur XP® method was calibrated with two serum based calibrators that adjust a master curve. Bio-Rad controls were used to assure proper performance. All methods were run in duplicate according to package insert directions. Data were evaluated using EP Evaluator™ software.

Results: Comparative results for samples from patients with normal kidney function agreed closely. For one set of samples from normal patients, mean y (Diazyme) = 10.1 µmol/L, mean x (Catch) = 10.0 µmol/L; y = 0.98x + 0.243, R = 0.975, SEE = 0.762, N = 43. For another set, mean y (Centaur) = 8.4 µmol/L, mean x (Catch) = 8.6 µmol/L; y = 0.95x + 0.18, R = 0.983, SEE = 0.566, N = 25. Comparative results for samples from ESRD patients also agreed well. Mean y (Diazyme) = 29.0 µmol/L, mean x (Catch) = 27.1 µmol/L, y = 1.14x - 2.03, R = 0.982, SEE = 2.008, N = 139. For a another ESRD patient set, mean y (Centaur) = 26.7 µmol/L, mean x (Catch) = 27.1 µmol/L, y = 1.02x - 0.76, R = 0.989, SEE = 1.596, N = 24. Visual inspection of the data revealed no outliers.

Conclusions: The three homocysteine methods evaluated here measure homocysteine comparably both in patients with normal kidney function and in those patients with ESRD. Levels of various sulfur containing amino acids while elevated in ESRD do not appear to bias homocysteine results to a significant degree.

A-89

Effects of EDTA and Fluoride as anticoagulants on plasma glucose concentration of healthy human: A case study in Central Ghana.

D. Adu-Gyasi, D. Kwame Dosoo, S. Kofi Tchum, B. Ackon, K. K. Dzasi, P. Dawon Bilson, K. Boateng, R. Owusu, E. Kwarah, S. Apanga, K. Poku Asante, S. Owusu-Agyei. *Kintampo Health Research Centre, Kintampo North, Ghana,*

Background The number of people with diabetes is increasing due to increasing prevalence of obesity and physical inactivity. The worldwide prevalence of diabetes for all age groups was estimated to be 2.8% in 2000 and 4.4% in 2030. A study in the Greater Accra Region of Ghana revealed a 6.3% crude prevalence of diabetes.

Measurement of plasma glucose concentration is key in the diagnosis of Diabetes mellitus which is characterized by hyperglycemia. For economic reasons some facilities use EDTA as anticoagulant for glucose measurement.

The aim of this study was to find out if EDTA could be used as anticoagulant for blood sugar measurement.

Methods Venous blood samples were randomly collected from 135 consented voluntary blood donors of ages 15years to 45years of both sexes over a period of nine months. Blood samples were collected separately from each of the fasting participants (fasting not exceeding 16 hours) into, 2ml Fluoride K_3EDTA (VACUETTE) and 4ml K_3E K_3EDTA 7.2mg (BD Vacutainer) test tubes. These volunteers were selected from the Kintampo North Municipality and South District using the existing and periodically updated Health Demographic Surveillance System (HDSS). Plasma was separated between 2 to 6 hours after collection. The glucose concentrations of the two types of samples were measured using glucose oxidase reagent (ELITECH, France) and Selectra E Chemistry Analyzer (Vital Scientific, The Netherlands). Data was analysed with GraphPad Prism version 5.0.

Results For the 135 samples, the minimum glucose concentrations for fluoride and EDTA were 2.6mmol/L and 1.7mmol/L and maximum values were 8.7mmol/L and 8.9mmol/L respectively. The mean glucose concentrations for the two types of blood samples, Fluoride and EDTA, were 4.7mmol/L (95% CI (4.6, 4.8)mmol/L) and 3.9mmol/L (95% CI (3.7, 4.0)mmol/L) respectively. The difference between the means of plasma glucose concentration of the two types of samples was statistically significant with P<0001.

Conclusions By the criteria of the American Diabetes Association to define diabetes, a cut-off concentration of Fasting Blood Sugar (FBS) of 7.0mmol/L and above, if confirmed, is used. The definition of conditions such as diabetes, which mostly presents emergency conditions and based on measurements of analytical parameters, such as plasma glucose concentration, should adhere strictly to the set and accepted standard operation procedures and requirements. Using EDTA as anticoagulant for glucose measurement will under estimate the right plasma glucose concentration of a sample. In our study, the mean concentration of plasma glucose estimated in EDTA was lower than that measured in fluoride with a P value of <0.0001. It is therefore the responsibility of Health Care Givers to select the appropriate anticoagulant for the estimation of plasma glucose concentration for patients.

As demonstrated in previous studies, fluoride should be used as the prefered agent to inhibit glycolysis for the estimation of plasma glucose concentration in blood.

Tuesday AM, July 27

Poster Session: 10:00 am – 12:30 pm
Management

A-90

Studies On The Improvement Of Critical Laboratory Value Notification Using A Failure Mode And Effect Analysis

S. Yenice, C. Maden, T. Esin. *Gayrettepe Florence Nightingale Hospital, Istanbul, Turkey,*

Objective: To identify potential failure modes, causes and effects concerning patient safety, implement and assess the sustained improvement acts using a failure mode and effect analysis (FMEA) technique in reporting of critical laboratory values (CLV) of clinical chemistry tests for emergency cases and inpatients. FMEA is a procedure that analyzes potential failure modes within a given system. Each failure mode is classified by severity to determine the effect of failures on the system. Most patient safety reporting systems concentrate on analyzing adverse events after an injury has taken place. Healthcare FMEA, in contrast to a root-cause analysis, offers users analytical tools that can enable a team to proactively identify vulnerabilities in a care system and deal with them effectively. In essence, FMEA was used as a systematic, engineering-based approach in this study to identify such system vulnerabilities in CLV notification process and to correct them before they occur.

Methods: A five-step process was used.

Step 1: Patient Safety Committee decided to study on the potential failure modes, causes, effects and improvement acts about the CLV notification process.

Step 2: A multidisciplinary team was assembled including experts and individuals from the departments of Clinical Biochemistry, Internal Medicine, Emergency Care, Adult and Newborn Intensive Care Units, Nursing Service and Quality Management.

Step 3: Team members developed processes and subprocesses, then verified a flow-process diagram.

Step 4: Focusing on the subprocesses, team members listed all potential failure modes to determine their severity, occurence and probability. The hazard scoring matrix was used to define the risk priority numbers (RPN) and probability of an event's reoccurence and its severity. The Decision Tree was used to determine if corrective actions should be taken.

Step 5: The team determined what the best course of action was to take. Outcome measures were identified to analyze results and rapid Plan-Do-Study-Act methodology was used to test redesigned processes. Statistical analysis were performed to compare the pre- and post-RPNs.

Results and Conclusion: Six processes and 31 subprocesses were identified. 66 potential failure modes, 97 potential failure causes and effects were determined. Improvement actions were performed. Pareto diagrams were used to compare the pre- and post-RPNs. FMEA is a potent and invaluable tool to trap the potential failures. Yet, process is complex, time-consuming, and requires an intensive labor input. Therefore, a good team effort and detailed planning should be reserved. Overall assessment of processes revealed a high level of improvement (66%) that most became the standard operating procedure.

A-91

A Study of Various Recommendations for Assessing Method Bias with Some Suggestions

D. Plaut[1], L. McCloskey[2], A. Mazzara[3], B. Friedt[4], W. McLellan[5].
[1]Unaffiliated, Plano, TX, [2]Thomas Jefferson College of Medicine, Philadelphia, PA, [3]Garden City Hospital, Detroit, MI, [4]Hurley Hospital, Grand Rapids, MI, [5]Unaffiliated, Hollywood, FL,

INTRODUCTION: In reviewing our protocols for assessing method bias, we found three organizations (AACC, CLIA and CLSI) that differ considerably in their suggestions for: 1) the number of sample pairs (20 to 200), 2) the number of runs (4 to no recommendation), and 3) the statistics to use in evaluating the data (regression, t-test, difference plot and no recommendation). In an effort to clarify the situation we undertook a rigorous study of these issues.

MATERIALS AND METHODS: Method comparison data from 8 analytes (including cyclosporine, free dilantin, BNP with a monoclonal antibody vs. a polyclonal antibody) on 4 different instruments from three laboratories were studied. The number of paired samples ranged from 23 to 80. We also simulated data (20 to 100 points) to extend the laboratory data in a number of additional experiments. The data were evaluated using slope-intercept, t-test (paired and unpaired), the correlation coefficient and the standard error of the estimate, per cent bias, both the difference and the analysis of means plots.

RESULTS AND CONCLUSIONS: The paired t-test is the most sensitive to a bias between methods (Table). The difference and the analysis of means plots are helpful visual tools that can confirm the t-test. Slope and intercept data are sometimes difficult to interpret and should be used cautiously. Because the correlation coefficient and standard error of the estimate are measures of random error, they play no part in the study of bias. When a bias of ≥2%, exists it can be detected with as few as 20 points using the paired t-test with assistance from the mean difference.

n =	100	80	60	40	20					
Mean	58.56	60.24	59.67	61.50	60.09	61.77	60.73	61.77	59.63	60.24
Min	21.79	23.52	21.79	23.52	31.68	30.04	33.15	32.40	36.24	33.69
Max	95.83	96.58	95.83	96.58	95.83	96.58	95.83	96.58	85.94	96.58
% diff	**-2.52**		**-2.69**		**-2.26**		**-2.11**		**-2.18**	
slope	1.06		1.06		1.09		1.09		1.11	
intercept	-1.82		-1.82		-3.53		-3.74		-1.82	
r	0.98		0.98		0.98		0.98		0.98	
Sxy	3.02		3.04		3.08		3.07		3.32	
t-test	0.411		0.448		0.541		0.653		0.736	
paired t	0.000		0.000		0.000		0.002		0.110	

A-92

A Glycohemoglobin Challenge across an Integrated Network

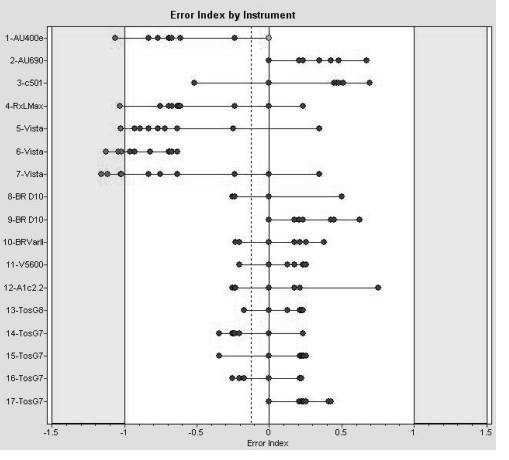
M. G. Rush, C. L. Wiley. *Providence Sacred Heart Medical Center and PAML, Spokane, WA,*

Background: The PAML Laboratory Network, an integrated network of laboratories, provides service to physicians and hospitals throughout the Northwest. The network is standardized for many aspects of laboratory practice and operation including reporting units, formulas for calculation of derived parameters, reference ranges. Standardization of major analytes is achieved by correlation and maintained by annual challenges administered by the Network Standardization Coordinator. Hemoglobin A1c is a standardized analytes whose importance is underscored by its recent endorsement as a tool to diagnose diabetes.

Objective and Methods: 17 network laboratories were challenged with 10 whole blood EDTA samples for Hemoglobin A1c testing. Results from all sites were within allowable error limits. Samples were collected from volunteers, aliquotted, refrigerated, and sent to each site by FedEx®. The samples were assayed for Glycohemoglobin using 2 methods and 12 different platforms: Immunoassay - Beckman Coulter AU 400e and AU690, Siemens RxL Max, Vista 1500 and 500, and HPLC Ion-Exchange - Bio-Rad D-10 and Variant II, Ortho Vitros 5600, Tosoh A1c 2.2, G7 and G8. In anticipation of the CAP's adoption of ±8% as the acceptable error limit, the data were evaluated using the new standard. 70% (7 of 10) is considered passing.

Results: The challenge data were analyzed using EP Evaluator® Release 9. In Figure 1 the mean of each sample is the 0 Error Index. Data points between -1 and 1 are within ±8% of the mean while points outside ±1 fail.

Figure 1:
Multiple Instrument Comparison



Conclusions: The allowable error for each of 10 samples is set as the group mean ± 8.0%. 95% of submitted results passed and all network laboratories passed, although the Siemens instruments achieved lower results. Even across different methods and many platforms the measurement of Glycohemoglobin is sufficiently standardized and accurate to pass the CAP's increasingly stringent standard.

A-93

Changes in specimen mislabeling rate with educational intervention

R. A. McPherson, C. Anderson, W. G. Miller. *Virginia Commonwealth University, Richmond, VA,*

Background: Mislabeling of blood specimens submitted for laboratory testing is an error that can result in adverse consequences to patients. Specimens in our institution are identified with bar-coded labels generated at the patient site by the hospital information system; these labels are read by instruments in the laboratory, so mislabeling of specimens occurs almost exclusively at the time of collection. This study examined the effect of educational efforts on the reported incidence of mislabeled specimens at our medical center over 6 years (2004-2009).

Methods: Patients having blood collected were identified by nursing staff with two unique identifiers (e.g., name and medical record number). Mislabeling events were identified predominantly by nurses who recognized the error after results were reported on incorrect patients. A small proportion of events were detected by laboratory personnel through comparison with prior test results on the same patients. All specimens included in a single phlebotomy were considered as one event. Data for each year were analyzed by month so each year had n=12 data points. Laboratory-nursing liaison meetings addressed specimen mislabeling in 2002 with implementation in 2004 of formal reporting of all events in the UHC Patient Safety Net system that communicates errors to nursing supervisors to make corrective actions. Specimen labeling was also made part of nursing competency training and annual assessment. In 2009 a mandatory 4 hour course in safety training was instituted for all employees (over 8000 individuals).

Results: The average reported incidence of mislabeling for the entire study period was 37.7 events/100,000 specimens (E/htS) for a process defect rate of 4.87 sigma. This rate dropped annually from 2004 through 2008 as follows [mean (95%CI)]: 42.5 (38.8-46.6), 40.2 (36.1-44.2), 37.9 (33.8-42.0), 36.0 (31.9-40.1), and 28.5 (24.5-32.6) E/htS. Over this 5 year period, the mislabeling rate dropped an average of 0.25 E/htS per month (n=60, r=0.525, p<0.0001) by least squares analysis. The rate then rose abruptly in 2009 to 41.1 (37.0-45.2) E/htS. Evaluation by month showed the largest mean month-to-month change occurred from June (mean 32.9 E/htS) to July (mean 38.2 E/htS); this difference was not statistically significant. A concurrent study of all improperly collected specimens (including clotted, QNS, other problems) showed an error rate that dropped drastically from 1785 E/htS in 2004 to 537 E/htS in 2009.

Conclusions: Mislabeling errors were reduced by combined processes of education and feedback when those errors occurred, although other more frequent improperly collected specimen problems showed greater improvement. Even at best performance, the mislabeling rate was improved by only one-third of highest value likely due to the difficulty of controlling low level sporadic errors in human behavior. A wide-spread effort to increase safety awareness apparently contributed to increased reporting of mislabeling events in 2009. Further reduction of mislabeling errors might require implementation of automated devices for positive patient identification.

A-94

Quantitative Fecal Fat Analysis by Nuclear Magnetic Resonance Spectroscopy: More Cost-effective and Efficient than Gravimetry

K. C. Quinones[1], L. Skodack-Jones[1], D. G. Grenache[2]. *[1]ARUP Laboratories, Salt Lake City, UT, [2]University of Utah Health Sciences Center, Salt Lake City, UT,*

Background: Identifying opportunities to increase efficiencies, decrease testing costs, effectively utilize personnel resources, and reduce environmental footprints are frequent concerns for clinical laboratories. The quantitative measurement of fat in feces is used clinically to identify malabsorption syndromes. Traditional gravimetric analysis is a time-consuming methodology and requires the use of organic solvents. In contrast, nuclear magnetic resonance (NMR) spectroscopy can be used to rapidly and accurately quantify the fat content of stool and does not require organic solvent extraction. Our laboratory performed gravimetric fecal fat analysis until November 2009 when NMR was validated and introduced as a replacement method.

Objective: To identify and quantify efficiencies gained by changing methodologies for quantitative fecal fat analysis from gravimetry to NMR spectroscopy.

Method: Quality assurance data from testing by gravimetric analysis was obtained for 5.5 months immediately prior to initiating NMR analysis (pre-NMR) and 2.5 months after making the method change (post-NMR). Specific metrics evaluated were, turn-around time (receipt of specimen to result reporting), chemical and material costs, labor costs, laboratory space required for testing, and analytical time.

Results: 1,175 specimens were tested during the pre-NMR period and 324 specimens were tested during the post-NMR period. The pre-NMR mean turn-around time for result reporting was 42.6 h (95% CI 41.6-43.5) which was significantly decreased by 23% to 33.0 h (95% CI 31.2-34.9) (p<0.0001) in the post-NMR interval. The mean cost of reagents and supplies increased by $4.60 per specimen after converting to NMR spectroscopy. Labor costs decreased by 73% in the post-NMR period, in part because the methodology allowed testing to be performed by a laboratory technician instead of a medical technologist. The physical space required to accommodate the gravimetric method reagents, supplies, and equipment occupied 21.28 ft^2 of prime laboratory space which decreased by 42% to 12.32 ft^2 in the post-NMR period. Based on our estimates, the time required to perform the analysis decreased from a mean of 8.6 h (pre-NMR) to 0.23 h (post-NMR).

Conclusion: Quantitative fecal fat analysis by NMR spectroscopy is more efficient and cost effective than gravimetric analysis. Turn-around time, labor costs, laboratory space, and analytical time were all reduced upon implementation of the NMR method. Reagent and supply costs increased but these were more than offset due to the savings in labor costs. The discontinued use of organic solvents is an additional benefit. It has a positive environmental impact and is in compliance with accrediting agency regulations for reducing hazardous wastes.

A-95

A Case Study in the Principles of LEAN: A Collaborative Approach to Improving Troponin Turnaround Time

M. K. O'Brien, R. McCallie. *Providence St Vincent Medical Center, Portland, OR,*

Background: The American College of Cardiology and American Heart Association publish guidelines that establish recommended time-to-treatment for patients who experience myocardial infarction. Door-to-needle with fibrinolytics is 30 minutes, while door-to-balloon treatment with percutaneous transluminal coronary angioplasty (PCTA) is within 90 minutes. Our facility established a turnaround-time (TAT) expectation for result reporting of ED troponin at 45 minutes, with a 95% consistency rate. Baseline average TAT for "specimen in-lab to result" was 28 minutes; however, that goal was only met 75% of the time.

Objective: Improve patient care by consistently meeting TAT expectations. A Lean assessment was performed with the following goals:

1. Identify process steps within sample flow that would reduce cycle time of "in-lab to result" TAT.
2. Identify limitations that prohibit meeting "in-lab to result" TAT.
3. Develop standard process flow(s) to be utilized by staff to meet TAT expectations.

Methodology: A work group of laboratory personnel to include management, medical technologists, and laboratory assistants participated in a Lean Kaizen event. The following Lean principles were introduced:

1. Value is specified from the perspective of the customer.
2. The current state value stream is defined.
3. The desired future value state is defined.
4. Waste in a value stream is identified and eliminated.
5. Value should "flow" on the basis of customer needs and demands.
6. Continuous improvement is pursued on the basis of the desired future state.

Team members measured each step of the current process in minutes. These measurements were incorporated into a current value stream map. Results indicated little waste, with potential opportunities based on improvements in reducing specimen time on the ADVIA Centaur® CP immunoassay system. A desired state value stream map was created. Distance traveled by personnel was measured to identify wasted motion. Prior to and following the Kaizen event, Siemens Healthcare Diagnostics spent time assisting in the investigation of instrument failure. Active participation included hardware improvements, software upgrades, and test methodology reagent upgrades. Education and maintenance training were also provided. Additional changes incorporated into the laboratory's workflow included reducing centrifugation time and utilizing separate specimens for basic chemistry testing and troponin analysis. Processing workflow was streamlined to allow specimens to "flow" to the technical area.

Results: The Kaizen team identified waste and prioritized improvements to workflow and the ADVIA Centaur CP system. Once changes were made, time and distance measurements were taken and a post-Kaizen value stream map was created. A statistical comparison of pre- and post-Kaizen practices demonstrated the total processing time improved to 92.6% of the total cycle time. Additionally, time traveled by laboratory personnel was decreased by 3.65 miles/shift, resulting in improved staff satisfaction.

Summary: Because of the collaborative efforts of the instrument manufacturer and clinical laboratory personnel, the department was successful in achieving the Lean Charter Goal and has maintained consistency in meeting the expected troponin result TAT of <45 minutes for >90% of ED specimens.

A-96

A Lean System Dynamic Transformation Model for Health Care Processes

R. Chadha[1], A. Singh[2], A. Nayar[1], J. Kalra[3]. *[1]University of Saskatchewan, Saskatoon, SK, Canada, [2]SD Mission Hospital, Rudrapur, India, [3]Royal University Hospital, Saskatoon, SK, Canada,*

Objectives: The critical nature of health care operations requires that some excess capacity should necessarily be stored in the system to provide required flexibility of response. It is well established that in health care, patient satisfaction and quality care are important indicators for the success of any health care enterprise. It is hypothesized that integration of Queuing theory and Lean methodology should improve the dynamic performance of the health care system. To prepare the health care organizations to meet the service demand, we developed a Lean Health-Care (LEAN-HC) model based on the integration of process transformations.

Methodology: We reviewed, evaluated and compared the emergency care system using the system dynamic model and redesigned the process using a lean value stream mapping approach to eliminate waste and achieve Just-In-Time (JIT) services. The study was carried out in SD Mission Hospital, India, and compared with similar studies of Penn State University USA, and Mercy Health Care, Iowa State, USA. The model included three primary steps: 1) Use of priority class queuing model to understand and to balance the demand with capacity. 2) Use of lean value stream mapping approach to identify the waste. 3) Following continuous improvement principles to modify and improve services: 5S- visual management, mistake/error proofing, service lead time reduction using one piece flow, JIT and standard operating procedures.

Results: Our data demonstrated that the implementation of the LEAN-HC model resulted in the following: 1) Improving process flow improved the capacity to serve patients. 2) Placing emergency physicians, near arrival with existing nursing and registration staff, optimal utilization of the electronic medical record and queuing theory, physicians directed care upon arrival and determined clinical care needs resulted in a 23% reduction in a key parameter of throughput efficiency, length of stay, for all patients. 3) Lean methods like 5S and value stream mapping were useful in uncovering significant improvement opportunities to increase the turnaround time (TAT) on lab results which have a direct bearing on patient wait time. 4) Once the process of care is re-mapped, the bottleneck in the process can be identified. There will always be one stage which will move slower than the others, but the goal is to even the flow by reducing variation as much as possible and plan the bottleneck for where it can be most effectively controlled.

Conclusion: These results demonstrate how to apply queuing theory, how to devise actions based on the derived results and the benefits that those hospitals which adopt this strategy will receive. It was found that not only can queuing theory deliver more efficient service at a higher quality to patients, but it also can reduce the waste and overhead costs experienced by many medical facilities. These changes were observed without any change in resource availability and without any evidence of safety or quality issues.

A-97

Utilizing Laboratory Metrics and "Dashboard-style" Reporting

S. E. Dawson, T. Ocasio. *Swedish Covenant Hospital, Chicago, IL,*

Objective: Our goal was to develop effective reporting tools that could concisely and effectively communicate results of key elements of performance, quality and service for use by our laboratory and the hospital's process improvement committee to assess overall operational status, while also helping to identify opportunities for improvement.

Method: Our laboratory developed and implemented the use of two graphic and color-coded metrics and dashboard-reporting formats. The KPI (key process indicator) Benchmarking chart utilizes metrics from a Solutions Action O-I™ report, to graphically display our lab's performance level for 6 key operational indicators comparing them to benchmarked data from similar hospital labs. Included are indicators of cost effectiveness, productivity, and utilization. The background color for our lab data reflects the performance level achieved for each indicator. The colors range from green (favorable, top 25%) to yellow (at the 50%) to red (highly unfavorable, bottom 25%) with variations of shading to reflect trends. Through this use of color, the viewer gets a quick, focused snapshot of these important areas of overall performance and can easily identify successful progress and/or areas requiring attention.

A second, more graphic management tool, is the Laboratory Dashboard. Resembling an automobile dashboard, 4 gauges and 4 data boxes provide a quick overview of operational and financial performance. The gauges display results for proficiency testing (accuracy), turnaround times for stat Emergency Department (ED) and 6am routine morning draws, and a percent excellent ranking for customer satisfaction (as measured by Professional Research Consultants, Inc.). The numbering on each dial is color-coded to indicate levels of achievement for easy interpretation. Beneath the gauges are four data boxes which display selected indicators of managerial and operational effectiveness listed in the KPI benchmarking chart above.

Application and Results: With the colored dashboards clearly highlighting areas for improvement, projects designed to address the issues are initiated. For example: Turn-around time was addressed with the implementation of automation, followed by autoverification, centrifugation changes, ED triage-based phlebotomy performed by laboratory personnel, a wireless patient and specimen identification system and most recently a real-time monitor screen displaying pending ED specimens. Done incrementally, the impact of each change was visible through the dashboard metrics. Over a two year span covering 2008-09, the turn-around time decreased 25% for chest pain patients and 29% for the general emergency department population. The financial investments made were validated through the improvement in operational metrics.

Failure to improve was also clearly visible. Over utilization of laboratory services and increases in supply costs caused metric numbers to change from green, to yellow-green, to yellow, making it easy to identify for both laboratory managers and administrators alike.

Conclusion: These two tools have proven to be important drivers for service and process improvement projects across disciplines in our laboratory. The KPI metrics chart and Laboratory Dashboard have enabled us to track and focus on continuous improvement efforts across key strategic areas while enabling us to achieve outstanding operational results (top 10 to 25th percentile) as compared to our peers.

A-98

The Role Of Six Sigma Metric To Improve The Analytical Performance Of Serum Folate Analysis

M. E. Mendes[1], R. G. Rotondaro[2], G. V. Fragoso[1], P. Romano[1], V. R. M. Batista[1], N. M. Sumita[1]. *[1]Central Laboratory Division & Laboratories of Medical Investigation (LIM-03) of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil, [2]Production Engineering Department of Polytechnic School of Universidade de São Paulo, São Paulo, Brazil,*

Objective: The aim of this work was to evaluate the application of six sigma metric using DMAIC methodology to reduce the analytical variation of Folate assay in a public tertiary hospital laboratory.

Material and methods: The Folate assay was performed by eletrochemiluminescence method on Elecsys 2010 (Roche Diagnostics). The DMAIC is described as follow.

Definition phase: we defined the responsabilities of the team, the SIPOC diagram was used to describe the process. The coefficient of variation of the PreciControl anemia level II (CVA2) was considered as critical to quality (CTQ). The upper limit of acceptability was 9%.

Measure phase: we measured the CTQ time series, descriptive statistic and normality test were applied. Tests of repeatability and reproducibility (R&R) of Elecsys 2010 and reproducibility among operators (R&R) were performed. We gathered the preliminary data to evaluate current process performance and its capability (defects per million opportunities).

Analysis phase: we applyed brainstorming, Ishikawa diagram, Failure Modes and Effects Analysis, Pareto analysis, trends analysis, study of the results of R&R, analysis of control charts and proficiency tests.

Improvement phase: the objective was to reduced the CVA2 through implementation of specific action plans.

Control phase: after 7 months the consolidation of the action plans were achieved and the process capability was recalculated.

Results: The frequency of corrective maintenances were reduced and improvement in the performance indicators of Elecsys 2010 were achieved. A new equipment (Cobas-Roche 411) was installed and the workload was redistributed between both equipments. Other employees were trained and their competence were enhanced. The workflow was revised and simplified. The operating procedure was revised. There were changes in the workflow. This rational use of the kit resulted in the reduction of waste and economy.

Phase	Mean of CVA2(%)	Process performance (PpK)/ Sigma Capability	Evolution of DPMO
Measurement	10.64	-0.10 / 1.21	112,485
Analyze	8.25	0.24 / 2.22	13,262
Improve	6.81	0.85 / 4.04	26
Control	6.07	1.29 / 5.60	0.04

Conclusion: The DMAIC methodology allowed to establish the factors that were interfering in the good performance of serum Folate assay.

A-99

Evaluation of the appropriateness of prostate-specific antigen requested by General Practitioners

M. Salinas[1], B. Lumbreras[2], M. Lopez Garrigos[1], J. Lugo[1], J. Uris[3]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]University Miguel Hernandez, San Juan, Spain, [3]University of Alicante, Alicante, Spain,*

Background: The purpose of this study was to compare the use of PSA testing between urologists and general practitioners (GPs), the variation in test ordering behavior between the different centers included in the study and the tendencies in PSA testing along the period of study (2002-2009).

Methods: We performed a cross-sectional study of the number of PSA tests ordered by GPs and urologists during the years 2002 and 2009 in the Health District in Alicante, Spain. This area includes primary care centers, where GPs can order tests without referring the patient to the main hospital, the San Juan Hospital (tertiary centre attending for 234,424 people), mainly attended by specialized doctors as urologists. All samples were analyzed in the Clinical Laboratory Department of this hospital. The different primary care centers and the hospital included in the study used similar ordering forms for laboratory tests. We collected the following variables: a) patient age (≤40; ≤50 and > 50 years); b) type of doctor petitioner on the referral form (urologist or GPs); c) center (primary care or hospital), and d) year. According to the clinical guidelines established in our setting, routine testing for PSA is recommended in patients older than 50 years and age younger than 50 for men with first degree relatives who had prostate cancer. We considered as reference PSA testing in patients older than 50 years and compared PSA testing in patients younger than 50 years and younger than 40 years with that reference category (index PSA<50/PSA>50 and PSA<40/PSA>50). We estimated these indexes according to the centre, type of doctor petitioner and year of study. Differences in test ordering data between centers and years of study were tested with the Kruskal-Wallis test.

Results: A total of 17,646 PSA tests were ordered by urologists and 51,640 by GPs. The value of the index PSA<50/PSA>50 was 0.027 when urologists ordered the diagnostic test in comparison of 0.076 when GPs did (p<0.001). In patients younger than 40 years, the value of the index was 0.006 in urologists and 0.015 in GPs (p<0.001). A large variation in both index was observed between the 10 different primary care centers (p<0.001). PSA testing in men younger 40 and 50 increased along the time in patients attended by both urologists and GPs. However, this variation was greater in men younger 40 than in men younger 50 and in urologists rather in GPs: PSA<50/PSA>50 increased 2.22 times in urologists and 1.33 in GPs in 2009 in comparison with the year 2002, and PSA<40/PSA>50 increased 2.55 times in urologists and 1.42 in GPs in 2009 in comparison with the year 2002 (p<0.001).

Conclusions: PSA testing in men younger than 40 and 50 years is higher in patients attended by GPs than in those by urologists, suggesting inappropriate use of PSA testing. There is an increasing tendency in the PSA ordering, particularly in men younger 40 years old. These data are suggestive for interventions focused on PSA testing and prostate cancer screening in primary care settings.

A-100

General Practitioners (GPs) and Endocrinologists HbA1c requesting patterns in a Health District of the Valencian Community (Spain)

M. Salinas[1], M. Lopez-Garrigos[1], B. Lumbreras[2], M. Gutierrez[1], J. Lugo[1], J. Uris[3]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]University Miguel Hernandez, San Juan, Spain, [3]University of Alicante, Alicante, Spain,*

Background: The glycation of haemoglobin was first used 30 years ago to assess the level of glycaemia in subjects with diabetes. Since then, assay of HbA1c has become established as a laboratory marker of increased risk for the long-term complications of diabetes. In July 2009, the international expert committee has determined that an HbA1C value of 6.5% or greater should be used for the diagnosis of diabetes.

The aim of this study was to examine GPs and endocrinologists HbA1c requesting patterns in 2008 and 2009.

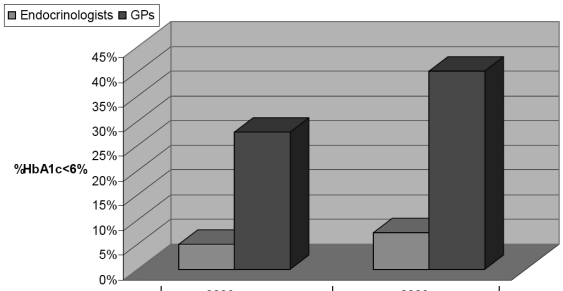
Methods: We performed a cross-sectional study of the number of HbA1c tests ordered by GPs and endocrinologists during the years 2008 and 2009 in the Health District of Alicante, Spain. This area includes Primary Care Centers, where GPs can order tests without referring the patient to the main hospital, the San Juan Hospital that serves a population of 234,424 inhabitants. We calculated the percentage of HbA1c results <6% with respect total requested for patients managed by GPs and endocrinologists. Differences in test ordering data between GPs and endocrinologists were tested with the Chi-square test.

Results: Total and HbA1c with results <6% requested by GPs and endocrinologists in 2008 and 2009 is shown in figure. The differences between GPs and endocrinologists were significant (p<0.001) in both years.

Conclusions: The percentage of HbA1c results <6% with respect total requested for patients managed by GPs is higher than in those by endocrinologists.

If we consider patients with HbA1c results below 6% as non-diabetics or very well controlled diabetic patients, the results may suggest:

- Diabetics patients managed by GPs are better controlled or
- GPs are requesting HbA1c in not diabetic patients, previously to be considered as a diagnostic test, suggesting inappropriate use. Study results may serve as a basis for designing new HbA1c requesting protocols.



	2008			2009		
	ECs	GPs	Chi-square test	ECs	GPs	Chi-square test
HbA1c total requests (absolute value)	1532	14459		1510	18665	
HbA1c<6% (absolute value)	77	4022		111	7482	
HbA1c<6% (%)	5,03%	27,82%	p<0,001	7,35%	40,09%	p<0,001

A-101

Achieving continuous improvement in stat laboratory: a seven-year experience

M. Salinas[1], M. Lopez Garrigos[1], B. Lumbreras[2], J. Lugo[1], M. Gutierrez[1], J. Uris[3], J. Uris[4]. *[1]Hospital Universitario San Juan, San Juan, Spain, [2]University Miguel Hernandez, San Juan, Spain, [3]University of Alicante, San Juan, Spain, [4]University of Alicante, Alicante, Spain,*

Background: Stat laboratories are under pressure to supply information as quickly as possible for the diagnosis and treatment of disease. The assessment of customer satisfaction is considered today as an important component of any stat laboratory quality program.

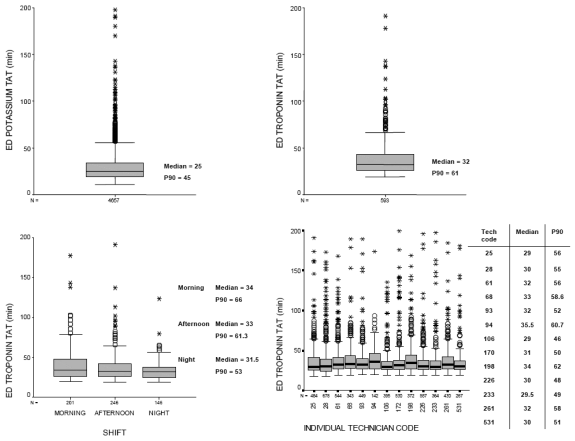
Objectives: Show how continuous quality improvement can be implemented by delivering a monthly quality report to stat laboratory personnel. Ascertain the emergency department clinician needs through surveys related to laboratory service.

Methods: Registers of Intra-laboratory TAT, workload, and staff productivity were collected since 2003 to 2009 automatically. The monthly report included troponin and potassium TATs, workload, and productivity. A monthly stat laboratory quality report has been delivered to the laboratory personnel. In January 2009 a physician satisfaction surveys assessed their satisfaction with the laboratory service overall and in relation to three specific issues. Evaluations were rated on a scale of 1 to 5 (1: poor; 5: excellent). Surveys addressed the intra-laboratory Perceived TAT satisfaction and Desired TAT.

Results: Workload and productivity increased from 2003 (58052 and 78952 requests, and 5.5 and 5.8 request/technician/hour in 2003 and 2009 respectively) while troponin TAT remained steady (period mean 30.86 min). Figure shows the box plots for ED troponin sample TATs (total, for all three shifts and individual-technician TATs for the last six months) and potassium TAT contained in December 2009 quality report.

The surveys showed an overall satisfaction of 3, with 3.6 for the reliability of test results, 3.9 for accessibility, and 3.3 for TAT. The Perceived TAT was 44.9 minutes and the Desired TAT was 21.7 minutes.

Conclusions: TAT results were maintained over time despite increased workload, possibly due to monthly report delivering to the laboratory personnel. There is need for teamwork with physicians to enhance their understanding of clinical laboratory science and appreciation of the real possibilities of meeting their laboratory TAT expectations.



A-102

A Method for Establishing and Evaluating Evidence-Based Laboratory Medicine Best Practices: The A6 Cycle

R. H. Christenson[1], S. R. Snyder[2], J. Derzon[3], C. Shaw[2], D. Mass[4], R. Black[3], P. Epner[3], E. Liebow[5]. *[1]Univ of Maryland Sch of Med, Baltimore, MD, [2]CDC Division of Laboratory Systems, Atlanta, GA, [3]Battelle Centers for Public Health Research and Evaluation, Seattle, WA, [4]Arizona State University, Tempe, AZ, [5]Battelle Center for Public Health Research and Evaluation, Seattle, WA,*

Objective: To develop a methodology for the systematic review of Laboratory Medicine (LM) topics and identification of pre- and post-analytic practices that are effective at improving outcomes.
Relevance: The Institute of Medicine attributes nearly100, 000 deaths each year to medical error. Most LM errors occur in the pre- and post-analytical testing phases. Although more than 200 evidence-evaluation systems have been proposed, none are designed to specifically evaluate LM quality improvement practices and outcomes. Also, there is generally insufficient published evidence in LM, such that new methods need to accommodate unpublished evidence.
Methodology: Validated evidence-based medicine (EBM) methods used by the USPSTF (2008), AHRQ (2002), CASP (1993) and others were adapted to LM quality improvement issues using the "A6 cycle," (ASK, ACQUIRE, APPRAISE, ANALYZE, APPLY and ASSESS) to develop "Laboratory Medicine Best Practices (LMBP)" systematic review methods. Key modifications allow inclusion of quality improvement study designs and unpublished evidence. The LMBP "A6 cycle" steps are: (1) ASK a focused question in the form of a problem statement; (2) ACQUIRE evidence by identifying sources and collecting potentially relevant studies; (3) APPRAISE studies by applying screening criteria (topic area, practices, and outcomes) to create an evidence base; (4) ANALYZE by standardizing and summarizing studies, and rating overall strength (study quality, effect size magnitude and consistency) using an expert panel to identify evidence-based "best practices;" (5) APPLY by disseminating evidence review findings via peer-reviewed literature and other media, educational programs, and guidelines as appropriate, and by actual practice implementation; (6) ASSESS practices to evaluate performance outcomes/ results, which may identify other quality issues to continue the cycle of improvement.
Example of LMBP Review: Pre-analytic patient specimen identification was a pilot topic. (1) ASK: "Are barcoding systems (using bar-coded patient armbands and scanners) effective at reducing specimen identification errors?" (2) ACQUIRE: 81 published and 6 unpublished studies were identified. (3) APPRAISE: Application of screening criteria resulted in a total of 10 studies being fully abstracted. (4) ANALYZE: 8 studies were included in the evidence base; 6 studies were rated "good" study quality and 2 were rated "fair," all with consistent effect sizes in favor of barcoding. Meta-analysis favoring barcoding versus no barcoding for avoiding ID errors average log odds ratio was 2.45 (95% confidence interval: 1.6-3.3). The Expert Panel rated barcoding systems' overall effect size "substantial," and its strength of evidence "high." On the basis of the evidence review, barcoding systems were recommended as effective at reducing patient specimen identification errors. (5) APPLY: Disseminate through peer-reviewed publications, educational programs and other media. (6) ASSESS: Ongoing data collection and evaluation will assure practice works and in what settings, and may reveal other issues/ questions for future reviews.
Conclusion: The developed LMBP A6 methodology, adapted from validated evidence-evaluation systems, can be applied to systematically review and evaluate quality improvement practices in LM. Practice evidence can be reliably identified, standardized and summarized to complete evidence reviews and establish best practice recommendations for improving patient outcomes and reducing medical error.

A-103

Improvement of specimen collection accuracy through the cooperation between laboratory personnel and nursing staffs with a quality improvement project

H. Ning. *Chang-Gung Memorial Hospital, Tao-Yan, Taiwan,*

Objective: To improve the specimen collection accuracy through the cooperation between laboratory personnel and nursing staffs in the Department of Cardiology which showed the highest sample rejection rate among the others at Chang Gung Memorial Hospital, Lin-Kou, Taiwan.
Methods: Several on-site observations and discussions were performed by the laboratory personnel and clinical nurses. Strategies that were generated to improve the specimen collection accuracy included 1) establishing a clear written policy and operation procedure to standardize the phlebotomy technique; 2) providing special trainings, such us using butterfly collection devices in patients from whom blood-drawing may be difficult; 3) labeling the drawing order and selection of collection tubes on the requisition forms; 4) offering nurses a portable blood roller mixer to reduce the clotting rate in anticoagulant tubes; and 5) performing a continuing education program and competency assessment of blood collection techniques annually among nursing staffs or within 3 months of employment for new nurses.
Results: The overall specimen rejection rate decreased significantly from 2.35% in 2006 to 1.62% in 2008.
Conclusions: The cooperation between laboratory personnel and nursing staffs appeared to improve greatly the sample collection accuracy. The same strategies may be implemented in other departments for the improvement of sample collection accuracy in the whole hospital. It is anticipated that such efforts may help to increase the overall patient safety and qualities of medical services in this hospital.

A-104

25-Hydroxyvitamin D assay: Evaluation of a semi-automated routine clinical service

L. A. Perry[1], M. Zaman[1], V. Lee[1], B. J. Molloy[2], L. J. Calton[2], S. D. Gillingwater[2], D. P. Cooper[2]. *[1]Barts & The London NHS Trust, London, United Kingdom, [2]Waters Corporation, Manchester, United Kingdom,*

Objective: In July 2009 we switched our routine 25-hydroxyvitamin D (25OHD) clinical service from an automated immunoassay platform (Diasorin Liaison) to a semi-automated tandem mass spectrometry method. We review the impact on our service 6 months later.
Methods: The semi-automated tandem mass spectrometry method quantifies both 25OHD2 and 25OHD3 after sample preparation involving solid-phase (Waters Oasis HLB µElution plate) extraction performed using a Tecan robotic sample handler. We process ~4000 samples a month using this system. The Tecan robotics affords some automation in the sample preparation stage by performing the following steps:
-read the sample bar code
-transfer sample (150µl) and internal standard (10µl) to a deep-well microtitre plate
-add the protein precipitation solution and mix the sample.
-prime the microtitre solid-phase elution plate
-transfer supernate to the primed elution plate
-perform further washing steps
-move the elution plate onto the assay microtitre plate into which is eluted the extracted sample.
The assay microtitre plate is manually sealed and loaded into the LC/MS/MS system for analysis. The Tecan takes 105min prepare 96 samples and can prepare three 96-well microtitre plates for 25OHD in a working day. If the assay was performed manually it would take a highly skilled lab operator approx 6h to prepare one 96-well plate.
Benefits of this service: Significant savings in service delivery have been realised that exceeded initial expectation:
-staff salary cost per day is halved leading to savings of approx $20,000 per yr
-the process is three times more efficient than the manual service
-errors are minimised
-assay sensitivity is increased
-25OHD2 and D3 are measured simultaneously
Problems experienced in service delivery: During the first 6 months of service there have been few minor errors and only one 'lost run'. The errors were:
-a 'short sample' that was rectified by modifying the SOP to include topping up the water reservoir
-a sample bar code read error that halts the analytical run

-a catastrophic power failure that lead to loss of stored bar code ID data. The data were manually re-entered after power was restored.

Summary: We have successfully introduced a semi-automated solid-phase extraction tandem mass spectrometry assay for 25OHD. This service has exceeded expectations in significantly reducing staffing and assay costs. We can offer both 25OHD2 and 25OHD3 reporting with increased sensitivity. The Tecan robot is an ideal platform that will facilitate the introduction and semi-automation of other assays that require similar sample preparation techniques.

A-105

Communicating Clinical Pathology (CP) Critical Laboratory Values (CLVs) in an Acute Care Hospital

T. C. Aw, Y. Liang, P. Zeng, S. Satheakeerthy, S. P. Tan. *Changi General Hospital, Singapore, Singapore,*

Background There is scarce data in the literature on the timeliness or reporting and of receipt of CLVs. Our 800-bed hospital's CP CLVs encompass clinical chemistry (glucose, sodium, potassium, calcium, and blood gases) and hematology (hemoglobin, WBC count, platelet count, aPTT, and INR). After analyses, all results are reviewed, certified and transmitted to the Lab Information System (LIS) before being printed locally at the various care locations. Each day we produce an average of 6000 CP results. As soon as a CLV is known, the technologist phones the caregiver to inform them of the abnormalities after providing two patient identifiers (TPIs - name and hospital ID number). The nurse reads back the critical results of the patient. The technologist records the reporting transaction in an electronic lab log; the nurse records the receiving transaction in the patient's file.

Method We conducted an audit of how the CP lab fared in communicating CLVs to our care-givers.

Results All reporting transaction logs over a 2-month period were reviewed and submitted to the hospital quality improvement unit (QIU). QIU randomly reviewed about 40% of the patient files for documentation of the transaction (identity of both caller and receiver) and availability of CLV results. There were 1409 CLVs in the reporting transaction log: 1142 (81.1%) were notified within 3 minutes, 242 (17.2%) between 4-6 minutes, 6 (0.4%) between 7-10 minutes, and 19 (1.3%) 11-20 minutes. Of the 561 files reviewed by the QIU, 225 (45%) had incomplete documentation (CLV results available but no transaction record - 39%, transaction recorded but CLV results missing - 27%, and transaction record and CLV result both absent - 34%).

Conclusion While the CP lab has communicated all CLVs diligently and in good time, the recording of CLVs by care-givers could be further improved.

A-106

University of Louisville Lean Laboratory Project

K. George[1], C. A. French[2]. *[1]University of Louisville, Louisville, KY, [2]Ortho Clinical Diagnostics, Bonita Springs, FL,*

Objective: The University of Louisville Laboratory was not meeting their turn-around time goals and wanted to improve customer service. They also wanted to decrease the cost per test while increasing quality.

Methodology: The laboratory formed a Lean Team of 6 laboratory employees and 1 Lean Consultant. The team learned lean tools and applied them to the each laboratory process. Each process was filmed and analyzed using lean methodology. The team used the analysis to create a Job Standardization Package (JSP) for each process.

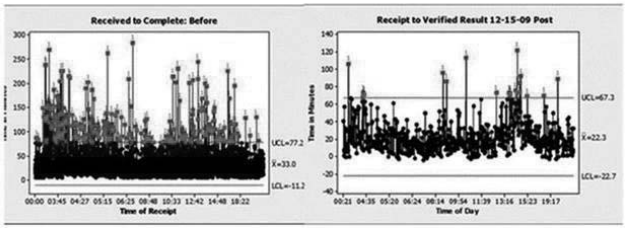
Validation: Baseline data was collected before any changes were made. This data was compared to data collected after standard work was implemented. Please see charts.

Description: The team developed JSP's and used them to pilot each laboratory process. The initial pilot was conducted in the lean room to ensure that the JSP was correct as written. Next the team did a pilot in the laboratory following the JSP, again making revision as needed. Each pilot was done for a period of time up to 24 hours. Data was collected after each pilot that monitored turn-around-time during the pilot period. Once improvement was confirmed final training of all staff and implementation of the new JSP's was completed.

Outputs: Job Standardization packages for laboratory processes, metrics to control the process, control plan and audit sheets for management.

Results:

- Turn-arount time reduced by 22%
- Decreased technologists by 7.4 or 21% reduction in staff
- Space savings of 432sq feet or 22%
- Standard work practices
- Reducton in errors and error potential
- Created daily performance measurement
- Cross-training of staff improved by 39% with an expected 100% by the end of the first quarter
- Inventory control system developed and implemented



A-107

Computing a patient-based sigma metric

L. Kuchipudi, J. Yundt-Pacheco, C. A. Parvin. *Bio-Rad Labortories, Plano, TX,*

Objective: Derive a new sigma-metric to more accurately reflect the capability of a test method with respect to its quality specification for the patient population served.

Relevance: The sigma metric widely used in clinical diagnostics is computed as (TE_a - |bias|)/CV and is treated as a constant, independent of concentration. This is rarely the case. Typically, when sigma metrics are computed at different concentrations, different values are obtained. Rather than a single sigma-metric, in reality there is a sigma-profile that varies over the concentration range of the analyte. We propose a method for computing a single sigma metric from the underlying sigma-profile which reflects the performance of a test method with respect to its quality specification for the patient population it is tested upon.

Methodology: Obtain a distribution of result concentrations for the patient population served. For each concentration in the patient population determine the TE_a (%), bias (%), and analytical imprecision (CV). Compute the sigma-metric at each concentration: (TE_a (x) - |bias(x)|)/CV(x) where x is the result concentration. Compute the patient-based sigma-metric as the weighted average of the sigma-metrics at each concentration (weighted by the probability of each concentration in the patient population).

Validation: The form of the patient-based sigma-metric is the same as the common sigma-metric, except that is uses point estimates for the TE_a, bias and CV at the individual result concentrations rather than a single estimate based on control values. This improves accuracy by capturing changes in the relationship between error specification and test method performance as a function of result concentration.

Conclusion: The patient-based sigma-metric reflects the performance of a test method with respect to its quality specification for the patient population it is tested upon.

Analyte	TE_a BV Desireable	CV L1	CV L2	QC σ L1	QC σ L2	Patient-based σ
Albumin	4.92	2.45	1.64	2.01	3.00	2.51
AlkPhos	13.90	2.57	2.01	5.41	6.93	5.57
Amylase	17.50	1.25	1.07	14.00	16.31	12.94
Bilirubin, Total	39.10	3.51	2.11	11.14	18.50	8.28
Calcium	3.06	2.02	1.81	1.51	1.69	1.56
Chloride	1.88	1.18	1.01	1.59	1.86	1.86
Cholesterol	10.30	1.77	1.50	5.82	6.85	6.38
CK	38.10	4.14	2.02	9.20	18.83	13.32
Creatinine	9.96	3.92	2.22	2.54	4.49	2.83
Glucose	8.88	1.68	1.43	5.29	6.23	5.57
Iron	39.70	1.97	1.00	20.15	39.83	17.64
Potassium	7.44	1.35	1.01	5.51	7.34	5.58
Sodium	1.12	0.90	0.81	1.24	1.38	1.27
Uric Acid	15.40	1.37	1.08	11.24	14.31	12.22

A-108

Repeating Critical Results in The laboratory: Tradition or Necessity?

R. Khoury, A. Gandhi, B. P. Salmon, S. Gibbs, P. Gudaitis, D. Gudaitis. *Aculabs, Inc., East Brunswick, NJ,*

Background: Critical results account for the majority of the repeats in laboratory practice, it is a practice inherited from archaic, historical testing methods in the laboratory. However, the accuracy of results have increased tremendously with the introduction of fully automated systems, clot detectors, and the ultrasensitive sensors, in addition to improving the sensitivity on the majority of the tests. The important question in the laboratory practice now is whether repeating critical results is a redundant waste of time and resources, or practices which must be continue to be followed.

Method: more than 500 samples with critical values from potassium (using Roche P module), glucose (using Roche Integra 800), hemoglobin (Beckman Coulter, LH750) and prothrombin time (Beckman Coulter, TOP) were repeated and the integrity of the specimens were verified and documented. The percentage and absolute difference between replicates were calculated for each of the analytes. Because of the wide range tested to cover both the critically low and high results, the outliers were based on the % difference.
Results: The minimum and maximum values tested, mean for the absolute difference, and the % outliers are listed in table 1.

Test	Min value tested	Max value tested	Mean of % difference	Mean absolute difference	% Outliers
Prothrombin time	33.2 Sec	111.4 Sec	1.3%	0.8 Sec	1.2%
Hemoglobin	6.2 g/dl	14.9 g/dL	1.4%	-0.01	1.2%
Glucose	22 mg/dL	488 mg/dL	1.5%	-1	0%
Potassium	2.1 mmol/L	11.3 mmol/L	2%	0.06	0%

Conclusions: Although the data suggested that repeat analysis is not necessary for the analytes tested, eliminating the repeat requires other criteria to be verified before the results are released to the care giver like checking instrument flags, delta check, and verifying the integrity of the specimen. Critical results for prothrombin time lead to critical INR>4.5, and because the reliability of INR> 4.5 is unknown, it is wise to repeat prothrombin time with INR>4.5 to confirm the results.

A-109

Customer relationship management (CRM):results of a program for the clinical analysis industry.

M. D. C. Freire, F. S. Souto, J. O. Garcia, R. Medina, C. M. G. Carvalho, C. M. A. Rangel, M. M. Marinho. *Sérgio Franco Medicina Diagnóstica, Duque de Caxias - Rio de Janeiro, Brazil,*

Background: Several studies show that client retention and fidelization strategies bring advantages such as profits with repetitive sales, lower operational and publicity costs. One retention strategy is customization of services, great challenge to companies in consolidated markets, and which profits are dependent of scale economy, such as clinical laboratories. The aim of this study is to present and discuss results of a retention strategy by customization of services in the clinical analyses industry, and its reflexes in a laboratory performance.
Methodology: From the idea of developing and managing customized individual relationships, in 2003, was implemented a Customer Relationship Management (CRM) program in its 4 basic steps: identification, differentiation, interaction, customization. We built a database of physicians that required tests, here called clients, updated at each new interaction with the company (identification). The clients were differentiated by his value for the company, in respect to the income generated and to his ability to influence other clients (differentiation). We selected the top 50 income generators from database and opinion leader physicians from the main medical institutions and associations of Rio de Janeiro. In 2008, the CRM actions were extended to the top 150 income generators. Yearly the results of incomes in the database are reviewed, as well as the status of the opinion makers, and the list of selected clients is updated. The customized interaction with the selected group of clients include: direct relationship with the medical and executive director; participation in scientific events promoted by the laboratory; subsidy to costs of clinical research tests; individualized follow-up of test results with reduction of promised TAT; priority in the advertisement of new services and scientific publications developed by the laboratory; office visitation by a medical staff to survey satisfaction; access to VIP rooms in drawing facilities; gratuity in domiciliary sample drawings and in express mailing of results; customized relationship actions in holidays and birthdays; training courses for the clients assistants and secretaries. The CRM program cost in 2009 was 0,4% of the total operational costs.
Results and discussion: The program was started in 2003, with 164 clients selected, growing only in number of opinion makers until 2008, when the top 150 income generators were included. In 2009, the clients selected were 907 - 7,5% of the database. The incomes from drawing facilities grew 291% from January 2003 to December 2008, while the incomes generated by differentiated clients grew 634%. By 2009, 21% of the drawing facilities income came from the selected clients. In the last satisfaction survey, on October 2009, the totality of the CRM group declared satisfied or very satisfied with the company services, and in market surveys done by an independent consultant (CVA Solutions), the laboratory brand was considered the one with higher attractiveness and lower rejection in the market of Rio de Janeiro, on 2008 and 2009. Thus, the results of the fidelization and retention program are consistent with business literature and reaffirm the potential financial and market share gains promoted by this marketing strategy in the clinical analysis industry.

A-110

Community Hospital Implements Lean Principles to Improve Delivery of Patient Care

J. Stanley[1], A. Peter[2], C. Kajila[3], R. Francisco[2], L. Downey[2], B. Rivera[2], D. Jones[2], S. Briggs[2], D. Tener[1]. *[1]ValuMetrix, OCD, Johnson & Johnson, Inc., Raritan, CA, [2]Ukiah Valley Medical Center, Ukiah, CA, [3]Ukiah Valley Medical Center, Ukian, CA,*

Background: Reduced reimbursement from private and public insurers is forcing hospitals to be innovative in reducing costs without jeopardizing quality and patient care. In addition, the challenge to replace staff vacancies due to retiring baby boomers or those leaving the field remains a constant struggle. According to the U.S. Department of Health and Human Services, only two new clinical laboratory scientists are entering the field for every seven facing retirement. Lean manufacturing is one method that can help reduce costs by eliminating waste and improving the delivery of care to patients with greater efficiency. Standardized work and continuous flow of processes also frees up staff for other responsibilities or may alleviate the need to hire replacement staff to complete the same workload.
Method: This 78 bed rural community hospital implemented a Lean project in the core laboratory to reduce operational costs. The project goals were to improve product flow, decreasing turn-around times, increase operator productivity, and reduce inventory supplies. The scope of the project included phlebotomy, specimen processing, chemistry, hematology, coagulation, immunoassay, and urinalysis. Product process flow and operator analysis was used to develop solutions in four critical areas: equipment layout and workstation design, standardization of work methods, visual performance measures, and material management. Isolated work cells were eliminated and instruments representing 80% of the test volume were strategically placed in the new layout to support continuous flow of product and operator. Staff was trained on standardized work processes to ensure repeatability and reproducibility of work. Work stations were designed to support each process and a material replenishment system was implemented to ensure continued supply availability without disruption of work. Samples were delivered and picked up for storage every 6 minutes to facilitate a first-in-first-out method of testing.
Results: The expected goal was a 25% reduction in turn-around time (TAT) from collect to verify, with a 35% stretch goal from baseline averages for specimens received from inpatients, outpatients, and the emergency department. During the first week of implementation the TAT for a chemistry metabolic panel, protime with INR, and urinalysis met the 25% goal for inpatients, and the TAT for CBCs with differential and troponin exceeded the 35% stretch goal. Additionally, a proposed annual $331,968 in staff savings is expected based on the post-project implementation. Savings includes a shift to unlicensed personnel for automated testing due to the inability to fill current vacancies with licensed clinical laboratory scientists. The challenge for the laboratory is sustainability and adherence to the new process. Daily performance metrics that monitor TAT and routine audits will help ensure compliance and provide a basis for continuous improvement.
Conclusion: Implementation of Lean standard operating principles can help improve operational efficiencies by improving process flow, improving quality of services that contribute to patient care, reduce costs, and increase testing capibilities to meet customer demand and current healthcare challenges.

A-111

The Practice Of Universal Precautions Among Medical Laboratory Scientist / Technicians / Assistants In Zaria,Nigeria : How Adequate Is It?

J. S. Ogboi[1], A. T. Olayinka[1], T. Aiyenigba[2], A. O. Adeleye[1]. *[1]Ahmadu Bello University, Zaria, Nigeria, [2]Lagos University Teaching Hospital, Lagos, Nigeria,*

Background and Objective: Universal precautions are simple infection control measures that reduce the risk of transmission of blood pathogens through exposure to blood or body fluids among patients and health care workers. Among these health care workers are medical laboratory scientists, technicians and assistants who are faced with professional hazards such as needle pricks and many blood borne infections in their routine day to day work in the laboratories. The purpose of this study is to find out how adequate the knowledge and practice of universal precautions is among these laboratory staff at various hospitals.
Materials and Methodology: This study was carried out among the medical laboratory scientists/technicians/assistants in different laboratories in Ahmadu Bello University teaching hospital (ABUTH), and Ahmadu Bello University (ABU), both in Zaria, Kaduna State, Nigeria between September and November, 2009.
The medical laboratory scientists, technicians and assistants in the different laboratories were selected using the stratified random sampling method and a cross sectional descriptive study design was used with 150 of them participating in the study.
Result: The mean age of the respondent is 31.74 ± 10.01. The male respondents

were 115(76.7%) while the female counterparts were 35 (23.3%). There was a poor knowledge of universal precautions among these laboratory personnel as only 38.6% of them had heard about universal precautions. Only 18.7% had correct general laboratory practice. There was a poor knowledge of disease transmission with handset (phone), as only 9(6%) laboratory personnel wash their hands before answering calls during routine work/analysis.
Conclusion: Our findings indicates that despite common contacts with blood and contaminated equipments, knowledge of universal precaution is unacceptably low, which shows that the existing strategies to control infections to health care workers have not been adequately addressed. There is urgent need to consider factors that enable people to change behaviour, and also the availability, cost and convenience of the preventive barriers. Thus a combination of strategies is required, including continuous education on universal precaution through hospital sponsored seminars. Also, it should be included as part of the orientation programme for all new employees and in the curriculum of medical laboratory sciences.

A-112

Improving Capillary Specimen Rejection Rates Through Lean Processes

G. A. Olsen. *Boys Town National Research Hospital, Boys Town, NE,*

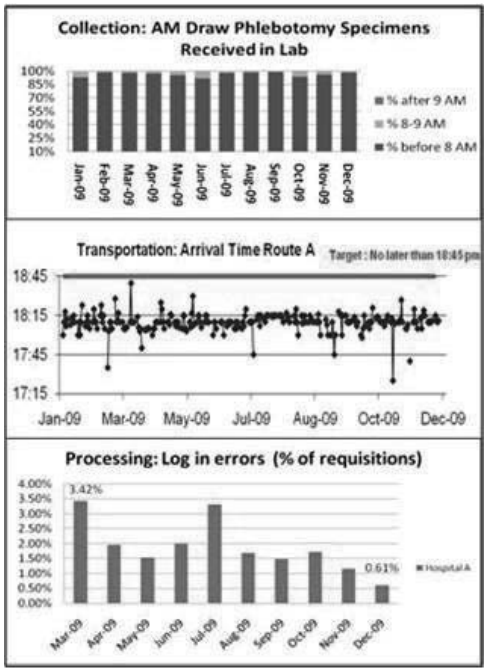
Background and Objective: During a three month time frame it was noted that capillary samples submitted for Capillary Lead testing were rejected at a rate of 11% due to the detection of clots. Clinician, patient care provider and patient dissatisfaction were voiced. A goal was established to reduce the rate to 2% or less.
Process Evaluation and Change: Due to the large variability of clotted sample rates between remote clinics, a root cause analysis of current processes at multiple sites was completed. The review identified a lack of a standardized collection process. Identified inconsistencies included: 1) multiple manufacturer collection devices in use, 2) lack of proper selection of age appropriate skin puncture devices, 3) improper collection techniques, and, 4) lack of proper mixing of samples during and immediately after collection. Analysis of rejection rates by collection device revealed that Becton-Dickinson (B-D™) device specimens had a 20% rejection rate while Sarstedt collection devices were rejected at a rate of 5%. Clinic rejection rates varied from 2% to as high as 30% indicating a high degree of collection technique variability. Implementation of the Lean principle of standardization of processes was utilized. The decision to require submission of all capillary lead testing specimens in a Sarstedt device was made and a training program created to educate existing clinic personnel on collection variables that impact the formation of clots in capillary samples. The training program included both face to face training as well as hand out materials.
Results and Conclusion: Following completion of training at all locations and standardization of a single collection device, the impact was measured. The first month following implementation, the rejection rate was reduced to 1.8% and a three month cumulative rate of 1.95% however in subsequent months the rejection rate increased to 3%. Further analysis revealed lack of compliance at two clinic locations. Subsequent feedback and retraining has resulted in sustained improvement throughout the organization. For the last three months of 2009 an average rejection rate of only 1.0% and a one month best of 0.4% was achieved. Standardization of a single collection device, appropriate selection of capillary puncture devices, in addition to the implementation of a comprehensive training program has had a significant impact on rejection rates for EDTA capillary specimens for lead screening. The training program has been incorporated into all new hire training for clinic personnel performing capillary collections. Implementation of Lean concepts and ongoing feedback can be easily incorporated into point of care processes that impact clinician and patient satisfaction.

A-113

A quality dashboard for specimen collection, transportation and processing

J. Su, M. Astion, W. Chandler, A. Edwards. *University of Washington School of Medicine, Seattle, WA,*

Background: A Quality Dashboard is a tool that summarizes quality metrics to help managers determine if key processes are in control. Quality dashboards are often incorporated into disciplined problem solving methods like Lean or Six Sigma.
Objective: To develop a Quality Dashboard for a Specimen Procurement Division in a large healthcare system in the United States. The division is responsible for specimen collection, transportation and processing.
Method: The quality metrics were chosen based on 1)Root-Cause-Analysis of errors, 2) literature review, and 3)comparison with two peer institutions that developed quality dashboards and achieved excellent performance on dashboard measures. The metrics for specimen collection included mislabeling rates and the fraction of the morning hospital phlebotomy collection received in the lab by a target time (8AM). Transportation metrics included the monitoring of on time arrivals for specimen couriers. The metrics chosen for specimen processing included work load as a function of time (hour of day, day of week, month), and percentage of paper test requisitions incorrectly logged to the laboratory information system. The latter measurement included specific error rates such as failure to enter the correct test(s) and failure to correctly enter patient information.
Results: Representative samples from the quality dashboard are shown below. Quality improvement (QI) projects were initiated when metrics were out of control. For example, after implementing a variety of interventions -double checking, enhanced supervision and accountability, and work load leveling --the percentage of paper requisitions erroneously logged into the LIS dropped from 3.4% of requisitions to 0.6% in one of the hospitals studied.
Conclusions: A quality dashboard was created to measure performance of a specimen procurement division. Future work will focus on expanding the dashboard and using it to measure the effect of QI interventions.



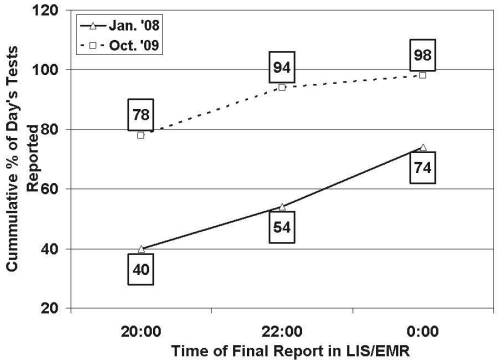
A-114

Decreased Turnaround Time for Critical Values Reporting Using Lean Principles

V. I. Luzzi, J. Zajechowski, D. Smith, R. Benitez, J. Carey, R. Zarbo, C. Feldkamp. *Henry Ford Hospital, Detroit, MI,*

Background: Lean process management has been successfully applied to many aspects of clinical and anatomic pathology services. These principles are applied to decrease the time between client request and product delivery, eliminate non-value added activities, empower the employees to propose and test changes, monitor changes for measuring success, and produce slow incremental changes. The Pathology and Laboratory Medicine laboratories at the Henry Ford Hospital support a 905 bed inner-city, quaternary-care, teaching-hospital, 26 regional medical centers, esoteric testing for 5 suburban hospitals and more recently an outreach program. Using Lean principles, we aimed to accommodate system growth, increase efficiency in a challenging economy, and improve turnaround time (TAT) for test results and critical value reporting.
Methods: We employed multiple Lean tools to redesign the laboratory work space and processes: standardized work and connections, load leveling, moved tasks/samples closer to workers, and reduced batch size. We used visual tools to reinforce and maintain changes (production and inventory kanbans and whiteboards to capture daily performance indicator charts and defects). Indicators used include TAT for results reporting, fraction of critical values reported by 12 midnight, and labor efficiency. The TAT goal for test results was set to 4 hours from time of specimen arrival, and before midnight of the same day for critical value reporting. Efficiency was defined as number of tests reported per full-time employee .
Results: After Lean process improvements, 95% of outpatient results are now reported within 4 hours of lab receipt (figure), 98% of critical values are reported before midnight, and our labor efficiency increased by 7% compared to the same period in 2008.
Conclusions: Using Lean principles to re-design the laboratory, we were able to

accommodate volume growth without increased staffing, decrease the results and critical value reporting TAT, and increase labor efficiency.



A-115

Audit of the requests of laboratory tests in the Emergency Departments of a seven hospitals Health Care System

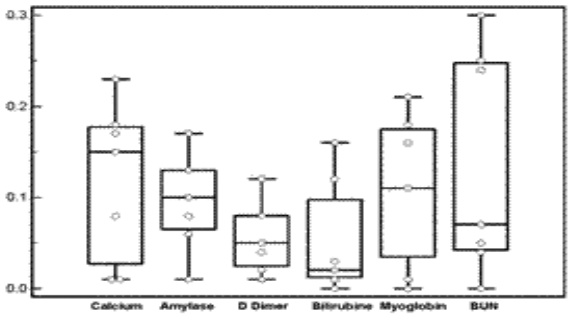
R. M. Dorizzi, L. Baldrati, L. Vascotto, S. Berlini, P. Masperi. *Laboratorio Unico di AVR, Cesena, Italy,*

Background: The consolidation process of the Health Care Service is accelerating also in Italy and the need to standardize the panel of the tests that are requested to the laboratory is increasing. It is becoming more and more frequent the finding of Emergency Departments (EDs) of the same Health Care System more or less recently consolidated showing dramatic differences in the pattern of the requests. The aim of this study is to compare the patterns of the requests in seven hospitals of a Network recently set up in Italy (AVR: Ravenna, Faenza, Lugo, Rimini, Riccione, Cesena, Forlì).

Methods. The 24 tests (including 95-98% of the total workload)
requested by the seven EDs in three successive trimesters (A=May-July 2009; B: August-October 2009; C=November 2009-January 2010) have been downloaded from the single LIS serving the entire Network and the Tests/Admissions (T/A) ratio has been estimated.

Results. The T/A ratios were respectively: 5.22, 4.86, 4.62, 4.54, 4.51, 2.33, 2.7. Moreover, we found that six tests were requested in a very consistent way: Complete Blood Count, Potassium, Creatinine, Sodium, Glucose, ALT (the ratios were between 0.29 and 0.38). While two EDs requested all the other tests with a much lower intensity, the other five EDs requested 6-12 further tests with a ratio around 0.30. Really, the T/A ratio of Total CK of the seven hospitals spanned between 0.33 and 0.00 and that of Fibrinogen and BUN between 0.30 and 0.00.

Conclusions. In the Autumn 2009 the data of the trimesters A and B have been presented to the prescribing clinicians and an audit started in the two successive trimesters demonstrating a dramatic fall in the requests of some tests (e.g fibrinogen and mioglobin in one hospital fell respectively from 3362 to 5 and from 2101 to 438). The trend will be monitored in 2010.



A-116

Creation of a study group to everyday problem solving and implementation of innovations in the clinical laboratory.

V. Fernandes, L. Rosato, M. Caixeta, G. Barra, L. Abdalla, J. Vaz, S. Costa. *Laboratório Sabin, Brasília, Brazil,*

Background: We describe the creation of a study group whose goals are to receive, discuss and propose solutions to everyday problems of a clinical laboratory, as well as to plan and implement technical innovations. This group, known as "innovation core", has, since its creation in the second half of 2009, developed and implemented four innovation projects/troubleshooting. Thus, its relevance to the clinical laboratory is characterized by bringing an analytical and impartial vision of different departments, aiming to generate improvements and innovate processes.

Methods: The innovation core encourages, via a computadorized system, the identification of everyday problems to be solved, news, and ideas from outside the laboratory that could be applied to the company. After receiving the problem or new idea, simple and direct questions are prepared and shared with all employees so that they can opine on the subject, send solutions and previous experiences. Then, the innovation core organize the returned messages, discusses its details, and generates quantifiable data and objective evidences. If feasible, a deployment project is written and its implementation is encouraged in sectors where innovation/solution applies.

Results: Five months after the creation of the innovation core, four innovation/problem solving projects were developed and implemented: 1) Replacement of Ethidium Bromide for a modern DNA dye (less toxic and with less expensive disposal); 2) Recycling of blank labels. After registering the patient and their exams, the tubes labels are printed. A single blank label is let between different patients to avoid misidentification. Thus, a blank label is generated for each patient and, in our laboratory, this results in thirty-five thousand blank labels wasted a month. In this project, a series of tips and ideas for reuse of these labels on the everyday work were produced by the laboratory staff; 3) Adjusting the automated DNA extraction system for whole blood. The automated DNA extraction system of our laboratory is excellent for specimen as serum, plasma, cervical swabs, but does not produce good results for whole blood. The extracted DNA can not be amplified by PCR, due to incomplete removal of sample inhibitors, such as hemoglobin. On completion of this project, a protocol for rapid pretreatment of whole blood was developed. It was based on hemolysis and separation of leukocytes by centrifugation, which allowed the automated extraction of good quality DNA; 4) Creation of the laboratory Twitter, an open and direct communication vehicle with the client, where doubts are resolved, and health tips and news are transmitted.

Conclusion: Thus, in this work, we describe the implementation of the innovation core to identify and solve the everyday problems of our clinical laboratory. The developed system involves the participation of all company employees via a computerized system. By sharing the problem and the active search for solutions with colleagues from different sectors, we generate more efficient and better solutions as a result of the collaborative thought generated within the company.

A-117

A Study of the 1974 CAP Suggested QC Rules: Are They Valid Today?

D. Plaut[1], W. McLellan[2]. *[1]Unaffiliated, Plano, TX, [2]Unaffiliated, Hollywood, FL,*

INTRODUCTION: The high degree of accuracy, precision and consistency of today's instruments, together with what seems a reluctance to move away **from** a SINGLE value outside the ±2 SD line as a rejection signal, we thought the QC rule system proposed by the **College of American Pathologists** (CAP) in 1974 merited a current evaluation. The CAP suggeted criteria: • Use two levels of control with each run • Accept the run if both controls were within ±2 SD • Accept the run if one control was within ±2 SD and the other between 2 and 3 SD. (A value outside 2 SD is acceptable) o Reject the run if one value exceeds ±3 S o Reject the run if both controls exceed the same 2 SD within the run o Reject the run if one control exceeds the same 2 SD limit on consecutive runs.

METHODS: Our study was in two parts: 1) Data from **individual** laboratories were studied to determine the probability of an analyte failing a CAP survey using the CAP rules. 2) The probability of ANY lab failing a survey using the CAP criteria was determined from inter-lab data. For this part we calculated the "total possible error" from the target using the formula: $\mathbf{TPE = 2*SD_{group\ means} + 2*SD_{group}}$ **[…… . … .X……^……..*……]** $_{\text{limits X = group (target mean)}}$, $^{\wedge} = \mathbf{2*SD_{group\ means\ *\ =\ 2*\ average\ SD\ group}}$ The SDgroup means was calculated using n individual means from an interlab report. The SD_{group} was the average SD of the individual SDs from labs reporting for the current month.

RESULTS for 1: From CAP survey data from chemistry (5 labs), coagulation (6 labs) and hematology (3 labs), we found only 2 analyte/ instrument situations in which 2 or more challenges had a Standard Deviation Index (SDI) greater than 2. In neither of these two did the laboratory exceed the CAP limits. There were **no** situations where there was a failure.

RESULTS for 2: With the formula for TPE, we found that better than 95% of the cases would have passed the survey. For example given group data from 56 labs reporting glucose on the Level I control on Instrument R for October, 2009, the group mean (thus the target mean) was 98. From this the TPE = (2*1.1) + (2*3.1) = 8.4%. The CAP limit for glucose is 10%.
CONCLUSIONS: These results are very likely due to the increase in precision and long term stability of the instruments operating today coupled with the current acceptable range per CAP (as well as other survey groups). We hope that these data will serve as encouragement to adopt the CAP suggestions which will reduce the number of false rejects (10% when two controls are used with a single value exceeding ±2 SD as a reject signal) while keeping error detection high.

A-118

Evidence-Based Evaluation of Practices to Reduce Blood Culture Contamination

D. Mass[1], S. R. Synder[2], C. Shaw[2], A. Patta[2], M. Washington[2], R. H. Christenson[3], J. Derzon[4], R. Black[4], E. Liebow[4]. *[1]Arizona State University, Tempe, AZ, [2]CDC Division of Laboratory Systems, Atlanta, GA, [3]University of Maryland Medical Center, Baltimore, MD, [4]Battelle Centers for Public Health Research and Evaluation, Seattle, WA,*

Objective: To identify evidence-based quality improvement practices to effectively reduce blood culture contamination rates by applying new systematic review methods developed by the CDC Laboratory Medicine Best Practices (LMBP) initiative.
Background: Patients with suspected septicemia have blood cultures drawn to identify causative pathogens and direct subsequent treatment. False positive results lead to unnecessary treatment and costs. According to the American Society for Microbiology, the rate of blood-culture contamination should not exceed 3%; however the average blood-culture contamination rate is 1.1% to 5.2%. Contaminated blood cultures constitute one half or more of all positive blood cultures in some medical centers. Applying the CDC LMBP review methods to published and unpublished data identified evidence-based practices that are effective at reducing blood culture contamination rates.
Methods: Since 2006, the CDC has sponsored a LMBP initiative to develop and apply transparent methods for systematically reviewing laboratory medicine quality improvement interventions to identify effective measures for improving healthcare quality outcomes consistent with the 6 Institute of Medicine aims (safe, timely, effective, efficient, equitable and patient-centered). These methods were applied to answer a focused question: "What practices are effective at reducing blood culture contamination?" The first step was to develop a supporting analytic framework which identified the scope of the review question, including a description of the quality issue/problem, improvability, potential quality improvement practices/interventions, and outcomes of interest. The following steps included: 1)acquiring published evidence available in electronic databases and through hand searching and unpublished evidence using a data collection form; 2) appraising pertinent studies by applying screening criteria appropriate to the review question and analytic framework (topic, practices, and outcomes) to identify the relevant body of evidence; 3)analyzing evidence (studies) by standardizing, summarizing, and systematically rating study quality and effect size (including statistical meta-analysis whenever possible), and consistency to rate the overall strength of evidence.
Results: Three aspects of practice were reviewed with the LMBP process: 1. Technique: venipuncture versus intravenous catheter; 2. Staff: dedicated phlebotomy team versus other personnel (i.e. nurses); 3. Collection Tool: preparation (sanitizing) kit versus no preparation kit. Published articles (n=1677) were identified for these three practices; 14 of which met the screening criteria. Seven unpublished studies were submitted; two met the screening criteria. Thus 16 studies were fully analyzed. The following odds ratios (OR) represent the estimated summary effect size for each practice; venipuncture- (7 studies, OR = 2.63, 95% CI 1.85-3.72); phlebotomy team- (6 studies, OR = 2.76, 95% CI 2.17-3.51); Preparation Kit- (4 studies, OR =1.1, 95% CI 0.99-1.41).
Conclusions: Based on the strength of evidence of effectiveness for each practice, the Blood Culture Contamination Expert Panel (7 members) identified as best practices to reduce blood culture contamination rates: the use of venipuncture as the preferred technique for sample collection where the option exist in the clinical setting, and dedicated phlebotomy teams to collect the blood culture.

Tuesday AM, July 27

Poster Session: 10:00 am – 12:30 pm
Infectious Disease

A-119

Addressing the challenges of the Giemsa stain in the diagnosis of malaria in an endemic area using the Partec CyScope®.

B. Nkrumah[1], A. Agyekum[1], S. E. K. Acquah[1], J. May[2], E. Tannich[2], N. Brattig[2], F. Hünger[1], S. Nguah[3], Y. Adu-Sarkodie[4]. *[1]Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana, [2]3. Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [3]Komfo Anokye Teaching Hospital, Kumasi, Ghana, [4]Clinical Microbiology Dept, School of Medical Sciences, KNUST, Kumasi, Ghana,*

Introduction One of the main interventions of the Global Malaria Control Strategy is the prompt and accurate diagnosis of malaria as it is the key to effective disease management (A global strategy for malaria control, 1993). It is thus of concern that poor diagnosis continues to hinder effective malaria control. The laboratory diagnosis of malaria has up to now relied nearly exclusively on microscopy, a valuable technique when performed correctly but unreliable and wasteful when poorly executed. A better utilization of microscopy and the development of alternative diagnostic techniques could substantially improve malaria control (Institute of Medicine, 1991).
Aim To evaluate the Partec CyScope® (PC) for the diagnosis of malaria in human blood from patients in an endemic area using an expanded gold standard.
Materials and Methods 263 samples from patients attending the Under Five Clinic and OPD of the Agogo Presbyterian Hospital in the Asante Akim North District, Ghana were examined with two different tests independently and blinded. The test methods employed in the study were: (i) Giemsa-stained blood smears (GS) (ii) PC
Outcome 107 (40.7%), 111 (42.2%) samples were positive for the presence of Plasmodia by GS and PC respectively. Compared to the expanded gold standard the sensitivity of GS and PC was 94.7% and 95.6% at 95% confidence interval. The specificity was 100% and 98% at 95% confidence interval. The positive predictive value was 100% and 97.3% and the negative predictive value was 96.2% and 96.7% respectively with good agreements k=0.95 and 0.94. The test performance of PC was very similar to GS. However, the PC had added advantages; it was faster, easier to use, less expensive in terms of cost per test and test equipment than the GS and has a standby battery making it ideal for field work and places where there is no electricity.The PC can therefore be used as an alternative method for GS.

A-120

Biomarker Discovery in HIV/AIDS using Proteomics

D. Haarburger[1], J. Bergström[2], T. Pillay[1]. *[1]University of Cape Town, Cape Town, South Africa, [2]University of Gothenburg, Gothenburg, Sweden,*

OBJECTIVES: The aim of this study was to use proteomic methods to analyse the serum of stage I HIV positive subjects, in order to identify novel protein changes that are induced in the human serum proteome following HIV infection. This information may be used for the development of novel biochemical markers to monitor the progression of this disease.
METHODS: Serum was collected from asymptomatic Xhosa males between 21 and 35 years of age. HIV testing was performed and the samples were separated into HIV positive and HIV negative groups, and subjected to 2D-gel electrophoresis. The gels were silver stained and analysed to detect significant differences between protein spots. Spot differences were considered significant if the mean ratio between the HIV positive and HIV negative groups was greater than two or less than a half, and the p value was less than 0.05. Significant spots were digested by trypsin and subjected to analysis using MALDI-TOF mass spectrometry. Database interrogation was performed with the Mascot algorithm using the Swissprot and NCBInr databases.
RESULTS: Eight HIV positive and six HIV negative 2D-gels were prepared. 7 659 spots were detected in these 14 gels and 562 matches were made. 11 spots were found to be significantly different between the two groups. Nine spots were significantly decreased and two spots were significantly increased in the HIV positive group compared to the HIV negative group. The proteins found to be decreased were identified as abnormal spindle-like microcephaly-associated protein, type II cytoskeletal keratin, zinc finger CCCH domain-containing protein 13, alpha-1-acid glycoprotein, haptoglobin, apolipoprotein A-I and hemoglobin beta chain. The proteins found to be increased were identified as coiled-coil domain-containing protein 49 and 60S ribosomal protein L4.
CONCLUSION: A number of proteins that undergo consistent changes have been identified and this information could be used to develop immunoassays to analyse protein changes in the clinical laboratory as an additional method of monitoring changes in HIV infection.

A-121

Effect of Low-Density Lipoprotein Levels on the Measurement of Hepatitis C Viral Load in Chronic Hepatitis C Patients

E. M. Abdalla, A. M. Nasar, A. El Hawary, N. Y. Abdel Razek, B. B. Hasan. *Faculty of Medicine, Suez Canal University, Ismailia, Egypt,*

Background: Hepatitis C virus infection is hyperendemic in Egypt, 20 to 30% of which progress to liver cirrhosis. Hepatocytes represent the primary site of HCV replication *in vivo*. Several HCV-binding proteins have been suggested to mediate viral entry of HCV into cells; these include the CD81 receptor and the low-density lipoprotein (LDL) receptor. Several studies supported the concept that LDLR can regulate the rate of hepatocyte infection by competing with the virus through the LDLR. Therefore, serum HCV levels can be regulated by the rise and fall of serum beta-lipoproteins. Unexplained difference in results of quantitative PCR for hepatitis C could be secondary to changes of LDL levels. We evaluated the effect of LDL on the measurement of hepatitis C viral load in chronic hepatitis C patients through estimation of the levels of viral load of hepatitis C in relation to variation of the corresponding LDL. Also, we evaluated the effect of difference in LDL levels on other liver function tests.
Subjects and Methods: The study is a cross sectional study, carried out in the internal medicine clinic in Suez Canal University Hospital in Ismailia. 30 patients, seropositive for HCV antibodies and HCV-RNA, attended the clinic for at least two times with LDL levels in at least 2 occasions, with a difference of levels more than 10% in a period of 2 weeks. Patients were not receiving anti-viral or anti-hyperlipidemic treatment or suffering from chronic liver disease due to causes other than HCV. Patients fulfilling the inclusion criteria; biochemical assessment of liver function test, direct determination of serum LDL levels and quantitative determination of HCV-RNA level using the reverse-transcription polymerase chain reaction (RT-PCR) assay were performed.
Results: The present study demonstrated an inverse statistically significant correlation between the absolute HCV RNA viral load differences and absolute LDL level differences or LDL percent differences. The lower the absolute LDL differences, the higher the absolute HCV RNA viral load differences. Also, there was an inverse statistically significant correlation between the absolute HCV RNA percent difference and ALT percent difference. The results support the concept that LDLR is a HCV receptor and that beta-lipoproteins competitively inhibit the infection of hepatocytes with HCV through the LDLR. From this data, our study creates an equation that we can use for calculation of the predicted HCV RNA difference level using a regression analysis; guided by the difference in LDL levels.

A-122

The Prevalence of the Four species of Human Malaria Parasites in Children at the Korle Bu Teaching Hospital, Ghana

F. A. Botchway[1], C. E. Lekpor[2], R. A. Botchway[3], D. K. Dosoo[4]. *[1]Department Of Child Health Laboratory, Korle - Bu Teaching Hospital, Accra, Ghana, [2]Department Of Pathology, Korle - Bu Teaching Hospital, Accra, Ghana, [3]University Of Ghana, Legon, Ghana, [4]Kintampo Health Research Centre, Kintampo, Ghana,*

Background: Malaria is caused by four different species of Plasmodium namely P. falciparum, P. malariae, P. ovale and P. vivax. Malaria accounts for 7 - 8% of all certified deaths and ranks 5th as the commonest cause of death in the 0 - 4 age group in Ghana. An infection with P. falciparum can proceed rapidly to severe disease with serious complication such as cerebral malaria. The high invasive power of P. falciparum compared with the other species leads to rapid destruction of erythrocytes. Cerebral malaria, the commonest cause of death from malaria is associated with the cytoadherence of infected erythrocytes to the endothelial lining of capillaries and venules of the brain. Patients suffer from headaches that gradually become more severe and last longer, eventually convulsions, coma and hyperpyrexia culminating in death. In Ghana, malaria infection is the commonest cause of childhood convulsions. Malaria infections produce partial immunity. Transplacental passage of IgG from a malarious mother provides the neonate with protection that diminishes in strength over about 12 weeks. It has also been established that breast milk of infected women also contain protective IgG antibodies. The risk of infection with or without complication is limited almost entirely to the non-immune and persons with low immunity. Consequently, the most vulnerable groups are infants beyond 3-6 months of age who have lost their maternal protective immunity and young children under 5 years of age.
Aim:The aim was to study the prevalence of the four different species of human malaria parasites in children at the child health department of the Korle Bu Teaching Hospital

(KBTH)
Methods: The study covered a period of 6 months(Feb - July 2009) and was carried out on 178 (104 males and 74 females) clinically and laboratory diagnosed cases as malaria patients ages between 1 day to 12 years at the child health department of KBTH .Thick and thin blood films were prepared and stained with Giemsa and Lieshman stains respectively. The thick film was used to estimate the parasite density and the thin film for the identification of malaria species. Statistical analyses were done by simple parametric methods.
Results: In the thick film examination, 24(13.5%) had parasitaemia in the age range 1day - 1 year, 96(53.9%) had parasitaemia in the ages ranging from 2- 5years, 46(25.8%) had parastaemia in the ages raging from 6 - 9 years and 12(6.7%) in the ages ranging from 10 -12 years.
In the thin film examination, 170(95.5%) were P .falciparum, 6(3.4%) were P. malariae, 2(1.1%) were P. ovale , 4(2.2%) were mixed infections of P.falciparum and P. ovale. There was no P. vivax seen.
Conclusion: The study showed that the most prevalent species of malaria parasites was P. falciparum (95.5%), followed by P. malariae (3.3%) and P. ovale (1.1%).There were 2 cases of mixed infections, made up of P. falciparum and P. malariae. There was however no P. vivax seen. This confirms the fact that P. vivax occurs mainly in the temperate regions and particularly absent in West Africans.

A-123

Identification of diagnostic biomarkers for Tibetan tuberculosis by proteomic fingerprinting of serum in China
B. Ying, L. Wang. *West China hospital, Chengdu, China,*

Background: The Tibetan ethnic group is one of the largest ethnic groups in China. This population originated primarily from QINGZANG plateau, which has some of the highest rates of TB in the world, as well as high levels of drug resistance. Therefore, early detection and diagnosis of TB are crucial for a better patient outcomes. The diagnosis of tuberculosis is traditionally based on chest radiography, tuberculin test, and mycobacterial staining/culture. However, about 40-60% of patients with pulmonary tuberculosis were undiagnosed by these methods. The development of new diagnostic tests to replace traditional diagnosis of sputum TB was emphasized by the World Health Organization (WHO). Therefore, the search for rapid and reliable diagnostic tests for TB based on the examination of sputum, blood, and other clinical specimens has been the focus of a number of studies. Serum proteomic analysis based on surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF MS) may provide a feasible platform for the diagnosis of pulmonary tuberculosis. This is a high-throughput technique for analysis of complex biologic specimens such as serum. In this study, we used SELDI with ionic (CM10) protein chip arrays to screen potential serum biomarkers for the detection of TB. By using an artificial intelligence classification algorithm, several novel biomarkers were selected to facilitate the optimal separation of patients with TB and non-TB controls.
Methods: A total of 80 serum samples from patients with active pulmonary tuberculosis and control subjects from a Chinese Tibet population were analyzed by SELDI-TOF MS. A training set of spectra derived from analysis of serum from 30 patients with active pulmonary tuberculosis and 22 non-tuberculosis controls were analysed by a machine learning algorithm called decision tree boosting. The discovered decision tree was then used to classify an independent testing set of 28 masked serum samples: 19 from patients with tuberculosis, and 9 from healthy volunteers or those with non-tuberculosis disorders.
Results: Compared with the control group, 36 protein/peptide peaks were noted in Tibet patients with tuberculosis. Of the 36 protein/peptide peaks, 30 peaks over-expressed among the patients with tuberculosis. In contrast, 6 peaks down-regulated in the TB patients. Among 36 differential peaks, 13 positive and 2 negative protein/peptide peaks have more than 2-fold statistically significant difference between the two groups average. The optimal decision tree model with a panel of one biomarker with mass: charge ratios (m/z) of 4821.9 determined in the training set could precisely detect 28 of 30 (sensitivity 93.3%) active pulmonary tuberculosis serum samples and 3 of 22 (specificity 86.4%) non-tuberculosis samples. Validation on an independent blinded testing set gave an accuracy of 88.6% (sensitivity 89.5%, specificity 88.9%) using the optimal classification tree consisting of the one biomarkers.
Conclusion: These preliminary data suggest a potential application of SELDI-TOF MS proteomic pattern analysis as a rapid and accurate method to diagnose individuals with active pulmonary tuberculosis in Chinese Tibetan population from corresponding control subjects.

A-124

Effect of renal transplantation on serum hepatocyte growth factor levels in hemodialysis patients with HCV infection.
L. N. Kamel[1], A. Kenawi[2]. *[1]Theodor Bilharz Research Institute, Giza, Egypt, [2]Kasr El Aini, Cairo, Egypt,*

Objective: The purpose of this study was to evaluate the effect of renal transplantation on serum hepatocyte growth factor levels in hemodialysis patients with HCV infection.
Materials and methods: Seventy four subjects with HCV infection were enrolled in the study, they had normal liver function tests and patients with active hepatic disease were excluded from the study. The 74 subjects were further subdivided into three groups: Control group (n=10), hemodialysis group (HD) (n= 30) and renal transplant recipient group (n=34). Serum hepatocyte growth factor (HGF) determination was performed using quantitative sandwich enzyme immunoassay.
Results: Our study revealed that serum levels of HGF in HD patients showed a significant increase as compared to control ($p<0.001$) and renal transplant groups ($p<0.001$). In HD patients serum HGF levels showed a positive correlation with both serum creatinine levels ($r =0.874$, $p< 0.001$) and urea levels ($r= 0.559$, $p< 0.001$) but did not correlate with ALT levels or duration of HD. Serum HGF values in renal transplant recipients showed no statistically significant changes as compared to controls and did not correlate with either creatinine, urea, ALT and cyclosporine blood levels.
Conclusion: Our results suggested that elevated serum HGF in HD patients might be attributed to its increased production in response to the chronic renal injury, the effect of heparin, or its reduced removal in CRF patients. Elevated serum HGF values in HD patients return back to normal in renal transplant recipients with good allograft function.

A-125

Evaluation of the Abbott ARCHITECT Toxo IgG, IgM and IgG Avidity Assays
G. T. Maine[1], A. Francisco[2], R. Stricker[3], R. Stricker[3]. *[1]Abbott Diagnostics, Abbott Park, IL, [2]Abbott Diagnostics, Baar, Switzerland, [3]Dianalabs, Geneva, Switzerland,*

Objective: Evaluate the performance of the ARCHITECT Toxo IgG, IgM, and IgG avidity assays in samples from women with documented seroconversion for toxoplasmosis during gestation and in samples from the Toxoplasma Serology Laboratory in Palo Alto, CA.
Relevance: Document performance of the new Toxo panel in the clinical laboratory relative to current methods in use.
Methods: Archived patient samples (n = 200) selected from pregnant women (n = 47) with documented recent seroconversion for toxoplasmosis were tested and the results compared to the Abbott AxSYM Toxo IgG and IgM assays. Archived patient samples (n = 400) from the Toxoplasma Serology Laboratory were tested and the results compared to the Sabin-Feldman Dye test, the differential agglutination test (AC/HS), and the Vidas Toxo IgG avidity assay.
Results: In samples from pregnant women undergoing seroconversion for toxoplasmosis the ARCHITECT and AxSYM Toxo IgG and IgM assays detected the same first bleed as Toxo IgG and IgM positive or equivocal in 42/47 and 45/47 patients, respectively. In the remaining patients either the ARCHITECT or AxSYM assay detected the patient as positive for Toxo IgG or IgM one bleed earlier. Detection of Toxoplasma-specific IgM before IgG occurred in 5/47 patients (11%). The clinical sensitivity of the ARCHITECT Toxo IgG avidity assay using a cutoff of 4 months post-seronegative bleed was (111/112) = 99.1% (95% CI = 95.1-100%). In samples from the Toxoplasma Serology Laboratory the agreement between the ARCHITECT Toxo IgG test and the Toxo IgG + IgM tests with the Dye test was (383/392) = 97.7% (95% CI= 95.7-98.9%) and (389/392) = 99.2% (95% CI = 97.8-99.8%), respectively. Agreement between the ARCHITECT and Vidas IgG avidity assays was (229/234) = 97.9% (95% CI = 95.1-99.3%). The agreement between the ARCHITECT and Vidas Toxo IgG avidity assays with the AC/HS test was (231/241) = 95.9% (95% CI = 92.5-98.0%) and (247/258) = 95.7% (95% CI = 92.5-97.9%), respectively.
Conclusion: The performance of the three fully automated Abbott ARCHITECT Toxo immunoassays was equivalent to the reference assays and easier for a laboratory to perform than the Dye test and AC/HS assay.

A-126

Performance Characteristics of the Access® Cytomegalovirus (CMV) Immunoglobulin M (IgM) Assay

M. L. Rawlins[1], S. L. La'ulu[1], L. Maxwell[2], J. C. Ritchie[2], W. L. Roberts[3]. *[1]ARUP Laboratories, Salt Lake City, UT, [2]Emory University Hospital, Atlanta, GA, [3]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT,*

Cytomegalovirus (CMV) is a member of the Herpesviridae family and is found throughout the world. The seroprevalence of human CMV virus ranges from 30% to 90% in developed countries and increases with age. Primary infection is usually asymptomatic in healthy individuals, after which the virus remains latent for life with periodic reactivation and rarely causes complications in these individuals. However, in the fetus, neonate, immunocompromised individuals, and transplant recipients, CMV infection can cause serious disease. Measurement of antibodies against CMV can be used to detect current infection, past infection in immunocompromised persons who are at risk for recurrent infection, pregnant women, and newborns suspected of having CMV infection. The imprecision and concordance of the automated Access 2 CMV IgM (Beckman Coulter, Inc.) and the mini VIDAS CMV IgM (bioMérieux, Inc.) assays were evaluated. Imprecision data are summarized in the table. Serum specimens submitted to the clinical laboratory for CMV IgM testing (n=1046), maternal serum testing (n=307), and specimens from immunocompromised subjects (n=75) and transplant recipients (n=216) were tested by the Access 2 and mini VIDAS methods. The mini VIDAS was used as the comparison method. Equivocal samples were excluded from all data analyses. For samples submitted for routine CMV testing, relative sensitivity, relative specificity, and concordance for the Access 2 assay were 87.3%, 97.2%, and 96.6%, respectively. For maternal serum samples, relative specificity and concordance were 99.7% and 99.7%, respectively. There were no samples positive by both methods for this group, so relative sensitivity could not be estimated. For immunocompromised samples, relative sensitivity, relative specificity, and concordance were 100.0%, 97.1%, and 97.2%, respectively. Relative sensitivity, relative specificity, and concordance for transplant recipients were 100.0%, 94.9%, and 95.0%, respectively. Based on these data, the Access 2 CMV IgM assay showed good analytical performance and is suitable for routine clinical testing.

Summary of imprecision data

Method	Sample	N	Mean concentration (S/CO)	Within run CV (%)	Between run CV (%)	Between day CV (%)	Total CV (%)
Access 2	L1	20	0.109	2.1	4.1	0.0	4.6
	L2	20	1.11	6.6	0.0	6.3	9.2
	L3	20	11.2	6.9	0.0	3.7	7.8
mini VIDAS	L1	20	0.145	13.2	0.0	4.1	13.9
	L2	20	1.10	6.1	3.3	0.0	7.0
	L3	20	1.76	4.0	0.7	0.7	4.1

A-127

Investigation of the relationship between apolipoprotein E gene polymorphisms and hepatitis B virus infection in northern China

C. Xiong[1], Q. Cen[2], Z. Yin[3], X. Zhou[1], J. Tu[1]. *[1]Zhongnan Hospital of Wuhan University, Wuhan, China, [2]Zhongshan Hospital of Wuhan University, Wuhan, China, [3]Beijing Chuiyangliu Hospital, Beijing, China,*

Background:Certain genetic polymorphisms can lead to differences on immunity function, thus affecting different clinical outcomes for HBV patients. The aim of this study was to investigate the association between apolipoprotein E gene polymorphisms and Hepatitis B infection status in northern Chinese population.

Methods:Genomic DNA was extracted by improved NaI method from the peripheral blood of 270 cases of hepatitis B patients with different infection models and 112 healthy controls. Multiplex Amplification Refractory Mutation System (Multi-ARMS) was performed to analyze Apolipoprotein E gene polymorphisms with three alleles (ε2, ε3, ε4) in the hepatitis B carriers and control cases. Chemiluminescence assay was used to detect serological markers for hepatitis B infection status.

Results:An improved PCR system for the detection of ApoE gene polymorphisms was established successfully. The frequency of ε2 allele in patients with different models of hepatitis B infection was higher than that in the normal control group (P<0.05). The ε2 allele, compared with the ε3&ε4 alleles, has positive correlation with the hepatitis B infection (OR = 1.735, 95%CI: 1.509-1.999, P<0.01; OR = 1.768, 95% CI: 1.554-2.011, P<0.01).

Conclusion:Our results indicated that the ApoE gene polymorphism was associated with the Hepatitis B virus infection and the ε2 allele had positive correlation with hepatitis B infection in northern China.

A-128

Evaluation of an Automated Immunofluorescent Assay for Procalcitonin

P. P. Chou, N. Matt, L. Song, K. Sisco, N. Sherman. *Quest Diagnostics Nichols Institute, Chantilly, VA,*

Introduction: Sepsis (systemic infection) is a daily challenge in intensive care units. There are various known therapeutic strategies to improve survival in patients with sepsis and early assessment is important for determination of the appropriate treatment. Procalcitonin (PCT) can be produced by several cell types and many organs in response to pro-inflammatory stimuli, in particular by bacterial products. In healthy adults, plasma PCT concentrations are found to be below 0.1 ng/mL. PCT levels rise rapidly within 6 to 12 hours after a bacterial infectious insult with systemic consequences. PCT levels can also be elevated independent of an infectious process, but the return to baseline is usually rapid. Such conditions include early onset of multiple traumas, major surgery, severe burns, or in the first two days of life in neonates. Viral infections, bacterial colonization, localized infections, allergic disorders, autoimmune diseases, and transplant rejection do not usually induce a significant PCT response (values < 0.5 ng/mL). Therefore, by evaluating PCT concentrations, the physician may use the findings to detect sepsis and to aid in the risk assessment for progression to severe sepsis and septic shock.

Method: The measuring principle of BRAHMS PCT is based on time-resolved amplified cryptate emission (TRACE®) technology, which measures the signal that is emitted from an immunocomplex between two highly specific cryptate- and XL665-labeled anti-PCT antibodies. The intensity of the signal is proportional to the amount of procalcitonin present in the specimens.

Results: This assay has a total CV of 4.9% (mean=0.3 ng/mL) and 1.9% (mean=10.6 ng/mL). This assay is linear from 0 to 175 ng/mL. Recovery studies using quality control materials were acceptable with bias < 5% at all levels up to 25 ng/mL. A correlation study using 68 serum specimens with known PCT concentrations yielded a linear regression line with a slope of 0.9595, a y-intercept of -1.07, and a correlation coefficient of 0.9986. A reference range of less than 0.1 ng/mL was established by analyzing both serum and EDTA plasma specimens collected from apparently healthy adult individuals. Both serum and plasma specimens yielded equivalent results in the reference range study. Stability studies show PCT has limited stability (<4 hours at room temperature and 5 days at refrigerated temperature), whereas PCT is stable for at least 43 days at frozen temperature in our study. Freeze and thaw cycles up to four times do not affect the results.

Conclusions: The performance of this assay is acceptable. This assay is fully automated, easy to perform, and provides rapid turnaround time. The reference range for both serum and EDTA plasma are in agreement with package insert provided by the manufacturer.

A-129

The clinical Laboratory's critical role in decreasing Methicillin-resistant Staphylococcus aureus (MRSA) hospital acquired infection (HAI) by implementing a rapid screening program in a community hospital.

D. L. Uettwiller-Geiger. *John T. Mather Memorial Hospital, Port Jefferson, NY,*

Background: Invasive MRSA infections occur in approximately 94,000 people annually and cause as many as 9,000 deaths per year, according to the CDC. HAIs cost the United States society between 4 and 5 billion dollars annually.

Objectives: To implement a rapid screening program to cost effectively detect MRSA colonized and/or infected patients using rapid polymerase chain reaction (PCR) in real time providing clinicians with key test results within one hour instead of days to reduce MRSA patient to patient transmission. An effective interventional surveillance program with along laboratory testing support will reduce the number of HAIs and the associated morbidity and mortality, thereby improving patient safety by reducing risks of infection and other adverse outcomes, while meeting the regulatory requirements for The Joint Commission, National Patient Safety Goals (NPSF), Goal 07.03.01.

Methods: A comprehensive, integrated, multi-disciplinary surveillance screening program was implemented in March 2008 using the new surveillance laboratory testing technology of PCR. The Cepheid GeneXpert System uses a single test cartridge delivering MRSA test results in less than an hour with minimal handling by a laboratory technologist. MRSA testing is provided on demand in real time during any shift, any day, and around the clock, allowing for fast interventions by clinicians and infection control preventists when MRSA is detected.

Results: Our surveillance screening strategy initially focused on risk populations of intensive care units (ICU), cardiac care unit (CCU), and Orthopedics and later expanded to the telemetry unit. In 2007, before rapid PCR MRSA screening, the infection rate was .90/1000 discharges and after implementation of rapid PCR MRSA screening in 2008

and 2009 the infection rate was .59/1000 and .29/1000, respectively. Comparing MRSA infection rates between 2008 and 2009 there was a 50% reduction in MRSA HAI with a corresponding 50% reduction in infection costs. MRSA surveillance screening costs in 2009 for Laboratory testing were $104,769 and based on the average cost of infection incurred during hospitalized medical care of $35,000 dollars per infected patient, we decreased the cost by $920,500 dollars in 2008 and by $847,000 in 2009, or almost $1.8 million dollars between 2007 and 2009.

Conclusions: Rapid surveillance screening for MRSA using automated molecular diagnostics of PCR reduces the time to diagnosis, treatment and cure, saving thousands of dollars in hospitalization and infection costs associated with HAIs, while enhancing patient safety and significantly reducing infection rates.

A-130

Biochemical and molecular characterization of *Mycobacterium avium* subspecies *paratuberculosis* and investigation of its relationship to Crohn's disease in humans

J. C. Uzoigwe[1], R. Kargbo[1], Y. Takahashi[1], G. R. Cook[1], M. L. Khaitsa[2], P. S. Gibbs[2]. *[1]North Dakota State University, Department of Chemistry, Biochemistry and Molecular Biology, Fargo, ND, [2]North Dakota State University, Department of Veterinary and Microbiological Sciences, Fargo, ND,*

BACKGROUND: Crohn's disease is a debilitating chronic infection of the intestine in humans. Although the etiology of Crohn's disease remains unproven, several studies have purported that *Mycobacterium avium subspecies paratuberculosis* (MAP) plays a causal role in Crohn's disease. MAP is the causative agent of Johne's disease in cattle, a chronic disease that in some ways resembles Crohn's disease. MAP has become a concern in laboratory medicine because, as reported by recent studies, DNA from this agent has been isolated from several patients with Crohn's disease, which suggests that it may be a factor in the causation of this disease. OBJECTIVES: The purpose of this research was to investigate the level of similarities between MAP isolates using molecular and biochemical methods, in order to improve our understanding of the possible differences in virulence between the isolates, and the possible role of some strains in the pathogenesis of Crohn's disease.

METHODS: Seventy five (44 asymptomatic and 31 symptomatic) isolates of cattle origin and 3 isolates of human origin, were characterized by utilizing molecular techniques of pulse field gel electrophoresis (PFGE) using SnabI endonuclease, and restriction fragment length polymorphism (RFLP) using BSTEII endonuclease , as well as biochemical techniques of mass spectrometry (MS) and high performance liquid chromatography (HPLC).

RESULTS: The HPLC analysis of mycolic acid yielded peaks at m/z 341, 399, 443, 487, 531, 575, 619, 663, 707, 751, 795, 839 and 883. Slight differences between symptomatic and asymptomatic groups were observed in the high mass region of the mass spectra, which showed that 4 (9.1%) of asymptomatic isolates and 24 (77.4%) of symptomatic isolates displayed additional peaks of longer chain length at m/z 910, 937, 940, 1037, 1045, and 1071. Of the three human isolates, two showed long chain mycolic acid similar to those of symptomatic cow isolates, while one displayed shorter carbon chain mycolic acid similar to those of asymptomatic cow isolates.

CONCLUSION: The results of this study provided new information on the possible role of long carbon chain length of mycolic acid in the virulence of some strains of MAP, in both bovine and human hosts, and together with the high level of similarity in PFGE and RFLP patterns of MAP isolates from symptomatic cattle and Crohn's disease patients, provided support that MAP may play a probable role in the etiology of Crohn's disease. Thus, it appears that particular MAP strains have developed the ability to infect a wide range of cattle, and possibly humans.

A-131

Evaluation of the RIDA®QUICK Norovirus Rapid Immunoassay versus real time RT-PCR for the detection of Norovirus in faecal samples

S. Day[1], C. Curtis[1], M. Lyon[2], A. Simons[3], P. Derrington[1]. *[1]Department of Microbiology, Pathology Queensland, Gold Coast Hospital, Gold Coast, Australia, [2]Public Health Virology Laboratory, Queensland Health Forensic and Scientific Service, Brisbane, Australia, [3]R-Biopharm AG, Darmstadt, Germany,*

Background: Noroviruses are the global leading cause of outbreaks as well as sporadic cases of acute gastroenteritis, estimated to affect 23 million people yearly in the United States. They are highly contagious and spread easily from person to person. Norovirus outbreaks occur in many settings such as schools, nursing homes, restaurants, hospitals and cruise ships and are associated with a high socio economic burden. Outbreaks result in health burden for individuals living or working in these settings and in significant cost for the institution. The total economic impact of a single outbreak in a 1,000 beds hospital was estimated to be $657,644. To prevent transmission, early diagnosis followed by appropriate hygiene interventions is crucial. The gold standard for diagnosis of norovirus from faecal samples is RT-PCR which is generally performed in a regional reference centre. Transport to these centres may result in delays in diagnosis and increased norovirus spread in an institution. A rapid and sensitive screening method would improve timely diagnosis and facilitate timely infection control measures. For this reason the performance and sensitivity of the RIDA®QUICK Norovirus Immunoassay (R-Biopharm) was evaluated versus real-time RT-PCR for detection of norovirus in faecal samples.

Methods: 159 frozen patient faecal samples were included in the study. Of these 87 (55%) were known positive and 72 (45%) were known negative by real-time RT-PCR. The samples were blinded to the operator and randomly tested in batches of six specimens representative of a norovirus outbreak. The RIDAQUICK Norovirus is a 15 minute, lateral flow through enzyme linked immunoassay for detecting genogroup I and genogroup II noroviruses in stool samples. Sample is filtered through a porous membrane bound with specific monoclonal antibodies directed against noroviruses. If present, norovirus antigen binds to the capture antibodies and is revealed by the addition of a labelled peroxidase conjugate. The positive reaction is made visible by the addition of a chromagen. Internal controls are included.

Results: The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the RIDAQUICK Norovirus was 82%, 100%, 100% and 82% respectively compared to RT-PCR. The results showed 90% agreement. At a CT value of 21 and below 98% of the PCR positive samples were RIDAQUICK Norovirus positive. Samples with a CT value of greater than 26 are confirmed by RIDAQUICK Norovirus in only 42% of cases.

Conclusion: The RIDAQUICK Norovirus is a simple 15 minute assay with a sensitivity of 82% and excellent specificity. A positive result correlates with high viral load and thus increased infectivity. If several samples are tested in an outbreak setting, the single test sensitivity can be further increased and in that case a single positive result precludes the need for a further testing of samples in that outbreak. The RIDAQUICK Norovirus is a simple and rapid assay which is easy to perform and is a suitable screening method for quick and reliable diagnosis of a norovirus outbreak to ensure timeous introduction of infection control measures.

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Evaluation of Two Commercial Enzmye Immunoassays for the Detection of Norovirus in faecal samples compared to RT-PCR

F. Schreiber[1], A. Simons[2], P. Derrington[3]. *[1]School of Biomedical and Physical Science, Griffith University, Gold Coast Campus, Gold Coast, Australia, [2]R-Biopharm AG, Darmstadt, Germany, [3]Department of Microbiology, Pathology Queensland, Gold Coast Hospital, Gold Coast, Australia,*

Background: Noroviruses are the leading cause of acute gastroenteritis worldwide and are commonly associated with large outbreaks in schools, nursing homes, restaurants, hospitals and cruise ships. Since noroviruses are highly infectious, a fast and reliable detection method is important for timely infection control measures. The highly sensitive and specific RT-PCR is the gold standard for norovirus diagnosis, but is also labour intensive, time consuming, require a high level of technological skill and is generally performed in regional reference centers. The transport to a regional center may result in a delay in diagnosis and thereby related delay in infection control measures. Enzyme immunoassays could be an attractive alternative or supplement to RT-PCR because they are rapid, simple and can be used in most diagnostic laboratories. The performance of two EIA kits, (RIDASCREEN®Norovirus 3rd Generation (R-Biopharm) and IDEIA™Norovirus (Oxoid)) versus RT-PCR was evaluated for the detection faecal samples.

Methods: In total 100 frozen faecal samples determined positive and negative by RT-PCR were tested by both EIAs. The samples were blinded to the operator and randomly tested in batches of six specimens representative of a norovirus outbreak. Both EIAs use a double antibody sandwich technique, while only RIDASCREEN Norovirus use a third enzyme labeled antibody to determine the presence of the "detector" antibody. The IDEA Norovirus utilizes specific monoclonal and polyclonal antibodies from genogroup I and II to detect these norovirus antigens. The RIDASCREEN Norovirus utilizes specific and cross reactive monoclonal antibodies for the detection of most common norovirus strains. The IDEIA kit requires a minimum processing time of 1.5 hours with one manual wash, and the RIDASCREEN kit requires a minimum processing time of 1.75 hours with two manual washes.

Results: Out of the 100 frozen samples included in the study 60 were known positive and 40 were known negative by RT-PCR.. Sensitivity and specificity versus RT-PCR was 76.6% (46/60) and 97.5% (39/40) respectively for RIDASCREEN and 66.6% (40/60) and 95% (38/40) respectively for IDEA. Compared to RT-PCR as standard reference the results showed an agreement of 85% for RIDASCREEN and 78% for IDEA. The PPV and NPV was 97.9 % and 73.6% for RIDASCREEN and 95.2 % and 65.5 % for IDEA respectively. Of the two EIA false positive results, one sample tested positive by both kits and one tested positive by IDEIA only - unfortunately the samples were discarded before RT-PCR could be repeated to confirm. The specificity may therefore be higher than reported.

Conclusion: The results suggest that EIAs could be implemented for norovirus screening as an adjunct to RT-PCR for rapid diagnosis of norovirus to allow timely implementation of infection control procedures in outbreak situations. The RIDASCREEN Norovirus 3rd Generation kit was superior in sensitivity and specificity compared to the IDEA Norovirus kit.

A-133

Preliminary quantitative assessment of serum HBsAg levels using the Access® HBsAg assay* on UniCel DxI 800

A. Pivert[1], S. Alain[2], M. Maniez[3], F. Lunel[1], F. Bouniort[4]. *[1]Laboratoire de Virologie, CHU Angers, Angers, France, [2]Laboratoire de Virologie, CHU Limoges, Limoges, France, [3]Etablissement Français du Sang Nord de France, Lille, France, [4]Beckman Coulter , Inc, Nyon, Switzerland,*

Background : Quantitative measurement of serum hepatitis B surface antigen (HBsAg) has been proposed as a predictor of response to antiviral treatment.
Objective : To evaluate the feasibility of quantification of HBsAg based on the qualitative Access HBsAg assay***.
Method : The Access HBsAg was tested with a documented quantitative panel (10 samples from 2.04 to 0.03 ng/mL) from EFS (Etablissement Français du Sang) and the WHO 00/588 diluted (from 100 to 0.09 IU/mL). A linear regression was calculated. Linearity was studied with a high-titer sample (5,000 ng/mL) diluted to a range of 0.04 - 500 ng/mL. All the samples were also tested with the quantitative ARCHITECT**HBsAg assay and correlation calculation was performed.
Results : The regression calculation with the EFS quantitative panel indicated a R^2= 0.9945 for Access HBsAg (the R^2 observed with ARCHITECT was 0.9804). The regression calculation with the diluted WHO standard showed a R^2 = 0.9999 up to 25 HBsAg IU/mL and 0.9813 up to 100 HBsAg IU/mL (the R^2 observed with ARCHITECT was 0.9954). A dilution study with HBsAg high-positive sample demonstrated excellent linearity of the Access HBsAg assay with R^2 = 0.9921 up to 50 ng/mL. The correlation coefficient between DxI HBsAg and Architect HBsAg ranged from 0.9898 to 0.9997 for the different panel (EFS quantitative panel) and diluted (WHO, EFS high-titer) HBsAg samples.
Conclusion : These preliminary data showed that the Access HBsAg assay, currently available as a qualitative assay for HBsAg, could be adapted for quantitative HBsAg determination. Additional investigation with clinical samples should support algorithm definition and performance with this new application.
*The Access HBsAg is not approved for use in the U.S.
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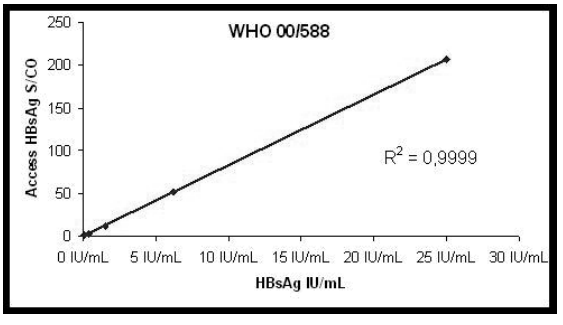

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Comparison of the Beckman Coulter Access® CMV IgG and CMV IgM Assays to the Roche Elecsys* CMV IgG and IgM Assays in Routine Laboratory

J. Van Helden[1], M. Martin[2], C. Vauloup-Fellous[3], F. Bouniort[4].
[1]Praxis für Laboratoriumsmedizin, Mikrobiologie und Humangenetik, Mönchengladbach, Germany, [2]Immunotech SAS, Marseille, France, [3]Hopital Antoine Béclère, Clamart, France, [4]Beckman Coulter , Inc, Nyon, Switzerland,

Background : The human cytomegalovirus (CMV) is a member of the *Herpes viridae* family transmitted person-to-person via close non-sexual contact, sexual activity, breastfeeding, blood transfusions, and organ transplantation. CMV infection is a serious concern for women of child-bearing age, because it is a leading cause of hearing and vision loss, as well as mental retardation among congenitally-infected children.
Objective : To compare the results of CMV markers (IgG and IgM) by two automated immunoassay methods.
Method : 500 blood (serum) samples (123 from transplant or immunosuppressed patients, 377 from pregnant women) were tested for CMV IgG and CMV IgM with the Elecsys IgG and IgM assays run on the Roche Modular E170 platform and with Access CMV IgG and IgM assays run on the Beckman Coulter UniCel DxI 800 platform. Samples showing discrepant CMV IgG results were tested with Vidas* CMV IgG assay. Samples showing discrepant CMV IgM results were further tested with different CMV IgG avidity methods (Liaison, Vidas and in-house avidity) assessing the sample status (primary or non primary infection). Concordance was calculated before and after resolution.
Results : The total positive and negative agreement of Access CMV IgG vs. Elecsys CMV IgG were 98.4% (492/500), 99.1% (416/420) and 97.4% (76/78), respectively. After resolution, the total positive and negative agreement of Access CMV IgG vs. consensus method was 100%. The total positive and negative agreement of Access CMV IgM vs. Elecsys CMV IgM were 92.8% (464/500), 33.3% (2/6 - not statistically significant) and 95.7% (462/483), respectively. Because the CMV IgG avidity index was high, indicating that the CMV IgM detected in both methods was not linked with a primary infection and can be considered as negative, the negative agreement after resolution was 94.2% (471/500) for Access CMV IgM and 96.6% (483/500) for Elecsys CMV IgM.
Conclusion : Based on this data Access CMV IgG showed better performance (relative sensitivity and specificity after consensus) than Elecsys CMV IgG and slightly lower relative specificity for CMV IgM (according to IgG avidity index). The Access CMV IgG and IgM assays are suitable for routine clinical use. *All trademarks are property of their respective owners.

A-135

Performance Characteristics of a Full-Length *HIV-1* Integrase Genotyping Assay by Sequence Analysis in the Siemens Clinical Laboratory

D. Maghakian[1], J. Turczyn[1], S. Cruz[1], L. Anders[1], L. Fisher[1], P. Joseph[1], S. Pandit[2], A. Uzgiris[2]. *[1]Siemens Clinical Laboratory, Siemens Healthcare Diagnostics Inc., Berkeley, CA, [2]Siemens Healthcare Diagnostics Inc., Berkeley, CA,*

Background: HIV-1 integrase enzyme is a three-domain monomeric protein (N-terminal, peptide chain, and catalytic core) that participates in the formation of the active quaternary structure. Catalytic core codons 51 to 211 mediate binding of magnesium ion and catalyze strand-transfer integration of HIV-1 proviral cDNA into the host cell genome. The HIV-1 integrase gene (IN) has no homologue in humans and is remarkably conserved with almost two thirds of its sequence under high selective pressure. Because of these characteristics, integrase inhibitors (DNA-binding, 3´-processing, nuclear-transport, and strand-transfer) are important therapeutics for further study. Of these, integrase strand-transfer inhibitors (STIs) such as raltegravir (MK-0518, Isentress®; Merck & Co Inc., Whitehouse Station, NJ, US) are particularly useful. Phase III BENCHMRK studies provided evidence for raltegravir therapy in combination with other antiretroviral agents in treatment-experienced patients demonstrating multidrug resistance. Furthermore, the phase III STARTMRK study demonstrated potential benefit for the use of STIs in treatment-naïve adult patients. Previously, HIV-1 reverse transcriptase (RT) and protease (PR) genotyping (TRUGENE®, Siemens Healthcare Diagnostics Inc., Deerfield, IL, US) has proven to be valuable in therapeutic management of the HIV-1-infected patient. Likewise, full-length IN genotyping in antiretroviral therapeutic drug monitoring for STIs is now the standard of care and will continue to provide valuable insight as a companion diagnostic for future integrase inhibitor drug development.
Objective: To validate a full-length HIV-1 integrase genotyping method by sequence analysis.
Methods: Viral HIV-1 RNA was extracted from 140 µL human plasma (QIAGEN QIAamp™ Viral RNA Mini Kit; QIAGEN Inc., Germantown, MD, US). Extracted specimens were reversely transcribed and amplified by PCR to yield a 1,165 bp amplicon spanning the IN gene (QIAGEN OneStep™ RT-PCR). Each specimen was sequenced using a series of eight BigDye™ terminator (Applied Biosystems BDT v3.1; Life Technologies, Carlsbad, CA, US) reactions according to standard protocol and detected by capillary electrophoresis (ABI 3130 and 3500xl genetic analyzers, Life Technologies). The 867 bp of contiguous bidirectional sequence generated was aligned against the NCBI reference sequence (LOCUS NC_001802 9181 bp ss RNA linear VRL 08-DEC-2008) and included sequence encoding for the entire mature IN peptide sequence. Treatment-associated mutations were visualized as discrepancies between the reference sequence and the patient sample.
Results: Within-run repeatability and between-run precision were both 100%. Analytical sensitivity of 898 target copies/mL with 100% accuracy was demonstrated across the entire 867 bp sequence (subtype B) and verified by in silico analysis. Full-length accuracy of well characterized HIV-1 group M subtype samples (A-H) was ≥98%. No contaminating sequence was detected in all negative control reactions (HIV-1-negative human plasma), with 100% specificity for HIV-1 IN sequence target. High-end viral load (>12,000,000 target copies/mL) indicated 100% genotype accuracy. The quality or accuracy of sequence

results were not compromised in the presence of potential interfering substances. Clinical studies indicated 100% concordance across a well-characterized and blinded sample set, with an estimated clinical sensitivity of 97.4%.
Conclusion: The full-length HIV-1 integrase genotyping assay is an analytically valid laboratory-developed method with demonstrated clinical utility.

A-137

Clinical evaluation of TRCRapid M.TB for the detection of *Mycobacterium tuberculosis* complex in respiratory and nonrespiratory specimens

E. Miyajima, H. Tanaka, H. Hirose, Y. Kato, S. Kida, H. Yonezawa. *Yokohama City University Medical Center, Yokohama, Japan,*

Background: The rapid and accurate diagnosis of tuberculosis is crucial to providing optimal treatment and reducing the spread of infection.
Methods: We evaluated respiratory and nonrespiratory clinical specimens using a new automated *Mycobacterium tuberculosis* complex (MTBC) ribosomal RNA detection kit (TRCRapid M.TB; Tosoh Bioscience, Tokyo, Japan), which is based on transcription-reverse transcription concerted reaction (TRC). TRC enables the rapid and completely homogeneous real-time monitoring of isothermal sequence RNA amplification without any postamplification procedures.
Results: The results were compared with those of *M. tuberculosis* culture. A total of 1,155 respiratory and 420 nonrespiratory specimens collected from 1,282 patients were investigated. Of the 45 specimens culture positive for MTBC, 42 were TRC positive, and of the 1,530 specimens culture negative for MTBC, 1,523 were TRC negative. Compared to the results of culture, the overall sensitivity and specificity of the TRC were 96.6% and 99.9%, respectively, for respiratory specimens and 87.5% and 98.5%, respectively, for nonrespiratory specimens. The sensitivity of the TRC was 100% for smear-positive respiratory and nonrespiratory specimens, 88.9% for smear-negative respiratory specimens, and 80% for smear-negative nonrespiratory specimens. No significant differences were observed in test performance between respiratory and nonrespiratory specimens.
Conclusion: The TRC method proved to be clinically useful for the rapid identification of MTBC in respiratory and nonrespiratory specimens and in both smear-positive and smear-negative samples.

A-138

Molecular Epidemiology of an Outbreak of Multidrug-resistant *Acinetobacter baumannii* in an Intensive Care Unit of victims of the Wenchuan earthquake

Y. Xie, m. kang, c. tao, h. fan, l. guo, y. ye, z. chen. *Division of Clinical Microbiology, Department of Laboratory Medicine, West China Hospital, ChengDu, China,*

Background: Multidrug-resistant (MDR) *Acinetobacter baumannii* are emerging as important nosocomial pathogens and were responsible for infection in some earthquake patients. During a two week period after the May 12, 2008 WenChuan earthquake, seven earthquake patients suffering from invasive infection caused by MDR *A. baumannii* were identified in the traumatic Intensive Care Unit (ICU) of West China Hospital SiChuan University (ChengDu, China). To study the nosocomial infection outbreak strains from these patients and from the hospital environment were collected and analyzed for identification, AST, and strain typing.
Methods: A total of 17 *A. baumannii* strains were isolated from earthquake patients and the environment in ICU. Eleven strains were isolated from seven different earthquake patients, Six strains were isolated from ICU medical equipment and family members of patients.
Results: Three different antibiogram profiles were found from the 17 isolates. Strain typing, using a commercially available strain typing system, DiversiLab, which employs the repetitive sequence-based PCR (rep-PCR) technology showed 5 different clusters of isolates: I (6 isolates), II (8 isolates), and III-V (1 isolate each).
Conclusion: MDR *A. baumannii* is one of the most important pathogenic microorganisms with a high incidence of hospital infection, especially in traumatic ICU after earthquake. DiversiLab System can be effective in tracking the outbreak of hospital infection caused by genetically related pathogens.

A-139

The Clinical Application of Serum Procalcitonin in Infectious Disease

L. Guo, M. Xiong, H. Fan. *Division of Clinical Microbiology, Department of Laboratory Medicine, West China Hospital, ChengDu, China,*

Background: to explore the clinical value of serum PCT in diagnosis and prognosis assessment of bacteria caused sepsis.
Method: A total of 80 inpatients admitted to our hospital for bacteria or fungi caused sepsis were selected. Another 20 cases of non-septic infections were chosen as controls. Fast venous blood samples were collected from each septic subject in the morning before breakfast on the 1st, 3rd, 5th days, and from non-septic subjects only on the first day. The samples were centrifuged and kept under -20°C. Enzyme linked fluorescent assay (ELFA) was employed to measure the serum PCT. CRP was determined by immunity-turidity method. The measurement data is expressed by mean±standard deviation. The comparison of two samples is make sure of t-test and the analysis of correlation is accomplished by linear correlation. Describe the receiver operating characteristics curve (ROC) to evaluate the capability of diagnosis and to account the area under the curve (AUC).
Results: Differences were statistically significant with serum PCT, temperature, heart rate, respiratory rate, WBC, PLT and CRP in septic group compared to non-septic group ($P<0.05$), with which the levels were markedly higher in septic group except PLT. Scatter diagrams of the correlations among PCT with temperature, PLT and CRP indicate positive correlations with degrees of correlation as following: CRP>PLT>temperature. Serum PCT level was much higher in subjects suffering septic shock, compared to which APACHE-II score has merely slightly elevated. The fluctuation of serum PCT level paralleled with the variation of septic condition, whereas CRP did not. Serum PCT levels were found to be markedly higher in patients with Gram negative bacteremia than in those with Gram positive bacteremia or fungi, regardless the severity of sepsis. A serum PCT level of 2.40 ng/ml yielded an 75.0% sensitivity value and a 63.9% specificity value for Gram negative related bacteremia in septic group.
Conclusions: Serum PCT is preferred to clinical symptoms,WBC,PLT and CRP in discriminating sepsis from non-septic infections. Serum PCT level is closely correlated with the severity of sepsis, suggesting its big value in assisting physicians with accurate and timely severity determination. In critically ill patients with clinical sepsis, the elevated PCT levels could be greater when the bacteremia is caused by Gram negative bacteria in comparison with Gram positive bacteria or fungi. And it might help to distinguish the type of bacteria causing sepsis, which may play an important role in guiding the therapy further on.

A-140

Detection and resistance analysis of mycoplasma in female genital tract inflammation

Q. Wu, Y. Li, M. Wang. *Renmin Hospital of Wuhan University, Wuhan, China,*

Background: To investigate the infectivity of mycoplasma in female genital tract inflammation, and monitor the resistance change so as to guide reasonable drug use in clinic.
Methods: We considered 1220 cases of patients with genital tract inflammation and 183 asymptomatic cases, aged between 18 and 55, females. Leucorrhoea specimens were strictly collected from vaginal fornix, which were soon inoculated in transport medium. Identification, enumeration and antibiotic of the pathogens were performed on the plate. Then incubated at 37°C, and observed for color changes at 24 and 48 h. Enumeration at one pathological level (≥10000 CFU/specimen). The antimicrobial susceptibility testing included tetracycline, doxycycline, erythromycin, azithromycin, clarithromycin, josamycin, ofloxacin, ciprofloxacin and pristinamycin. The absence of red colour on the relevant part of the strip provided an index of resistance or susceptibility to each antimicrobial agent.
Results: The positive rate of mycoplasma in genital tract inflammation was 70.9%, higher than 22.4% of asymptomatic cases, the difference was significant ($P<0.05$). Single Ureaplasma urealyticum (Uu) infections were high resistant to ciprofloxacin and ofloxacin by 71.1%, while susceptible to other seven antimicrobials (<10%). Resistance rates of the complex infections of Uu and Mycoplasma hominis (Mh) were high (>10%) to most drugs. Only susceptible to josamycin, doxycycline, pristinamycin, erythromycin (<10%).
Conclusion: It is important to perform mycoplasma detection for clinical diagnosis and reasonable use of antibiotics on patients with genital tract inflammations.

A-141

EBV Serology: new fully automated chemiluminescence assays

G. Vignati, B. Osnaghi, C. Giraldo, L. Motta, E. Pastori. *"G. Fornaroli" Hospital, Magenta (MI), Italy,*

Backgroung The Epstein-Barr virus (EBV) is a ubiquitous herpesvirus. It is recognized primarily for its etiological role in infectious mononucleosis (IM), but it is also involved in many diseases, and many forms of immunodeficiency. Diagnosis of IM is based upon clinical symptoms, in conjunction with haematological signs (pronounced lymphocytic response) and serological markers (presence of Heterophile antibodies and/or antibodies to EBV-specific proteins). Because mononucleosis can be confused with other infectious diseases (e.g. CMV, *Toxoplasma gondii*, hepatitis viruses and HIV), with similar clinical symptoms, the serological detection of antibody reactivity for various EBV antigens, including viral capside antigen (VCA) of both IgG and IgM classes, the early antigens (EA), and members of the Epstein-Barr virus nuclear antigen (EBNA) family, is fundamental for a correct diagnosis of EBV infection.

Methods New chemiluminescence EBV assays on Immulite XPi (Siemens Healthcare Diagnostics, Milan) have been evaluated using 123 routine non-selected sera and results have been compared to the method currently used in our laboratory (Liaison, DiaSorin, Saluggia). All discordant results have been tested in duplicate and then confirmed with western blot testing (EBV IgM/IgG; Alifax, Milan).

Results and conclusions All results are illustrated in table 1. Both relative (Immulite XPi *vs* Liaison) and absolute (Immulite XPi and Liaison *vs* Immunoblotting) sensitivity and specificity have been calculated for each parameter under analysis. The two methods showed an agreement of 83%, when considering all the markers, clinical data and results of the confirmatory tests. VCA IgG assays showed major differences, mainly due to the fact that different proteins are used as the solid phase (p18 synthetic peptide and gp125 for Liaison and Immulite respectively). Antibodies against gp125 are produced early during primary infections, while antibodies against p18 are produced later, which could explain the differences.

Table 1 - Summary (comparison data between Immulite XPi vs Liaison)

Marker	Samples tested			Immulite Xpi				Liaison	
	Total	Concordant sera	Discordant sera	sensitivity		specificity		sensitivity	specificity
	n	n (%)	n (%)	relative	absolute	relative	absolute	absolute	absolute
VCA IgM	**123**	**103 (83.7%)**	**20 (16.3%)**	**78.0%**	**95.0%**	**93.3%**	**93.0%**	**94.0%**	**96.0%**
negative		84							
equivocal		1							
positive		18							
VCA IgG	**122**	**106 (86.9%)**	**16 (13.1%)**	**93.0%**	**93.0%**	**58.0%**	**57.0%**	**100%**	**87.0%**
negative		7							
equivocal		0							
positive		99							
EBNA IgG	**121**	**116 (95.9%)**	**5 (4.1%)**	**99.0%**	**100%**	**92.0%**	**93.0%**	**100%**	**93.0%**
negative		23							
equivocal		0							
positive		93							

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Genetic study of two nucleotide variations within corresponding microRNAs and susceptibility to PTB in China

D. Li, T. Wang, X. Song, B. Yang, J. Zhang, J. Wang, c. Tao, B. Ying, L. Wang. *Division of Clinical Microbiology, Department of Laboratory Medicine, West China Hospital, ChengDu, China,*

Background MicroRNAs (miRNAs) are thought to play important roles in the pathogenesis of tumors or autoimmune diseases. Single nucleotide polymorphisms (SNPs) within miRNAs can change their characteristics via altering their target selection and/or expression, resulting in functional and/or phenotypic changes. We decided to investigate these variants in pulmonary tuberculosis (PTB) individuals, because such information is currently unavailable.

Methods In this study, two selected miRNAs SNPs (hsa-mir-146a rs2910164 G>C, and hsa-mir-499 rs3746444 T>C) were genotyped by PCR-RFLP: mismatch to demonstrate their association with susceptibility to PTB in 227 PTB cases and 483 normal controls. Some bioinformatics database has been searched to support the association between between miRNAs and the susceptibility to PTB.

Results There was no link between rs3746444 and PTB risk ($P > 0.05$),but subjects carring C allelincreased PTB risk, OR=1.483 (95%CI,1,094~2.010). A polymorphism (rs2910164 G>C) indicated an association with PTB risk in both genotype ($P = 0.000$) and allelic analysis ($P = 0.023$). Subjects carrying G allele of rs2910164 (C allele in miR-146a) were more susceptible to tuberculosis, with an odds ratio of 1.299 (95%CI, 1.037~1.1.727), especially those of homogenous GG genotype with an odds ratio of 1.72 (95%,1.043~2.837) when compared with individuals of CC genotype and adjusted for other factors,such as gender and nationality.

Conclusions This is the first study to suggest that common SNPs in miRNAs may contribute to the susceptibility of PTB, and further functional analysis of the SNP and its impact on mRNA targets is required to confirm the relationship between genotype and phenotype.

A-144

The prevalent situation and mechanism of aac(6′)-Ib-cr and qepA genes for plasmid-mediated quionlone resistance

H. Yu, C. Li, S. Hao. *Renmin Hospital of Wuhan University, Wuhan, China,*

Objective: To know the popular situation and resistance characteristic of plasmid mediated quinolone drug-resistant genes aac(6′)-Ib-cr and qepA genes in clinical common pathogens of Wuhan university hospital.

Methods: Susceptibility test to 18 kinds of antibiotics were tested by K-B in virto. aac(6′)-Ib-cr and qepA genes in quinolone drug-resistant pathogens were detected by PCR. MICs of Ciprofloxacin and Levofloxacin were determined by agar dilution technique in qepA positive strains. Use statistical methods to compare and analyse the data for understanding the drug resistance of positive strains. Use Plasmid Conjugation Experiments to discuss the genetic transmission of aac(6′)-Ib-cr and qepA.

Results: 213 isolates of the Clinical common pathogens were quinolone drug-resistant. Test the aac(6′)-Ib-cr and qepA genes of the 213 isolates by PCR amplification. The aac(6′)-Ib-cr and qepA genes were present in 17.4% and 2.3% respectively. All positive isolates were suspectible to imipeem and some to cefoperazone/sulbactam, but resistance to some other drugs. The paired t-test showed the MIC of ciprofloxacin is higher than levofloxacin for positive strains(The group of aac(6′)-Ib-cr positive: $t = 5.3481$, $P < 0.05$;The group of qepA positive:$t = 3.0186$, $P < 0.05$). The transfer of plasmid is successful for all of qepA positive strains and 7 of aac(6′)-Ib-cr positive strains from the random 10 aac(6′)-Ib-cr positive strains.

Conclusions: The prevalence of aac(6′)-Ib-cr and qepA genes is comfirmed in HuBei province and they are multiple drug-resistant. Positive strains is main mediated the resistance of hydrophilic quinolonewhich can spread thoug the way of horizontal transmission. So we should strengthen the monitoring of these genes in the future.

A-146

Clinical Evaluation of Rapid Antigen Tests for 2009 Pandemic Influenza A Virus

K. Tsao[1], S. Chao[2], T. Yang[3], C. Huang[1], Y. Huang[1], E. Chan[4], S. Shih[4].
[1]*Laboratory Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan,*
[2]*Laboratory Medicine, Chang Gung Memorial Hospital, Keelung, Taiwan,*
[3]*Laboratory Medicine, Chang Gung Memorial Hospital, Chiayi, Taiwan,*
[4]*Medical Biotechnology & Laboratory Science, Chang-Gung University, Taoyuan, Taiwan,*

Objective: A novel swine-origin influenza A H1N1 virus (2009 H1N1) was identified as the cause of the 2009 pandemic outbreaks of febrile respiratory tract infection. It is urgent to find the best rapid antigen test available for early detection of 2009 H1N1 virus. In this study, analytical sensitivity and clinical diagnostic efficiency of four commonly used rapid antigen tests for influenza virus were evaluated. These four tests include Medi-Pro cassette, QuickVue, Meridian and BinaxNOW.

Materials and results: To evaluate the analytical sensitivity of rapid antigen tests between different influenza virus subtypes, four reference isolates including three subtypes of influenza A (2009 H1N1, seasonal H1N1 and H3N2) and influenza B viruses were quantified first by real-time RT-PCR (rRT-PCR) and then assessed by different rapid antigen tests. The results showed that the sensitivity from different test kits vary significantly, ranging from the highest of $5x10^4$ RNA copies/*u*L for Medi-Pro cassette, $1x10^5$ for both QuickVue and Meridian, to the lowest of 2×10^6 for BinaxNOW. However, there is minimal difference in sensitivity for different virus subtypes from the same test kit. (e.g. Medi-Pro cassette had sensitivity of $5x10^4$, $6x10^4$ and $9x10^4$ for 2009 H1N1, seasonal H1N1 and H3N2 virus, respectively). This represents the fact that although these kits were initially designed for general flu detection, they can be also be used for 2009 H1N1 virus with similar sensitivity.

To verify the clinical diagnostic efficacy of rapid antigen tests, 218 respiratory tract specimens from patients with flu-like syndrome were collected in August 2009. These specimens were tested with the QuickVue kit and also compared to standard rRT-PCR assay of 2009 H1N1 virus. The positive rates were 26.1% and 63.7% respectively. Out of the 139 rRT-PCR positive specimens, only 41% showed positive by rapid antigen test. This study also indicates that the positive rate for rapid antigen test could reach 60% in

higher virus titer of positive rRT-PCR specimens with Ct value under 30. Furthermore, the specimens collected from children had higher positive rate (59.3%) comparing to adults (28.0%) among the 139 rRT-PCR positive cases. This reflected the fact that children's specimens typically contain higher viral load than adult. As we all know younger people are more susceptible to 2009 H1N1 virus; the rapid antigen test can really help in this populations group.
Conclusions: The study showed that out of the four rapid antigen test kits available today, the Medi-Pro cassette is most sensitive to all the influenza subtypes. Although the positive rate of the rapid antigen test is only about 40% and may produce false negative results, they can still reach 60% for patients with higher virus titer of 2009 H1N1 (Ct value under 30). This will provide valuable clinical importance to the timely treatment and management during a pandemic influenza A H1N1 virus outbreak. Younger population with higher viral load can benefit from these tests as they are usually affected by 2009 H1N1 virus more than other groups and the illness is typically severe.

A-147

Correlations between body composition and hormones in adolescents vertically-infected by HIV maintained on chronic highly active antiretroviral therapy

A. Schtscherbyna[1], C. H. Torres[2], L. M. C. Mendonça[3], C. Gouveia[4], E. S. Machado[4], S. V. Argolo[5], M. F. C. Pinheiro[6], M. L. F. FARIAS[7]. *[1]Serviço de Endocrinologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2]Serviço de Endocrinologia, Universidade Federal do Rio de Janeiro, Rio de janeiro, Brazil, [3]Serviço de Reumatologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [4]Serviço de Doenças Infecciosas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [5]Sergio Franco Medicina Diagnótica, Rio de Janeiro, Brazil, [6]Sergio Franco Medicina Diagnóstica, Rio de Janeiro, Brazil, [7]Serviço de Endocrinologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,*

Background: Both HIV infection and highly active antiretroviral therapy have been related to alterations in hormones and body composition in adults with AIDS but very little is known about HIV-infected adolescents.
Methods: We evaluated percent body fat and lean body mass by dual energy X-ray (DXA-Prodigy-GE) and biochemistry (Calcium, Phosphorus, 25-hydroxyvitamin D, Parathormone (PTH), Sex Hormone Binding Globulin (SHBG) and Cortisol in forty-seven HIV vertically-infected adolescents on HAART. Mann-Whitney test and Pearson's coefficient correlation were used (SPSS 13; $p<0.05$).
Results: Males and females did not differ in relation to age (17.4 ± 1.6 vs 17.4 ± 2 yrs), weight (55.9 ± 8.5 versus 51.4 ± 8 kg), BMI (20 ± 2 versus 21.3 ± 3.4 kg/m^2) but males were taller (1.67 ± 0.08 versus 1.55 ± 0.05 m), had more lean mass (46.7 ± 6.8 versus 33.6 ± 3.2 kg) and less % body fat (11.9 ± 4.3 versus 28.6 ± 7.5%). Eleven patients had severe vitamin D deficiency and some of them had secondary increase in PTH levels. Correlations were found between: weight and SHBG ($r = -0.38$, $p = 0.013$), height and SHBG ($r = -0.40$, $p = 0.009$), lean mass and SHBG ($r = -0.46$, $p = 0.002$), fat mass and cortisol ($r = 0.41$, $p = 0.029$).
Conclusions: Vitamin D deficiency might be related to the overall nutritional state of the patients. The increasing levels of androgens and muscle mass along puberty might explain the negative association of SHBG with height, weight and lean body mass. Both highly active antiretroviral therapy and hypercortisolism are related to alterations in body fat distribution, but Cortisol values were normal in all patients; thus, the association between this hormone and body fat seems unrelated to the disease and its treatment. We found a high prevalence of vitamin D deficiency in vertically HIV-infected adolescents on HAART. Cortisol was a positive influence in body fat in this population.

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Performance Evaluation of a Prototype Syphilis Assay Method on the ADVIA Centaur® System.

S. A. Patibandla, S. Shin, K. Yokoyama, S. Chowdhury, L. Baker. *Siemens Healthcare Diagnostics, Tarrytown, NY,*

Objective: Syphilis is a disease caused by the spirochete *Treponema pallidum* (TP). Serological diagnosis of syphilis is usually done by nontreponemal tests (Venereal Disease Research Laboratory [VDRL] test and rapid plasma reagin [RPR] test) and treponemal tests (hemagglutination for TP and fluorescent treponemal antibody absorption test), which are labor intensive and subjective. We report the evaluation of a fully automated syphilis assay* in a chemiluminescence immunoassay format on the ADVIA Centaur® system (Siemens Healthcare Diagnostics, Deerfield, IL, US). The assay uses recombinant TP antigens for the detection of TP IgG and IgM antibodies in human serum or plasma.
Methods: The diagnostic sensitivity of the antigens was evaluated with 814 samples, obtained from multiple vendors, that were positive by Abbott Architect Syphilis TP* (Abbott Park, IL, US), EIA, VDRL or RPR assays and that included IgG and IgM samples. Specificity was determined by testing 2738 normal/paid blood donor samples. Mixed-titer panels from commercial vendors were also tested. Samples from ANA, SLE, CMV, EBV, HBV, HCV, HIV, Lyme and RF were tested for cross-reactivity. The results are reported in index values and as reactive (index ≥ 1.1), equivocal (index 0.9-1.1), or nonreactive (index < 0.9). Discordant samples were tested by either the IMMULITE® 2500 Syphilis screen or the ICE* Syphilis assay (Abbott Murex, Dartford, UK).
Results: All the positive samples showed reactivity by the ADVIA Centaur Syphilis assay, resulting in 100% (814/814) sensitivity. The assay was reactive to all positive samples in the Zeptometrix mixed-titer panel and the BBI mixed-titer performance panel. Specificity determined by testing normal donor samples was 99.8% (2732/2738). Six false-positive samples were negative by the reference assays. Additionally, 198 samples from other patient populations, including pregnancy samples, dialysis patient samples, and pediatric samples, were tested. Thirteen of these samples were positive by the ADVIA Centaur assay and the reference assays. One sample was equivocal by the ADVIA Centaur assay and negative by the reference assays. The ADVIA Centaur Syphilis assay had a total %CV of less than 6.5% over the assay range in a 20-day study. The assay was evaluated for potential cross-reactivity with other viral infections and disease state specimens, and no change in clinical interpretation was observed.
Conclusion: The results of this study show that the prototype ADVIA Centaur Syphilis assay is a reliable and accurate, fully automated method to screen for the presence of TP antibodies in human serum or plasma.
* Not released for sale in the US.

Keywords: Syphilis, *Treponema pallidum*, Centaur

A-149

Performance Evaluation of the Elecsys® Anti-HCV Assay on the Roche Cobas and Modular Platforms

S. E. Melanson[1], M. J. Conrad[1], D. Sun[2], R. Ostlund[3], G. Shore[4], P. Jarolim[1]. *[1]Brigham and Womens Hospital, Boston, MA, [2]South Bend Medical Foundation, South Bend, IN, [3]Washington University School of Medicine, St. Louis, MO, [4]Unilab of Dade, Ft. Lauderdale, FL,*

Background: The prevalence of the hepatitis C virus (HCV) is increasing in many parts of the world. HCV can cause acute and chronic hepatitis, cirrhosis and extrahepatic complications; all of which contribute to morbidity and mortality. The objective of this study was to demonstrate the safety and effectiveness of the Elecsys® Anti-HCV immunoassay.
Methods: The protocol included testing of 2,096 prospectively and retrospectively collected specimens from 813 symptomatic and 1,283 high risk asymptomatic subjects. Five U.S. sites participated in the study performed on the cobas® e 601 and e 411 analyzers and on the MODULAR ANALYTICS E170 analyzer. Reference and supplemental testing was performed using the Vitros Anti-HCV assay on the Vitros ECi Immunodiagnostic System (Ortho Clinical Diagnostics), Chiron RIBA HCV 3.0 SIA (RIBA), and Cobas Amplicor Hepatitis C Virus Test, Version 2.0 (HCV-RNA). Potential co-infection by hepatitis A and B viruses were evaluated using the Ortho ECi HBsAg and the Siemens ADVIA Centaur Anti HAV IgM assays. The Elecsys Anti-HCV assay provides qualitative detection of IgG and IgM antibodies to the Hepatitis C virus. It is a two-step double antigen sandwich assay, allowing the presence of antigen to be measured by electro-chemiluminescence. Results are reported as reactive, non-reactive and borderline. Using human serum pools and controls, within run precision and day to day reproducibility over five days was performed with multiple reagent lots. The seroconversion sensitivity of the Elecsys assay was determined using twenty commercially available panels.
Results: The results are listed in the sequence: e411, (c601, E170). When results were identical for all three analyzers, only one number is shown. Within the site reproducibility varied from 4 to 25% (4-22%, 2-21%) and between site reproducibility was less than 19% (16%, 16%), with the majority of the variance arising from the between-lot component. Comparison of the Elecsys and Vitros assays with supplemental RIBA and HCV-RNA for the overall clinical population yielded 99.35% (99.57%, 99.57%) positive and 97.24% (97.12%, 96.88%) negative agreement. The prospectively collected population was tested for co-infection of hepatitis A and B. Fifty samples were infected with HBV and twelve samples had evidence of HCV-HBV co-infection. Positive agreement between Elecsys and Vitros was 100% (12/12), while the negative agreement between the same assays was 88.37% (38/43). In the retrospectively collected population with potentially interfering disease states, concordant results were obtained in 96.11% (96.50%, 96.92%) specimens. Of the 11 (10, 9) discrepant samples, 6 (6, 5) were in agreement with the Elecsys interpretation, three were in agreement with Vitros interpretation and two provided an indeterminate RIBA result. Ten of the seroconversion panels had equivalent detection between the Elecsys and Vitros assays, six series showed earlier conversion in Elecsys, two series showed earlier conversion in Vitros, and two series were non-converting.
Conclusions: Accurate diagnosis of HCV is important to decrease morbidity and

mortality. The Elecsys Roche Anti-HCV assay has excellent performance on the cobas and MODULAR platforms in high risk and symptomatic patients, patients with interfering disease states, patients with concomitant infections and early converters.

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A Multicenter Evaluation of the Analytical and Clinical Performance of the ADVIA Centaur® aHBe and HBeAg Assays

P. W. Dillon[1], C. Saxton[1], E. Schiff[2], M. D. de Medina[2], E. Chapman-Montgomery[1]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]University of Miami Center for Liver Diseases, Miami, FL,*

Background: New assays for HBe antigen (HBeAg) and antibodies to it (aHBe) have been developed for the Siemens ADVIA Centaur® Immunoassay System.* With the other ADVIA Centaur hepatitis assays (aHAVM, HAVT, aHBcM, HBcT, aHBs, HBs, HBs Confirmatory, and aHCV), the ADVIA Centaur system now supports a wider menu of hepatitis immunoassays.

Objective: To evaluate the clinical performance and precision of the ADVIA Centaur aHBe and HBeAg assays in clinical laboratories.

Methods and Materials: A total of 1708 patient samples (935 from high-risk individuals, 668 from individuals with signs and symptoms, 105 from dialysis patients) were collected prospectively at three sites: the University of Miami, the University of Texas Southwestern Medical Center, and BioCollection Worldwide, Inc. Prospective sample HBV classification (e.g., acute, chronic, etc.) was done serologically. One hundred twenty-five retrospective (acute or chronic) samples were purchased from commercial vendors. ADVIA Centaur aHBe and both DiaSorin (DiaSorin s.r.l., Saluggia, Italy; DiaSorin Inc., Stillwater, MN) methods have equivocal zones. A sample initially recovering in this zone is reassayed in duplicate. It is positive if both repeats are positive, and it is negative, if both repeats are negative; otherwise, it remains equivocal. ADVIA Centaur HBeAg has a retest zone. Such samples are repeated in duplicate, but all three results are tested against a single cutoff using a 2-out-of-3 rule. All were assayed by the ADVIA Centaur and DiaSorin methods at either of two US clinical laboratories. 3x3 and 2x3 tables were prepared to compare ADVIA Centaur aHBe and HBeAg results to DiaSorin results. Positive and negative agreements for both methods (DiaSorin equivocals not considered) and, for aHBe, overall (including equivocals on both) agreement were calculated. Calculations were done over all sites and study populations but also stratified by site, population and disease classification. Precision was evaluated using negative and positive controls, low and high calibrators run as unknowns, and sample pools (4 for aHBe, 5 for HBeAg) following a CLSI EP-5A-like protocol at both patient sample testing sites and in Siemens' Tarrytown laboratory using three reagent lots at each site. Hierarchical precision analyses (replicates in runs, runs in days) were done for each site and lot, and across all three sites for each lot and across all sites and lots. Separate analyses were done for each product.

Results: Over all sites, populations and classifications, these agreements (95% confidence intervals) between the ADVIA Centaur and DiaSorin methods were observed:
aHBe: Positive 95.9(93.3-97.8)%, Negative 92.7(91.3-94.0)%, Overall 92.7(91.4-93.8)%
HBeAg: Positive 94.8(89.0-98.1)%, Negative 96.4(95.4-97.2)%.
Excluding the low index negative control, negative pool, and low calibrator, the within-run, and within-site-and-lot CV imprecisions pooled across sites were 1.6%-9.0% and 1.8%-22.0% for aHBe, and 1.3%-13.1% and 1.7%-23.3% for HBeAg.

Conclusions: The ADVIA Centaur aHBe and HBeAg assays perform well both clinically and analytically. Their availability means that a broader suite of hepatitis serological assays is supported on the ADVIA Centaur Immunoassay System.

* The ADVIA Centaur aHBe and HBeAg assays are not available in the US.

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Procalcitonin as a predictor of blood culture results in critically ill patients

S. Ng[1], J. D. Faix[1], P. W. Park[2]. *[1]Stanford University, Stanford, CA, [2]Gachon University Gil Hospital, Inchon City, Korea, Republic of,*

Background: Procalcitonin (PCT) is part of the abnormal systemic immune response to infection that leads to sepsis. Over the past decade, numerous studies have investigated the diagnostic accuracy of PCT compared with other inflammatory markers, especially c-reactive protein (CRP). PCT has been shown to be useful in identifying patients who are at risk of developing sepsis. There has also been great interest in using PCT levels to help decide whether to start or discontinue antibiotic therapy in patients with known or suspected infection. Blood cultures are frequently drawn in patients suspected of systemic infection, but the results may be delayed. We sought to investigate whether PCT results, which may now be obtained quickly in the hospital laboratory, could predict blood culture results.

Methods: The VIDAS Brahms PCT assay (bioMerieux, Inc; Durham, NC) is an automated non-competitive immunoassay using solid-phase mouse monoclonal antibody and alkaline phosphatase-labeled mouse monoclonal antibody to capture PCT, and 4-methyl-umbelliferyl phosphate as substrate for fluorescent detection. The assay was linear (R-squared = 0.99) over the measurable range of 0.1 - 20 ng/ml. Intra-assay precision (% cv, n=10) was 2.4 and 2.7 and inter-assay precision (% cv, n=5) was 3.1 and 3.6 at 1.5 and 15 ng/ml, respectively. According to the manufacturer, PCT levels <0.2 ng/ml make systemic infection very unlikely, while at PCT levels >10 ng/ml, it is very likely. During a one-month period, we retrieved the heparinized plasma specimens drawn simultaneously with initial blood cultures from patients being admitted to the medical intensive care unit. These specimens (n=14) were frozen and, following the collection period, thawed and assayed for PCT. The PCT results were compared with the results of the blood cultures. This study was approved by the hospital's Human Subjects Research review board.

Results: Of the 14 patients whose PCT level could have been known at the time that the initial blood cultures were drawn, 5 had positive blood cultures (either initially or within the next four days) while 9 had negative blood cultures. All of the PCT results for the patients with positive blood cultures were >6 ng/ml (mean, 10.6; s.d. 8.1; range 6.2-22.7) with the exception of one patient (Candida fungemia). All of the PCT results for the patients with negative blood cultures were <3 ng/ml (mean, 1.4; s.d. 1.2, range, 0.1-2.7) with the exception of one patient (extensive cranial surgery the day before admission to the intensive care unit).

Conclusion: The results of this pilot study indicate that a cut-off for PCT between 3-6 ng/ml may help to identify patients whose blood cultures will be positive. This is significantly higher than previously reported (Chirouze C et al, *Clin Inf Dis* 2002; *35*:156-161), perhaps reflecting our focus on critically ill patients rather than patients admitted to hospital with acute fever. False negative results (in patients with non-bacterial sepsis) and false positive results (in the absence of septicemia in patients with recent trauma or surgery) will need to be addressed.

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Serological HBeAg Quantification in patients with HBV Infection Using Paul-Ehrlich Standards

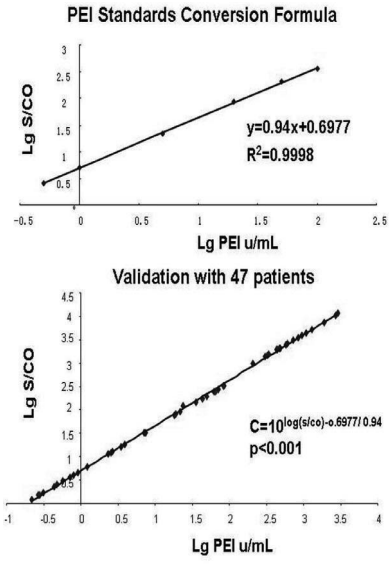
J. Xu[1], H. Ji[1], L. Huang[1], G. Qian[2], Z. Lu[1]. *[1]No.3 People's Hospital Affiliated to ShangHai Jiao Tong University School of Medicine, ShangHai, China, [2]Abbott Diagnostics Division, China, Shanghai, China,*

Objective: HBeAg quantification is indicated as important predicting marker for antiviral therapy and clinical outcome. By using Paul-Ehrlich international (PEI) reference standard, we studied the feasibility to adopt quantitative measurement on Architect platform for clinical application.

Methods: Standards and serum samples were measured in signal/cutoff (S/CO) value on Architect platform. Linear range and quantitative measurement were defined by a panel of serial dilutions of PEI standards and corresponding linear regression.

Results: The assay was validated using a cohort of 47 HBeAg positive patients. It showed excellent correlation between LogPEI and Log S/CO ($y=0.94x+0.6977$, $r^2=0.9998$, $p<0.0001$). The linear range was 0.5-100 PEI unit/mL and 1-350 for S/CO. S/CO could be converted to PEI unit using formula $C=10^{\log(s/co)-o.6977/\ 0.94}$ PEI U/ml. Samples with S/CO>350 were diluted in fetal calf serum at 1:50.

Conclusion: Qualitative HBeAg measurements offer wide linear range and close correlation to PEI standards at different concentrations. The S/CO value can be converted to quantitation results in PEI units on Architect platform. It could be widely used in clinical therapeutic monitoring and outcome prediction.



Figure

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Detection and identification of seven pathogenic vibrios in a single real-time PCR

J. Zhang[1], Q. Huang[1], J. Niu[2], Q. LI[1]. *[1]XMU, Xiamen, China, [2]Centre for Diseases Prevention and Control, Xiamen, China,*

Background Food consumption-associated *Vibrio* infections are becoming increasingly common in the world. We present a new real-time PCR platform for rapid and high throughput detection of pathogenic vibrios.

Method: The detection scheme was based on the Homo-Tag Assisted Non-Dimer (HAND) system and Multicolor Combinational Probe Coding (MCPC) technology. In HAND system, tagged primers were used to target rfbN for *Vibrio cholerae* O1, wbfR for *V. cholerae* O139, collagenase gene for *V. alginolyticus*, vvh (*V. vulnificus* specific hemolysin gene) for *V. vulnificus*, tdh (thermostable direct haemolysin) for pathogenic *V. parahaemolyticus*, toxR for total *V. parahaemolyticus*, *V. fluvialis* and *V. mimicus*, respectively. In the assay, modified molecular beacon(s) with same sequence but labeled with one or two fluorophore(s) hybridized to the specific amplicon, generating a characteristic fluorescence combination profile.

Results: The new platform allowed identifying a specific target from a broad spectrum of suspected pathogens. The detection limits for these vibrios ranged from 2 copies to 100 copies of genomic DNA per reaction. The specificity assay with 145 strains demonstrated 100% accuracy. The assay showed quantitative ability within a wide range (1pg to 100ng DNA per reaction for *V. fluvialis*, 100fg to 100ng DNA per reaction for the rest).

Conclusion: The assay shows great potential in identifying a pathogen from a number of suspected objects.

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Validation of the ADVIA Centaur® B•R•A•H•M•S PCT Immunoassay for the Risk Assessment of Critically Ill Patients in the ICU for Progression to Severe Sepsis and Septic Shock

E. H. Gluck[1], M. Levy[2], R. H. Christenson[3], F. S. Apple[4], A. H. Wu[5], S. Dawson[1], B. Plouffe[6], J. Freeman[6], J. DiCanzio[6], G. S. Martin[7], M. D. Siegel[8], A. P. Wheeler[9]. *[1]Swedish Covenant Hospital, Chicago, IL, [2]Rhode Island Hospital and Brown University Medical School, Providence, RI, [3]University of Maryland School of Medicine, Baltimore, MD, [4]Hennepin County Medical Center and University of Minnesota, Minneapolis, MN, [5]University of California, San Francisco, CA, [6]Siemens Healthcare Diagnostics Inc., Tarrytown, NY, [7]Emory University, Atlanta, GA, [8]Yale School of Medicine, New Haven, CT, [9]Vanderbilt University School of Medicine, Nashville, TN,*

Objective: To validate the performance of the ADVIA Centaur B•R•A•H•M•S PCT immunoassay* (Siemens Healthcare Diagnostics, Deerfield, IL, US), an assay intended for the quantitative measurement of PCT in human serum or plasma (lithium-heparinized, sodium-heparinized, or EDTA).

Relevance: Severe sepsis and septic shock are among the leading causes of death in critically ill patients in the intensive care unit (ICU). Procalcitonin (PCT) is an emerging biomarker of sepsis proposed as an aid in the diagnosis and prognosis of severe bacterial infection. The blood concentration of PCT rises rapidly and significantly in response to a systemic bacterial challenge. Integrating PCT into the diagnosis and management of septic patients may shorten the time to detection and increase the accuracy of diagnosis of severe sepsis, allowing earlier and more effective treatment of this condition.

Method: The ADVIA Centaur B•R•A•H•M•S PCT assay is a one-pass, 18-minute antibody sandwich immunoassay with a solid phase containing monoclonal antibody to fluorescein covalently linked to paramagnetic particles. The ancillary reagent contains two antibodies to procalcitonin labeled with fluorescein. The Lite reagent contains a monoclonal antibody to procalcitonin labeled with acridinium ester. The assay requires a sample volume of 100 µL. A direct relationship exists between the amount of PCT present in the patient sample and the amount of relative light units (RLUs) detected by the system. The assay dynamic range measures PCT concentrations between 0.02 and 75 ng/mL. External validation studies were performed at three clinical sites and included assessments of reproducibility and method comparison. Assay reproducibility was assessed in accordance with CLSI protocol EP5-A2.The reproducibility study was performed over 10 days using six pools with PCT values distributed across the range. The method comparison study evaluated the correlation of the assay with the B•R•A•H•M•S PCT sensitive KRYPTOR® assay, using 218 ICU patient samples with PCT concentrations ranging from 0.02 to 50 ng/mL, and obtained from six collection sites. In addition, internal validation studies were conducted at Siemens and included the assessment of functional sensitivity, limit of detection (LoD), analytical sensitivity, interference, cross-reactivity, dilution linearity, and range of expected values in apparently healthy subjects.

Results: All within-run, between-run, between-site, and total imprecision estimates were no greater than 5.9%, 3.6%, 6.1%, and 9.1%, respectively. Correlation evaluated by Passing-Bablok regression analysis showed a slope of 0.84 and an intercept of 0.002. The Pearson correlation coefficient (r) was 0.97. Internal validation studies demonstrated a minimum detectable concentration (zero standard + 2 SD) of 0.01 ng/mL and a functional sensitivity concentration of ≤0.04 ng/mL at a total CV of 20%. The dilution linearity study using 10 patient samples with PCT concentrations across the range demonstrated a mean recovery of 101% (range 89% to 116%). **Conclusion:** The ADVIA Centaur B•R•A•H•M•S PCT assay shows acceptable sensitivity and precision for the quantitative determination of PCT.

*This assay has not been cleared by the FDA and is not available for sale in the US. This assay is CE marked.

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Increased Prevalence of Gram-Negative Bacteria in Blood Cultures in Long-Term Care Facilities

R. Khoury, S. Gibbs, B. P. Salmon, A. Gandhi, P. Gudaitis, D. Gudaitis. *Aculabs, Inc., East Brunswick, NJ,*

Background: Bloodstream infection is one of the major causes for morbidity and mortality in the United States. The rate for hospitalization due to septicemia has doubled in the elderly population in recent years and it is estimated that every minute more than 2 people in the US die from severe sepsis. Rapid detection and identification is essential, but the treatment of such infection is complicated by the presence of antibiotic resistant organisms, which are on the rise in Long-Term Care Facilities (LTCF), specially the gram-negative resistant organism which responsible for the most serious nasocomial infections.

Design: 1170, 1048 and 1471 sets of blood cultures collected from residents in Long-Term Care Facilities during 3rd quarter 2007, 2008 and 2009 respectively. Every set included two vials (aerobic and anaerobic) which were incubated in a Bactec instrument. Positive cultures were subcultured and then identified using Microscan96 Walkaway conventional panels. They were segregated further by organism isolates.

Results: 11.2%, 9.1% and 14.1% of the cultures were positive for 2007, 2008 and 2009 respectively. Drug resistant organism accounted for 19.8% (2007), 18.9% (2008) and 25.1% (2009) of the total positive cultures. The most common drug resistant organism for gram positive isolates was MRSA, and Klebsiella Pneumonia for gram negative.

	2007	2008	2009
Total blood culture	1170	1048	1471
% positive culture	11.2%	9.1%	14.1%
% gram positive/total positive	77.9%	62.1%	73.4%
% Drug resistant/total positive culture	19.8%	18.9%	25.1%
% gram positive Drug resistant/total drug resistant culture	92.3%	44.4%	48.1%
% gram negative Drug resistant/ total drug resistant culture	7.7%	55.6%	51.9%

Conclusions: Although gram positive bacteria remain the most common organism

identified in positive blood cultures, multidrug resistant gram negative organisms are increasing in LTCF and account for more than half of the total drug resistant organism in 2008 and 2009. This increase raises concern about the therapeutic options available, the need for ongoing surveillance for antibiotic susceptibility, and the need for infection control practices to identify and limit the spread of resistant organisms among LTCF residents.

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Prevalence of serologic markers of bloodborne viral infections among health care workers in Mongolia

B. -. Tsetsegsaikhan[1], T. Khosbayar[2], M. Purevdorj[2], T. Gerelmaa[2], E. Azzaya[2], B. Suvd[2], W. Choi[2], J. Sarantuya[2], N. Bira[2], S. Enkhtsetseg[3].
[1]Ministry of Health, Ulaanbaatar, Mongolia, [2]Health Science University of Mongolia, Ulaanbaatar, Mongolia, [3]World Health Organization, Ulaanbaatar, Mongolia,

Health-care workers are at increased risk of infection due to occupational exposure to blood and other body fluids, contaminated sharps etc. However Mongolia is a highly endemic country of bloodborne viral hepatitis (HBV, HCV and HDV) infections, there is a limited data on sharps injury burden among HCWs in Mongolia.
The present study was designed to: (a) to evaluate the prevalence of serological markers of hepatitis B, C, D and HIV in HCWs; (b) to compare the prevalence of these markers between the physician, nurses and housekeeping workers; (c) to identify risk factors for the HBV and HCV in this population; and (d) to correlate the presence of bloodborne viral infection and sharps injury.
A total of 354 HCWs from primary, secondary and tertiary level hospitals were enrolled in this study and the number of the physicians, nurses and housekeeping workers were 94, 183 and 77, respectively. A total of 7 serological markers (anti-HBc, anti-HBs, HBsAg, HBeAg, anti-HDV, anti-HBC, anti-HIV1/2) were determined by ELISA.
Anti-HBc, anti-HBs, HBsAg and HBeAg seropositivity were determined 83.3%, 36.4%, 9.6%, 0.3%, respectively. Sera of 24 out of the 354 health care workers (6.8%) were positive for anti-HDV. The prevalence of anti-HCV was 20.1% (71/354) and none of the HCWs were determined anti-HIV1/2. 12.7%, 35.1%, and 85.1% of physicians, 9.29%, 38.8%, and 85.8% of nurses and 6.49%, 32.5% and 75.3% of the housekeeping workers with sharps injury risk were seropositive for HBsAg, anti-HBs and anti-HBC, respectively. A total 86.8% of workers were injured by sharps, and after the injury 28.6% of physicians, 26.7% of nurses, 30.2% of housekeeping workers were informed about their injury state. The average frequency of the sharps injury was determined 3.9 per year and HCWs with sharps injury history showed significant high prevalence of serologic markers of bloodborne viral infection when compared with HCWs without history of injury.
This study demonstrates that the high prevalence of bloodborne pathogen infections was determined among HCWs in Mongolia. On the other hand, the frequency sharps injury in the HCWs, is relatively high and this condition is more serious in nurses than physician and housekeeping workers. Finally, it is very important, especially for health providers and policy makers, to recognize the burden of the sharps injury among HCWs and implement effective preventive programs.

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Evaluation of the Human Immunodeficiency Virus Type 2 in Brazilian Samples Presenting HIV-2 gp36 Reactivity

I. Bendet[1], K. Otsuki[2], R. F. M. Souza[1], D. G. Amaral[1], A. C. P. Vicente[2].
[1]Sérgio Franco Medicina Diagnóstica, Rio de Janeiro, Brazil, [2]Instituto Oswaldo Cruz, Rio de Janeiro, Brazil,

Background: Human immunodeficiency virus (HIV) antibody tests have been developed to identify the two virus types HIV-1 and HIV-2. In Brazil the sequential algorithm to HIV diagnosis determines screened for HIV by using enzyme immunoassay (EIA) followed by Western blot (WB) or immunofluorescence as a confirmatory test. From November 2008 to May 2009, 15 samples from patients submitted to HIV testing presented in HIV blot 2.2 (MP Diagnostics) all major diagnostic HIV-1 bands plus the gp36 HIV-2 band. The objective of this study was to investigate the possibility of co-infection HIV-1 and HIV-2 in those Brazilian samples presenting HIV-2 gp36 reactivity.
Methods: The samples were submitted to HIV-2 specific WB: HIV-2 Western blot 1.2 (MP Diagnostics) and INNO-LIA HIV I/II Score (INNOGENETICS); the rapid tests: HIV 1&2 BiSpot ImmunoComb II (Orgenics) and GENIE II (BIO-RAD). HIV-1 and HIV-2 in house PCRs targeting protease, envelope, gag genes and LTR region were performed.
Results: All 15 samples were positive to gp80 and ten were also positive to gp36 HIV-2 envelope proteins in HIV-2 W. blot 1.2. Using INNO-LIA HIV I/II Score nine samples presented the gp36 HIV-2 band reactivity. Seven samples were positive to gp36 HIV-2 on both WB confirmatory tests. All samples were confirmed to be HIV-1 infected in the WB as well in the rapid test. However all of them were not reactive to HIV-2 in both rapid tests.
Concerning the identification of HIV-1 and 2 genome by PCR we had positive results to HIV-1 in all samples and to HIV-2 we get protease gene amplification in eight samples, one of them have also LTR amplification. All amplicons were sequenced confirming the identity with HIV-1 and HIV-2.
Conclusions: Since the beginning of HIV epidemic/pandemic in the 1980s, only one report showing the presence of HIV-2, confirmed by molecular biology (amplification and sequencing of HIV-2 protease gene), demonstrated the presence of this virus in Brazil. Here, we analyzed samples with serological evidences of HIV-2. Applying a set of specific serological tests to HIV-2 we failed to obtain a complete pattern of bands to confirm, by serological means, the HIV-2 infection. However, we succeeded to show the presence of HIV-2 genes (protease and/or LTR) in eight samples that were also positive to HIV-1. The lack of a total agreement with the serology and the molecular assay can reflect the sensitivity and specificity presented by different reagents to HIV-2 and the level of antibodies to both virus, HIV-1 and HIV-2, in co-infections. Therefore, we demonstrated the presence of HIV-1/HIV-2 co-infection in Brazilian samples presenting HIV-2 gp36 reactivity.

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Evaluation, Validation and Clinical Appraisal in ICU Patients with Suspected Sepsis: a Comparison of Two Procalcitonin Assays

J. Penman, J. Galbraith, G. Hoag. *Vancouver Island Health Authority, Victoria, BC, Canada,*

BACKGROUND: Procalcitonin (PCT) is the peptide precursor of calcitonin, secreted by the medullary cells of the thyroid. The pro-molecule is secreted by cells throughout the body in the course of most bacterial and some fungal infections. PCT may assist in distinguishing sepsis from other causes of systemic inflammatory reaction syndrome (SIRS). PCT has a very high negative predictive value for sepsis. There have been several publications on the value of PCT to assist in the early detection or exclusion of sepsis, monitoring of patient progress, predicting prognosis, and optimizing antimicrobial management.
OBJECTIVE: To evaluate and validate both Roche and BioMerieux platforms for a PCT (Brahms calibrated) assay. Secondly, to clinically assess in patients with, and without, suspected sepsis the value of PCT testing in the intensive care unit (ICU) with the intent to develop a clinical algorithm.
METHODS: The test systems (Roche e601:BioMerieux VIDAS) were compared and validated, and serial samples collected over 72h from 24 ICU patients were clinically appraised. The PCT assays were both calibrated to a reference standard. The results obtained for PCT were correlated with each other and to C-Reactive Protein (CRP). These were assessed relative to the patients' clinical course. A clinical utilization algorithm was developed based on a protocol reported in the literature and adapted to our conditions in discussion with the ICU, emergency room (ER) and other stakeholders.
RESULTS: Both the Roche and BioMerieux methods demonstrated acceptable precision (<10%) and their results correlated well (r=0.99). The Roche method results were 20 to 30% lower than the corresponding BioMerieux results across the assay range. The PCT results did not show a statistically meaningful correlation with the corresponding CRP results. A review in the ICU patients with, and without, suspected sepsis confirmed clinical utility. Both sets of PCT results classified the patients identically or within one sepsis-category of each other, and both paralleled the clinical course of the patients. CRP values were less sensitive to clinical changes and some moved contrary to the clinical change. A clinical-utilization algorithm was agreed to by physicians in the ICU and ER and implemented as the standard of care.
CONCLUSIONS: The Roche and BioMerieux methods showed acceptable precision across all assay levels and excellent correlation. The two PCT methods showed equal ability to classify patients and generally changed in parallel with clinical status. The clinical utility was confirmed in an ICU setting and a clinical algorithm was developed for management of patients with suspected sepsis and implemented as a standard of care in the ICU and ER. The potential utility for PCT includes early detection of the onset of sepsis; avoidance of antibiotic treatment in patients without PCT evidence of sepsis/infection; the monitoring and management of sepsis patients; and the possibility of earlier discontinuation of antibiotic treatment.

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Clinical feasibility of a rapid, prescreen PCR test for candidate MRSA colonized patients using an *Staphylococcus aureus* (SA) real-time PCR assay

S. M. O'Hara, B. I. Sodowich, D. R. Zweitzig, N. M. Riccardello. *Zeus Scientific Inc, Raritan, NJ,*

Objective: To test the feasibility of a nasal swab *Staphylococcus aureus* (SA) PCR prescreen assay to accurately identify MRSA candidate carriers. The cost of MRSA

colonization testing is a significant hospital expense. Since the majority of tests will be MRSA negative due to a historical MRSA/SA prevalence range of 20-30% for SA, it might be more prudent and cost effective to prescreen for MRSA candidates using a rapid low cost SA-PCR test prior to the more expensive MRSA PCR confirmatory test. This strategy should significantly reduce total MRSA testing costs and only add one hour of addition time to obtain the final MRSA result.
Method: 104 ICU nasal swab specimens were tested analyzing for agreement between the BD GeneOhm™ MRSA IVD PCR Assay and a new internally controlled (IC) SA real-time PCR assay. The ICU nasal swabs were eluted, bead mill lysed, and stored frozen prior to testing in both PCR assays on a SMART cycler. The BD GeneOhm MRSA test was performed according to the manufacturer's instructions. The SA real-time PCR test is a TaqMan based FAM labeled probe targeting a 160bp amplicon of the S*taphylococcus aureus* specific *nuc* gene amplified in combination with an internal control and IC-probe labeled with LC610.
Results: **Sensitivity**: Using the SA PCR amplicon we input 20 copies into 80 separate PCR reaction tubes in parallel on a 96 well plate and 80/80 were detected as positive. **Specificity:** 14 different SA strains (3 MSSA, 11 MRSA) all tested positive down to at least 10 genome equivalents. All non SA species tested to date, including human, were negative including, 9 closely related coagulase negative staph (CONS) species and 9 non-*Staphylococcal* species when tested with an input of 10,000 genome mass equivalents. All specificity results were confirmed by gel analysis.
Clinical data: All SMART cycler runs included positive and negative controls which were scored as valid. The BD GeneOhm MRSA assay showed 3/104 (3%) unresolved due to IC failure, the SA-PCR assay showed 1/104 (1%) unresolved due to IC failure. Of the 104 nasal swab specimens obtained from ICU, 23 (22%) were detected as positive by the BD GeneOhm MRSA PCR test. We found these same 23 (22%) MRSA positive specimens to be in 100% complete agreement with the corresponding SA real-time PCR test results. The remaining 81 samples were scored negative in the BD assay and for the new SA assay scored 62 (60%) negative and 19 additional SA positives. The SA prevalence in this ICU population was 42/104 (40%).
Conclusions: 60% of these ICU nasal swabs did not need to be tested by expensive MRSA PCR tests. Using hospital populations outside of ICU where the SA prevalence drops to 20-30% the unnecessary MRSA testing increases to 70-80%. These data support the opportunity to develop a more cost effective MRSA screening strategy, based on MRSA/SA prevalence ranges by employing an upfront lower cost SA PCR assay as a pre-screen for any downstream MRSA-PCR confirmatory test such as BD MRSA and Cepheid Xpert MRSA.

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Detection of High Risk HPV in urine of high and low risk populations in India

D. J. Robbins[1], D. Garg[2], J. Li[3]. *[1]TrovaGene Inc., San Diego, CA, [2]Immucor Inc., Warren, NJ, [3]Ambion Inc., Austin, TX,*

Background: Infection with High Risk human papillomavirus (HPV) is involved in 90-100% of cervical cancers. HPV detection in cervical cells, used as a supporting test for Pap smear positive patients, are much more sensitive than cytology but less specific for ongoing cervical cancer. Cervical cancer is one of the most common female tumors in countries lacking a screening program. Barriers include cultural and logistical problems with collecting cervical cells. Past attempts to alleviate these issues with testing of urine DNA had poor sensitivity, possibly due to inappropriate DNA isolation methods.
Objectives: In this study we investigate the feasibility of a urine-based HPV DNA test. We test an HPV genome region in which the sequence appears to distinguish High Risk types, potentially eliminating the need for manufacturing and operating a multiplexed assay.
Methods: Matching urine and cervical samples were collected in India from 270 High Risk subjects from STD clinics or district brothels, and 50 Low Risk subjects with no known predisposition to disease. The urine-based HPV DNA test involves isolation of DNA from urine and specific PCR amplification of the HPV E1 region to detect the presence of High Risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Results were compared to the digene ® HC2 High-Risk HPV DNA Test (QIAGEN). Discordant samples were further analyzed by DNA sequencing.
Results: Of 320 patients, 248 samples (77.5%) were concordant between the urine-based and HC2 assays. Of the discordant samples reactive by the urine-based test but nonreactive by HC2, 31/38 (81.6%) contained high risk HPV by DNA sequencing. 10 out of 34 (29.4%) of samples detected by HC2 but not by the urine-based test were shown to contain high risk HPV. Using DNA sequencing as the gold standard, the urine-based test false negative and false positive rates were 10/180 (5.6%) and 7/140 (5.0%) respectively.
Conclusions: HPV DNA can be isolated from urine samples and detected with sensitivity and specificity equal to or better than current assays based on cervical scraping. The urine-based test employs a single pair of primers that discriminates High Risk from Low Risk HPV DNA.

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Clinical Feasibility of a ToxB (*tcdB*) PCR test for detection of *Clostridium difficile* from loose stool samples in patients suspect of having Clostridium difficile-associated disease (CDAD)

S. M. O'Hara, D. R. Zweitzig, B. I. Sodowich, N. M. Riccardello. *Zeus Scientific Inc, Raritan, NJ,*

Objective: To perform a PCR comparison between BD GeneOhm™ Cdiff IVD Assay and a new Cdiff real-time PCR assay in an effort to improve cost effective choices for rapid, high performance *Clostridium difficile* (Cdiff) CDAD PCR tests.
Method: 33 fresh loose stool specimens suspected of CDAD from a local hospital were obtained and tested in parallel within 24hrs of collection. Specimens were tested analyzing for agreement between the BD GeneOhm™ C.diff IVD PCR Assay and a new internally controlled (IC) TaqMan based C.diff real-time PCR assay. The BD GeneOhm C.diff test was performed according to the manufactures' instructions. The new C.diff real-time PCR test was run in parallel on a SMART V1.7b Cycler system using an aliquot from the exact same swab sample eluate as was used for BD test. The C.diff real-time test is a TaqMan based FAM labeled probe targeting a 238bp amplicon of the ToxB *tcdB* gene amplified in combination with an internal control -probe labeled with LC610.
Results: **Sensitivity**: Using the new Cdiff PCR amplicon we input 20 copies into 80 separate PCR reaction tubes in parallel on a 96 well plate and 80/80 were detected as positive. 6 of 6 replicates at 10 copies gDNA -equivalents for 13 different representative toxinotypes (including the hypervirulent toxinotype III) tested positive using the new Cdiff assay.
Specificity: A total of 45 Cdiff toxigenic strains tested positive (composed of a combination of 2 Cdiff reference sets from CDC & ECDC) and 4 non-toxigenic Cdiff strains that tested negative using the new Cdiff assay. To date, a total of 28 non-Cdiff species (including *C. sordellii* and Human) tested negative when 10,000 gDNA equivalents were placed into the new Cdiff assay. All specificity results were confirmed by gel analysis.
Clinical: All runs included positive and negative controls which were scored as valid. Neither test showed any unresolved test results in this sample set. Of the 33 fresh stool specimens, 10 (30%) were scored positive in the BD GeneOhm C.diff test. These exact same 10 specimens also scored positive in the new Cdiff real-time PCR assay. The remaining 23 samples were scored as negative in both assays.
Conclusions: These data demonstrate a 100% agreement between BD GeneOhm Cdiff PCR assay results and the new Cdiff real-time PCR assay results.

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Development of a rapid and sensitive Group B streptococcus (GBS) PCR test that can be used to test prepartum or intrapartum women

S. M. O'Hara, D. R. Zweitzig, N. M. Riccardello, B. I. Sodowich. *Zeus Scientific Inc, Raritan, NJ,*

Objective: To perform a comparison between BD GeneOhm™ StrepB IVD PCR assay and a new GBS real-time PCR assay in an effort to improve performance and cost effective choices for rapid high performance GBS PCR tests.
Method: 22 adult female volunteers subjects performed self swabbing according to the BD GeneOhm StrepB vaginial /rectal specimen collection protocol. These specimens were tested in parallel for agreement between the BD GeneOhm StrepB PCR test and the new internally controlled (IC) GBS real-time PCR test. After specimen collection the Stuarts swabs were kept at room temperature and tested with in 24hrs. Swabs were processed according to the instructions found in the BD GeneOhm Strep B product insert. The new GBS real-time PCR assay was run in parallel using an aliquot from the exact same sample bead mill lysate tube as was used for the BD GeneOhm StrepB test. The GBS real-time test is a TaqMan based FAM labeled probe targeting an 114bp amplicon of the *cfb* gene amplified in combination with an IC detected by an LC610 TaqMan probe. For each specimen prepared, both PCR tests were amplified in parallel using the SMART V1.7b Cycler system.
Results: **Sensitivity**: Using the new GBS PCR amplicon we input 20 copies into 80 separate PCR reaction tubes in parallel on a 96 well plate and 80/80 were detected as positive.
Specificity: A total of 39 GBS strains, representing 9 different serotypes, tested positive at 20 copies of gDNA-equivalent using the new GBS PCR assay. To date, a total of 36 non-GBS bacterial species (including n=4 CAMP factor containing Streptococci) as well as Human tested negative when 10,000 gDNA -equivalents were placed into the new GBS assay. All specificity results were confirmed by gel analysis. Comparison study between the BD GeneOhm GBS test to the new GBS real-time PCR test showed no unresolved test results. All runs included positive and negative controls which were scored as valid. Of the 22 fresh swab specimens 6 were scored positive in the BD GeneOhm GBS test. These same 6 specimens also scored positive in the new GBS real-time PCR assay. The remaining 12 samples were scored as negative in the BD assay but, for the new GBS

assay 3 of these 12 scored as very weak positive signals that are consistent with Poisson distribution target levels as no IC inhibition was present.
Conclusions: The GBS prevalence rates of 6/22 (30%) and 9/22 (41%) are in agreement with the literature reported range of 20-40% positivity among this test population. If we exclude the 3 very weak GBS assay positives based on Poisson random sampling then the new GBS real-time PCR assay shows complete agreement with the FDA approved BD GeneOhm™ GBS IVD PCR Assay.

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Validation of an 8-cytokine muliplex assay on the Luminex platform for use in lithium-heparin plasma

A. L. Pyle, A. Woodworth. *Vanderbilt University, Nashville, TN,*

Background: Sepsis is a deadly disease with complex pathophysiology, largely regulated by cytokines. Early detection and treatment reduces mortality. Currently, there is no single biomarker with sufficient diagnostic strength for routine use in identifying sepsis in ICU patients. A combination of biomarkers related to pathobiology, detected in a single assay, may more accurately identify sepsis than any one marker alone.
Objective: To validate a panel of cytokines measured on the Luminex multipelx platform for use in lithium-heparin plasma samples.
Methods: Eight cytokines, IL-1β, IL-6, IL-8, IL-10, GM-CSF, INFγ, MCP-1, and TNFα, from the Luminex human cytokine kit (Millipore Corp, MA) were validated. Known amounts of cytokine standard resuspended in negative plasma were spiked into negative plasma pool. All patient samples were residual Lithium-Heparin plasma collected for physician-ordered metabolic panels from ICU patients (n=37). Recovery, linearity, and intra-assay precision studies were performed by measuring three concentrations of standard ten times each, and three intermediate concentrations in triplicate. Minimum detection limit (MDL) was determined by adding 2 standard deviations to the mean of 10 replicates of negative plasma pool.
Results: Each cytokine assay was linear (GM-CSF showed bi-modal linearity) though all but MCP-1 had a slope, due to poor recovery (see table) Intra-assay precision was <12%, <11%, <13%, <11%, <13%, <14%, <16%, and <18% for IL-1β, IL-6, IL-8, IL-10, INFγ, GM-CSF, MCP-1, and TNFα, respectively. The range of values in plasma from ICU patients began below the MDL for IL-1β (<0.17-5.7), IL-6 (<0.70-1942.2), IL-8, (<2.71-423.3), IL-10 (<3.98-898.9), INFγ (0.0-40.5), GM-CSF (<4.54-160.9), MCP-1 (<315.49-7413.3), and TNFα (<3.31-200.8pg/mL).
Conclusions: The Luminex assay can be used to measure IL-1β, IL-6, IL-8, IL-10, INFγ, GM-CSF, MCP-1, and TNFα in Li-Heparin plasma, as the assays are precise and linear. Future studies will examine the diagnostic utility of this panel to detect sepsis in an ICU population.

Expected Conc (pg/mL)	% Recovery								
	n=	IL-1b	IL-6	IL-8	IL-10	INFg	GM-CSF	MCP-1	TNFa
20.00	10	37.95	37.43	45.72	74.08	46.65	1161.0	35.72	27.05
200.00	10	40.25	37.99	46.45	73.87	51.27	793.84	91.07	28.01
800.00	3	42.27	37.98	41.09	65.96	47.04	357.33	94.31	25.09
2000.00	10	49.51	40.80	51.83	78.17	49.99	227.24	78.15	31.35
5000.00	3	33.47	30.45	37.41	73.76	36.33	112.80	104.63	22.35
10000.00	3	47.16	48.88	61.20	66.64	49.35	77.68	97.91	18.19

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Helicobacter Pylori DNA In Liver Samples From Egyptian Patients With Chronic Hepatitis C

M. A. Mahmoud, L. A. Tag Elden, M. A. Awad, H. A. Haile. *Suez Canal Medical School, Ismailia, Egypt,*

Background: Hepatitis C virus (HCV) is considered the most common etiology of chronic liver disease in Egypt, where its prevalence has been estimated to be around 14%. Chronic hepatitis may progress to cirrhosis and hepatocellular carcinoma (HCC). The association between Helicobacter pylori (H. pylori) infection and liver cirrhosis with or without HCC has been documented in different parts of the world however, the frequency of H. pylori in chronic hepatitis C (CHC) was not thoroughly investigated in Egypt. The aim of this study was to determine whether H. pylori DNA was associated with chronic hepatitis C in Egyptian patients.
Methods: Fifty-two Egyptian CHC patients were enrolled in this study. Liver biopsies were tested for presence of Helicobacter DNA by polymerase chain reaction (PCR), using two sets of primers specific for Helicobacter 16S rDNA. PCR products of positive samples were further identified by DNA sequencing. A commercial enzyme linked immunosorbent assay (ELISA) was used to detect anti-H pylori (IgG) antibodies in plasma.
Results: Helicobacter 16S rDNA was detected in 6 (11.5%) out of 52 CHC patients. Positive cases was more frequent in patients with high stage liver fibrosis (33.3%) than in those with low stage fibrosis (2.7%) (P = 0.006). Anti-H. pylori IgG was detected in 31 (59.6%) patients however, all H. pylori DNA positive cases were positive for H. pylori antibodies. There was no association between the presence of H. pylori DNA in the liver and age, gender of patients, duration of the disease, liver function tests, viral load or AFP levels.
Conclusion: These data confirm the presence of H. pylori DNA in liver of CHC Egyptian patients and suggest an association of this bacteria with progression of liver fibrosis. Further studies are needed to ascertain whether H. pylori plays a role in the development of HCC.

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The prevalence of Hepatitis B and C viral infections among blood donors. A retrospective study from rural Ghana.

B. Nkrumah[1], H. Owusu Frempong[2], P. A. Acquah[2], P. Averu[2]. [1]*Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana,* [2]*Agogo Presbyterian Hospital, Asante Akim North, Ghana,*

Introduction Hepatitis B and C viruses (HBV) and (HCV) are infectious diseases that cause millions of infections worldwide annually and high rates of development of liver cirrhosis (Kleinman et al., 2003). HBV infection is endemic in blood donors in Ghana with a prevalence of 10.8% (Allain et al., 2003) but 8.4% HCV prevalence (Ampofo et al., 2002). HCV is recognized as the primary cause of transfusion-associated non-A- non-B viral hepatitis worldwide and is endemic in West Africa (Jeannel et al., 1998).
Aim •To determine the prevalence of HBV and HCV in blood donors in a rural area over the past three years (2006-08)
Specific Aims
•Establish the prevalence rate for HBV and HCV among donors
•Establish the co-infection rate for HBV and HCV.
Study Design *Sampling:* Participants aged between 18-60 were involved in this study after they gave their consent. 3ml of blood samples from 2802 blood donors in a rural hospital were taken for HBsAg and Anti HCV screening.
Method: All samples were examined using DiaSpot® One Step Hepatitis test (DiaSpot Rapid Diagnosis, Pondok Kelapa 13450, Jakarta Indonesia). Tests were carried out according to the manufacturer's instructions and confidentiality was observed.
Outcome The HBV and HCV prevalence rate was 13.8% and 9.2% respectively in 2006. In 2008 it's 6.8% and 6.9% respectively at 95% confidence interval. Co-infection of HBV and HCV among the blood donors was 1.5% in 2006 and 2.1% in 2008 at 95% confidence interval. The prevalence of HBV was relatively higher in females and vice versa for HCV. Transmission was more among young adults aged 26-35.
The decreasing rate of HBV and HCV suggests a horizontal rather than vertical transmission (Cisneros-Castolo et al., 2001) and a decreased exposure to risk factors in conjunction with improving sanitary, socioeconomic conditions and adherence to good life style practices. Even though HBV and HCV infection among blood donor in rural Ghana is reducing there is the need for more sensitive and stringent screening algorithm for blood donations to ensure safety. Education and sensitization for HCV must be carried out to ensure that people are furthur enlightened about the infections. HBV vaccines must be widely made available.

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Development and evaluation of a multiplex real-time PCR assay using melting curve analysis for the detection of vancomycin-resistant enterococci.

J. Kim, C. Cha, H. An. *Gangneung Asan Hospital, University of Ulsan College of Medicine, Gangneung-si, Korea, Republic of,*

Background: We developed a multiplex real-time PCR assay using melting curve analysis that allows simultaneous identification of vancomycin-resistant genotypes and of clinically relevant enterococci. It's utility was evaluated.
Methods : The specificity of the assay was tested on 4 reference strains of vancomycin-resistant enterococci (VRE) and on 2 reference strains of vancomycin susceptible enterococci. 93 clinical isolates of enterococci with different glycopeptide resistance phenotypes were genotyped and identified by a multiplex real-time PCR assay using melting curve analysis.
Results : Representative melting curves for *Enterococcus faecium*, *Enterococcus faecalis*, *vanA*-containing *E. faecium*, *vanB*-containing *E. faecalis*, *Enterococcus gallinarum*, and *Enterococcus casseliflavus* were obtained. For 82 enterococcal isolates, phenotypic and genotypic results were the same. Glycopeptide resistance phenotypes of 4 isolates were discordant with glycopeptide resistance genotypes and discrepancies occurred in the

species identification for other 4 isolates. 3 isolates with mixed strains detected by the assay could not be correctly identified with phenotypic methods.
Conclusions : VRE genotyping and identification of clinical relevant enterococci were rapidly and correctly performed by the multiplex real-time PCR assay using melting curve analysis.

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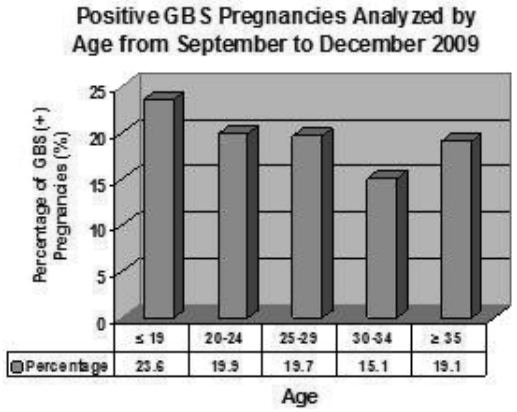
Prevalence of Positive Group B Streptococcus in Pregnancy by Age

X. Liu[1], B. H. Durham[1], M. Cao[2]. *[1]Saint Louis University and St Mary's Health Center (rotation), Saint Louis, MO, [2]St Mary's Health Center (rotation), Saint Louis, MO,*

Background: Since 1970, Group B Streptococcus (GBS) has been identified as the number one cause of life threatening infections in newborns. The vast majority of GBS infections are acquired during childbirth when the infant comes into direct contact with the bacteria carried by the mother. GBS causes an estimated 12,000 infant infections and 2,000 deaths in the United States each year. Many patients are left mentally and/or physically handicapped. Meningitis is the primary disease that evolves from the GBS infection, which has long term effects on the baby's central nervous system. However, 98-99% of all babies born to infected mothers will not become infected, if treated promptly. Even though GBS infections are much more common than other screenable diseases during pregnancy, including rubella, Down's syndrome, and neural tube defects, it remains generally unknown to the public. Understanding and awareness about GBS infection in pregnancy and its prevalence have become more critical. This study is designed to analyze the GBS rates among pregnant mothers.
Method: There are a total of 1,062 deliveries in a local hospital from September to December in 2009 included in this study. The research data is analyzed and categorized based on different age groups: equal or younger than 19, 20-24, 25-29, 30-34, and equal or older than 35 years of age. Each age group's data is tabulated based on the total delivery number and the GBS positivity, which are presented as a percentage.
Results: Among all the deliveries, 19.7% on average are GBS positive. The study demonstrated that the group of 20-24 years of age has the highest delivery number, 347. The equal or younger than 19 group seems to have a higher prevalence rate of GBS positivity than average, 23.6%.
Conclusion: Teenage pregnancies may have a higher positive GBS prevalence than the other age groups.



A-169

Analysis about hepatitis B patients with HBV pre-C A1896 mutation and BCP T1762/A1764 pairs of mutations and clinical correlation

C. LIU, Q. WANG, C. XIAO, J. WANG, Q. XIE, W. HOU. *The First People's Hospital of Zaoyang, Zaoyang, China,*

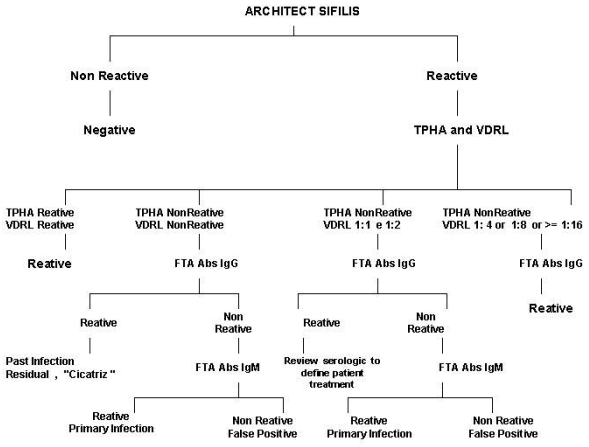
Objective To explore the relationship between hepatitis B virus (HBV) pre-C region / the basic core promoter (BCP) mutation and the copy numbers of HBV-DNA. Identify the significance of mutation detection.
Methods Conventional PCR was done and pre-C region of HBV-DNA was amplified, the PCR products was sequenced directly. 31 cases of hepatitis B patients' peripheral blood were drawn and A1896 mutation in HBV pre-C region and the BCP T1762 / A1764 double mutations both detected by sequencing. SPSS 16.0 software was used to analysis the relationship between HBV-DNA mutation and the numbers of HBV-DNA.
Results 31 cases of hepatitis B serum HBV-DNA occurred nt 1896 nucleotide GA point mutation in 18 cases; nt1762-bit AT occurred in 16 cases; occurred nt 1764-bit G> A in 18 cases; occurred nt1762-bit AT and 1764 G-A pairs of mutations in 16 cases; 1762 A-T1764-bit GA pairs of mutations and variations of the joint 1896 G-A mutation frequency was significantly higher than the individual variation. Using SPSS 16.0 analysis mutation and HBV-DNA load relationship. Pre-C, A1896 mutation, BCP T1762/A1764 variations are related to HBV-DNA load was not statistically significant differences.
Conclusions The copies of HBV-DNA can reflect the real-time virus number only, pre-C A1896 mutation and BCP T1762/A1764 dual variation could be helpful for determining the infectious status and the development of anti-viral treatment.

A-170

Laboratory Diagnosis of Syphilis: a proposal of a new fluxogram

R. C. S. Salles[1], P. V. Bottini[2], M. Fiorito[1], M. P. D. Novo[1], S. A. A. Bueno[1], F. C. Paganelli[1]. *[1]Public Health Care Service of Campinas, Campinas, SP, Brazil, [2]University of Campinas, Campinas, SP, Brazil,*

Infectious syphilis re-emerged as a significant public health problem in the late 1990s. Primary syphilis is the most challenging of the stages of early syphilis to detect with current serological screening tests giving lower sensitivity than other early stages. In Brazil, current guidelines for the management of syphilis recommend a screening with a non treponemical test (VDRL). A new highly automated syphilis assays based on chemiluminescence (CLIA) are now available.
The aim of this study was to evaluate the new Abbott Architect syphilis assay for the detection of antibodies to *Treponema pallidum* and propose a new diagnostic fluxogram to be applied in a public health care system.
In a one month period of 2009, we analyzed 2650 unselected samples submitted to the Immunology Service of the Public Laboratory of Clinical Pathology of Campinas, SP, Brazil, for routine screening for syphilis. All samples were initially screened by the new Architect Syphilis Chemiluminescence Assay. Non reactive samples were defined as negative for syphilis. Specimens with RLUs greater than the cut-off were considered positive. Positive samples were analyzed by a non treponemical test (Veneral Disease Research Laboratory Test -VDRL) and by the *Treponema pallidum* particle agglutination test (TPHA). Samples with TPHA and VDRL both reactive were considered positive for syphilis. If one of these confirmatory tests were non reactive, we submitted the samples to an additional assay (FTA-Abs).
Based on the screening test, 2386 samples (90%) were considered negative. The 264 remaining samples (10%) were tested by VDRL and TPHA. In 108 samples we observed a disagreement between the tests, so the samples were submitted to the FTA-Abs assay. Architect Syphilis Chemiluminescence Assay showed sensitivity and specificity >99%, with a predicitve negative value of 100%Based on our data we proposed a new fluxogram for the laboratory diagnostic of syphilis (figure below).



Tuesday PM, July 27

Poster Session: 2:00 pm – 4:30 pm
Endocrinology/Hormones

B-01

Evaluation of the body fat in women with Subclinical hypothyroidism.

T. Helou[1], A. Bergamin[1], P. F. S. Teixeira[1], M. Mainenti[1], F. Palha[1], V. B. Braulio[1], A. P. Cony[1], M. Vaisman[1], D. M. V. Gomes[2], M. L. L. Moreira[2]. *[1]University Federal of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Diagnostic Laboratory Sérgio Franco, Duque de Caxias, Brazil,*

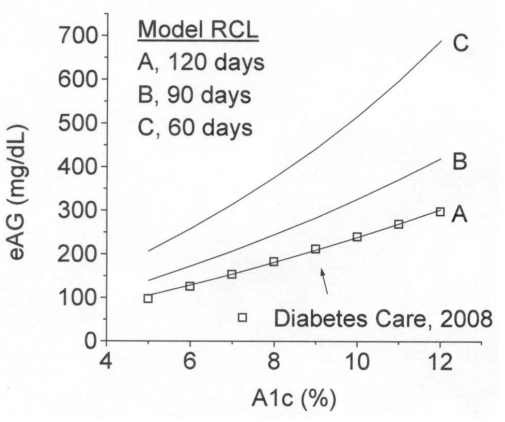
Objective: Subclinical hypothyroidism (SH) is a common problem, with a prevalence of 4% to 10% in the adult population. The prevalence of SH increases with advancing age and is higher in women. SH occurs when the serum thyroid-stimulating hormone (TSH) level rises above the upper limit of normal (>4,0 mUI/mL) despite a normal serum free thyroxine (FT4) concentration (>0,8 and <1,9 ng/dL). There isn't consensus for initiating levothyroxine replacement therapy in all patients with HS. Considering the possibility to occur alterations of the amount of body fat in carriers of HS, this study aimed to quantifie the body fat in these patients in comparison to a group without thyroid dysfunction, also called Euthyroid (EU) group. The EU group had no history of thyroid disease or thyroid antibody and a normal serum TSH concentration (>0,3 and <4,0).
Methodology: A cross-secional study was performed with two groups of the women: one with SH (two measurements of TSH >4,0 and < 12 mUI/mL with normal FT4), and other with EU, normal TSH (>0,3 and <4,0) and normal FT4. The TSH, FT4 and Thyroperoxidase antibodies (TPO) had been dosed by Chemiluminometric Immunoassay, DPC (Diagnostic Products Corporation). The Leptin was measured by Double antibody (RIA), Kit DSL-23100ACTIVE. The amounts of fat body were measured by anthropometric evaluation [cutaneous folds, waist (cm), BMI (kg/m^2) and %F-SIRI], and Bioelectrical Impedance Assessment (BIA). The BIA equipment was the Biodynamics Body Composition model 310, supplying the percentage of body fat (%BF), the mass of body fat in Kg (BF) and the index fat mass (IFM), dividing it BF for the height.
Results: There were 40 women included (25 HS and 15 EU). The two studied groups were similar in average of age, BMI and frequencies of sedentary life style. There were no significant differences between the two groups in respect to amounts of fat mass. Also there were not differences in the % body fat, measured by SIRI formula (23.9 ± 6.3 %, in EU, vs 26.1 ± 4.2; p= 0,242) or by the BIA (29.8 ± 4.6 vs 30.8 ± 6.4, repectivelly; p=0,450). However serum leptin concentration was higher in SH group (24.6 ± 19.7 vs 36.7 ± 20.0) and correlated positively with serum TSH levels (rs=0.341; p=0,022). The were adequate positive correlation between the different methods of fat mass measurement and leptin correlated positively and adequate with BMI (rs=0.563) and body fat [by BIA (rs=0.651) or anthropometric evaluation (rs=0.662), (all p<0,001)].
Conclusions: The total amounts of body fat or the %BF are not higher in women with SH, in comparison to another group, without thyroid dysfunction, with same age, BMI and sedentary life style. Despite no differences in the amounts of body fat, measured by different and concordant methods, we detected higher levels of leptin levels in SH, suggesting a direct effect of thyroid hormones on secretion or degradation of leptin. Therefore, we did not get results favorable for introduction of the treatment with Levothyroxine for reduction of the corporal fat in patients with subclinical hypothyroidism.

B-02

Modified A1c-derived estimated average glucose (eAG(A1c)) for reduced red cell lifetime (RCL), calculated using a kinetic model for A1c formation consistent with eAG(A1c) for normal RCL

D. F. Stickle[1], J. D. Landmark[2]. *[1]Jefferson University Hospital, Philadelphia, PA, [2]University of Nebraska Medical Center, Omaha, NE,*

Background: A1c-derived estimated average glucose (eAG(A1c); Nathan et al., Diabetes Care 2008;31:1473-8) is not intended for use under circumstances of abnormal red cell lifetime (RCL) as may be operative, for instance, in the presence of certain hemoglobin variants. However, a modified eAG(A1c) can in principle be predicted for any given RCL based on a kinetic model for A1c formation that successfully predicts eAG(A1c) for normal RCL. We determined kinetic parameters yielding the established eAG(A1c) according to the simplest model for glycation for normal RCL, and then calculated predicted alterations in eAG(A1c) for circumstances of decreased RCL.
Methods: Kinetics of A1c formation were characterized by the following model: A↔B→C, where A is native hemoglobin (fraction of total), B is the reversibly glycated hemoglobin fraction, and C is the irreversibly glycated hemoglobin fraction (A1c). A↔B was characterized as a function of glucose (G) by B/(A+B)=G/(G+*K*). B→C was characterized by dC/dt = *k*B. For a given red cell over time, C(t) for constant G is given by C(t)= 1-exp(-αt) (Eqn.1), where α = *k*G/(G+*K*). Across a population of cells of age 0-n days, the average C for constant G was computed analytically from Eqn.1. We determined *K* and *k* such that average C as a function of G using normal RCL (n=120) was compatible with the eAG(A1c) established for normal RCL. Using the same *K* and *k*, we then computed a modified eAG(A1c) for reduced RCL (n<120).
Results and Conclusions: Model predictions for A1c(G) compared well to eAG(A1c) for normal RCL (120 days) using *K*=1260 mg/dL and *k*=0.010/day (Figure; r^2>0.99). Using the same *K* and *k*, model-predicted eAG(A1c) was increased when RCL was decreased (Figure). The results provide a model-consistent prediction of eAG(A1c) for reduced RCL, and indicate the likely extent of underestimation of average glucose by normal-RCL eAG(A1c) when RCL is reduced.



B-03

Validation of a Second Generation Assay for Anti-Mullerian Hormone

C. M. Preissner, D. E. Morbeck, R. P. Gada, S. K. Grebe. *Mayo Foundation, Rochester, MN,*

Background: Anti-mullerian hormone (AMH), a glycoprotein dimer, is produced by testicular Sertoli cells and ovarian granulosa cells. Measurement of AMH is useful in evaluating testicular function in infants and children, monitoring ovarian granulosa cell tumors, and assessing ovarian reserve during infertility workup.
The Active® MIS/AMH ELISA (Gen I) kit was obtained from DSL-Beckman Coulter, Inc. In mid-2009 a modified version, the Active® AMH Gen II ELISA (Gen II) assay, became available. The major change was standardization to the Immunotech-Beckman Coulter (IOT) AMH assay.
Objective: To compare the Gen II kit to the original Gen I kit and validate it for routine clinical use.
Method: The Gen I and Gen II kits both used monoclonal antibody coated microtiter wells, with sequential incubations of patient sample, biotinylated monoclonal antibody, streptavidin-HRP and TMB chromogen. Patient results were available within 3 hours.
Validation and Results: The Gen I kit contained six standards plus a zero standard. The concentrations of three standards (0.05, 0.10, and 0.25ng/mL) were below the functional sensitivity of the assay (~0.5ng/mL) and had interassay absorbance CVs of >25%. The Gen II assay had the same number of standards but at higher concentrations. There was only one standard (0.16ng/mL) below the functional sensitivity (~0.25ng/mL) and its interassay absorbance readings varied by only 13.6%. The calculated LOD for Gen I was 0.056ng/mL and for Gen II was 0.032ng/mL.
Interassay imprecision of the two kit controls in the Gen I assay was ≤5.2%. Running an in-house serum control with a mean concentration of 0.14ng/mL was discontinued because of a 30.8% CV. It was replaced with a pool at 0.51ng/mL (CV 10.1%). Recoveries of 0.16ng/mL AMH spiked into 4 patient samples ranged from 65.6 to 85.9%. Recoveries of spikes of 1.6 and 8.2ng/mL AMH were within +/-20% of expected.
In the Gen II assay, interassay CVs were ≤3.4% and 8.1% for two kit controls and an in-house serum control (0.57ng/mL), respectively. Mean recovery of three levels of AMH spiked into 4 patient sera was 92.3%. Average agreement of 7 serially diluted samples with expected results was 96.5%. No significant measurement bias was observed for Heterophile Blocking Tubes (Scantibodies) in 30 samples, indicating these tubes can be used in cases of suspected heterophile interference. Comparison of 206 samples (0 - 289ng/mL) assayed in both kits yielded the equation Gen II = 1.53x Gen I - 0.77, r = 0.994. The 161 samples with AMH values <20ng/mL had a slope of 1.33, similar to the 1.34 slope reported by Beckman Coulter in 180 samples ≤18ng/mL. The normal reference

values obtained from 202 male (109 pediatric and 93 adult) and 198 female (99 pediatric and 99 adult) samples in the Gen II assay were generally higher than the values obtained with the Gen I assay and those established by Beckman Coulter.
Conclusions: When compared to the Gen I assay, the Gen II assay has improved precision and recoveries at low levels which should make it a more robust assay.

B-04

Validation of a Second Generation Assay for Inhibin B

C. M. Preissner, D. E. Morbeck, R. P. Gada, S. K. Grebe. *Mayo Foundation, Rochester, MN,*

Background: Inhibin B is a dimeric glycoprotein composed of α and βB subunits secreted by ovarian granulosa cells and testicular Sertoli cells. Inhibin B complements CA 125 as a tumor marker for ovarian cancers, being particularly effective in granulosa cell tumors, and is also useful in assessment of ovarian reserve and male infertility.
Objective: To validate the DSL-Beckman Coulter Active® Inhibin B Gen II ELISA kit (Gen II) for routine clinical use. The older Active® Inhibin B ELISA (Gen I) assay was used for comparison.
Method: The Gen II assay, which unlike the Gen I assay does not require sample pretreatment or an overnight incubation, can be performed in <4 hours. The monoclonal antibody-coated wells are sequentially incubated with sample, biotinylated antibody, streptavidin-HRP conjugate, TMB substrate and stopping solution.
Validation and Results: The standard curve extended from 10-1000pg/mL. Assay imprecision of three in-house serum controls with concentrations from 40.3 to 300.4pg/mL was ≤3.5% using three different lots of reagents. This was in contrast to the precision studies of Gen I kits in which two serum pools with mean concentrations of 17.0 and 77.8pg/mL had CVs of 53.4% and 26.8%. The two buffer-based kit controls (~100 and 400pg/mL) gave acceptable interassay CVs with both the Gen I (≤5.6%) and Gen II (≤2.7%) assays. The LOQ of ~10pg/mL in the Gen II assay was determined by running a sample with 12.9pg/mL Inhibin B in 18 assays resulting in a CV of 10.6%. The LOD calculated as 2.5SD above the zero standard was 1.2pg/mL. When five samples with Inhibin B levels from 102-854pg/mL were serially diluted, recoveries ranged from 82.3 to 121.4%. Recovery of 2 levels of Inhibin B spiked into three patient samples ranged from 92.2-107.4%. Method comparison of 71 patient samples with Inhibin B concentrations from 20-308pg/mL yielded the equation: Gen II = 1.115 x Gen I + 76.998, r = 0.773. When only samples from 20-100pg/mL were included, the results were: Gen II = 2.164 x Gen I + 25, r = 0.794. The effect of Heterophile Blocking Tubes (HBT, Scantibodies) on Inhibin B Gen II was tested to determine if the tubes could be used for troubleshooting possible future assay interferences. A total of 36 samples were tested with and without HBT treatment with a mean difference of 2.5% (range -8.3 to 19.4%). In contrast, in the Gen I assay, 11 of 39 samples (28.2%) tested with HBT had differences >20%. Reference intervals were established from 238 pediatric (121 male, 117 female) and 211 adult (111 male, 100 female) samples. Values in females were consistently lower than in males in all age categories and changed with phase of the ovulatory cycle.
Conclusions: The Gen II Inhibin B assay is more precise and is much easier and faster to perform than the Gen I assay.

B-05

Comparisons of six automated serum cortisol immunoassays with Abbott Architect i2000 using specimens from critically ill patients

B. K. De[1], E. Jimenez[1], S. De[1], M. L. Rawlins[2], S. L. La'ulu[2], W. L. Roberts[2]. *[1]University of Arizona Health Sciences Center, Tucson, AZ, [2]University of Utah Health Sciences Center, ARUP laboratories, Salt Lake City, UT,*

Cortisol is the major steroid hormone secreted by the adrenal cortex gland. It regulates carbohydrate metabolism, electrolyte and water distribution, and shows anti-inflammatory and immunosuppressive activities. Serum cortisol measurements can be used to diagnose both adrenal excess and insufficiency. The latter is especially important in critically ill patients who may benefit from cortisol therapy. However, the decision levels for response to stimulation tests were established long ago with assays that are no longer in use today. Also, other steroids can cross-react variably with different cortisol immunoassays. Our objective was to measure serum cortisol concentrations by commercial assays using samples from 136 critically ill subjects from a medical intensive care unit. The immunoassays used were the Abbott Architect i2000, Abbott AxSYM, Beckman Access, Siemens Centaur, Siemens Immulite, Roche E170, and Ortho ECi. The Architect i2000 was selected as a comparison method since the manufacturer claims to correlate well with liquid chromatography-tandem mass spectrometry. All six immunoassays showed variable positive biases by regression analysis (Table A) with the AxSYM and Access methods showing the lowest biases. Bland -Altman analysis indicated positive mean biases for all six assays (Table B). The 95% limits of agreements were wide for all assays suggesting large inter-individual variation of immuno-reactive species compared to the Architect i2000 assay. Variability of total serum cortisol immunoassays can confound the diagnosis of adrenal dysfunction in critically ill patients. We conclude that these assays need to be more specific for cortisol in order to reliably classify critically ill patients' adrenal status. Further studies using an LC-MS/MS comparison method are necessary to confirm these observation.
Comparison Method : Abbott Architect i2000; n = 136

A. Deming Regression Analysis					**B. Bland Altman Analysis (µg/dL)**		
						Limits of	**Agreements**
Method	**r**	**Slope**	**intercept**	**Sy/x**	**Bias**	**Lower (95%)**	**Upper(95%)**
Access	0.94	1.36	1.43	6.1	8.69	-4.54	21.93
AxSYM	0.96	1.11	2.82	4.0	4.94	-2.97	12.85
Centaur	0.95	1.12	4.75	4.6	7.11	-1.91	16.14
E170	0.90	1.34	2.69	7.5	9.39	-5.39	24.18
ECi	0.88	1.98	-1.55	12.0	18.06	-8.86	44.98
Immulite	0.95	1.17	4.27	4.8	7.72	-1.83	17.27

B-06

Rapid fractionation of serum prolactin isoforms by HPLC

M. Fahie-Wilson[1], S. Gaston[2], M. Minihan[2], N. Carolan[2], T. P. Smith[3]. *[1]Southend Hospital, Westcliff-on-Sea, United Kingdom, [2]Beckman Coulter, Lismeehan, Ireland, [3]St. Vincent's University Hospital, Dublin, Ireland,*

Background: Circulating prolactin exists in a variety of forms, principally monomeric prolactin (23kDa), big prolactin (60kDa) and macroprolactin (150kDa). However, in contrast to monomeric prolactin, big prolactin and macroprolactin are devoid of bioactivity *in vivo*. To prevent misdiagnosis and mismanagement of hyperprolactinemic patients it is essential to allow for the contribution these high molecular mass bio-inactive forms make to the total serum prolactin concentration. Gel filtration chromatography (GFC) has traditionally been employed as a reference procedure to separate serum prolactin isoforms with subsequent quantitation of the various molecular forms present by immunoassay. However, current GFC methods are slow, labour intensive and not particularly suited to routine use.
Objective: To set-up and validate a rapid, automated and high throughput HPLC method to fractionate serum prolactin isoforms.
Methods: Sera, from healthy individuals, from patients with true hyperprolactinaemia, from individuals with macroprolactinaemia together with a monomeric prolactin standard (National Institute for Biological Standards and Controls, United Kingdom) were subjected to chromatography. Conventional GFC was carried out using an AKTA chromatographic system (Pharmacia Biotech). Serum, 0.2-1.0ml, depending on prolactin concentration, was applied to the column (Superdex SD-75, 60cmx1.6cm) and eluted with TRIS buffered saline, pH 7.4 at a flow rate of 1.0 ml/min. The prolactin content of the fractions, 1.4ml, was measured using an AIA 1800 (Tosoh). For HPLC fractionation, we employed a Waters 1525 system fitted with either a 30cmX0.75cm TSK G3000SW (Tosoh) or BioSep-SEC-3000 (Phenomenex) column. Serum, 0.025ml, applied to the column was eluted with phosphate buffered saline, pH 6.8 at a flow rate of 0.35ml/min. The prolactin content of the fractions, 0.175ml, was measured using an Olympus AU3000i. Both Tosoh and Olympus immunoassays react with all forms of circulating prolactin.
Results: Initial studies revealed that the TSK column was capable of superior fractionation of serum prolactin isoforms in terms of recovery and resolving power relative to the BioSep column. Comparison of the pattern of immunoreactive prolactin isoforms determined by HPLC to that of conventional GFC revealed similar elution profiles. Three main immunoreactive species were identified and corresponded to monomeric prolactin, big prolactin and macroprolactin. The three isoforms were clearly resolved permitting acurate quantitation of the relative contribution of each molecular species to the total serum prolactin concentration. In contrast to a conventional GFC run, which may take up to 120 minutes, the HPLC method can fractionate a sample in under 17 minutes with comparable results. Additional benefits include excellent reproducibility, enhanced sensitivity (<2mU/L) and the ability to fractionate small sample volumes (<100µl).
Conclusions: We have developed and validated a rapid, reproducible and automated HPLC method capable of separating serum prolactin isoforms with greatly enhanced sensitivity and throughput. This methodology will facilitate more precise and specific quantitation of the biologically active and clinically relevant 23kDa monomeric form of prolactin in serum.

B-07

Methylenetetrahydrofolate reductase genotypes can predict impaired glucose tolerance

K. Nakatani, K. Noma, Y. Sakamoto, J. Nishioka, T. Nobori. *Mie University Graduate School of Medicine, Tsu, Mie, Japan,*

[Objective] Homocysteine (Hcy) is a sulfur-containing amino acid formed during the metabolism of methionine. Elevated levels of Hcy are leading to impaired endothelial through induction of the oxidative stress. The oxidative stress has also been shown to impair insulin action and secretion. Methylenetetrahydrofolate reductase (MTHFR) is an enzyme of to convert Hcy to methionine and its activity is closely related with Hcy levels. Single nucleotide polymorphism (SNP) of MTHFR 677C>T, which is located within the enzyme catalytic domain, has association with MTHFR activity and levels of Hcy.
In this study, we investigated association between Hcy, glucose metabolism and MTHFR genotypes. Moreover, whether MTHFR genotypes could be a marker of impaired glucose metabolism.
[Subjects and Methods] 451 Japanese volunteers (245 males and 206 females) were recruited in the hospital for medical check. After obtaining the written informed consent of each subject, anthropometry, 75-g oral glucose tolerance test (OGTT), blood pressure determination and biochemical examination were performed early in the morning after fasting overnight. SNP677 of MTHFR gene was genotyped by the single primer extension method. Insulin resistance was assessed using the homeostasis model assessment for insulin resistance (HOMA-R).
[Results] (1) Plasma Hcy levels showed significant gender difference (male, female: 10.7±3.9 μmol/L, 8.6±2.0 μmol/L) and further analysis was performed within each gender. Plasma Hcy levels showed a weak correlation with HOMA-R in female but no correlation in male.
(2) Plasma Hcy levels were significantly associated with MTHFR 677TT genotype in both male and female, but no association with adiponectin levels which were associated with insulin resistance. male: CC: 10.1±2.6 μmol/L, CT: 10.3±2.0 μmol/L, TT: 14.2±8.1 μmol/L, female: CC: 8.0±1.8 μmol/L, C: 8.6±1.8 μmol/L, TT: 10.2±2.8 μmol/L.
(3)Of male subjects with each MTHFR genotypes, no differences were observed in OGTT. However, female subjects with MTHFR 677TT genotype showed significantly elevated plasma glucose after glucose loading in comparison with other genotypes.
[Discussion] Insulin resistance was slightly influenced by plasma Hcy concentration but not by MTHFR genotypes. However, MTHFR genotypes were significantly associated with plasma Hcy in both female and male. Moreover, MTHFR genotypes were significantly associated with impaired glucose tolerance in female. Taken together, Hcy could affect glucose metabolism through impaired insulin secretion in female. In conclusion, MTHFR genotype could be a predictive marker of impaired glucose tolerance in female.

B-08

ARCHITECT® 2nd Generation Testosterone Immunoassay, an automated direct immunoassay giving good agreement with LC/MS/MS for both male and female specimens.

S. Blincko[1], E. J. Doran[2], A. Weerakoon[2], J. M. Ramp[3], R. C. Doss[3], B. G. Keevil[4], P. Wong[5], P. M. Sluss[6]. *[1]Abbott Diagnostics, Wiesbaden-Delkenheim, Germany, [2]Abbott Diagnostics, Dartford, United Kingdom, [3]Abbott Diagnostics, Abbott Park, IL, [4]University Hospital of South Manchester, Manchester, United Kingdom, [5]Toronto General Hospital, Toronto, ON, Canada, [6]Massachusetts General Hospital, Boston, MA,*

Background: In view of the well documented problems associated with testosterone immunoassays several LC/MS/MS methods have been developed. However all LC/MS/MS methods require extraction, extraction and derivatisation or precipitation prior to the sample being introduced to LC. As such they do not have the ease of use that an automated immunoassay offers nor are widely available. Clearly there is a need for an automated direct immunoassay that is both sensitive and specific enough to measure male, female and paediatric specimens accurately. We present results from an accurate, automated, direct immunoassay for testosterone.
Methods: Both SST serum samples (n =195) and UK NEQAS (n=198) specimens were measured by ARCHITECT® 2nd Generation Testosterone Assay and LC/MS/MS. The method of Gallagher et al measured the SST specimens and the data from the all lab trimmed mean (ALTM) for LCMS methods for testosterone was used for the UK NEQAS specimens.
The ARCHITECT® 2nd Generation Testosterone Assay is a delayed one step chemiluminescent microparticle immunoassay. 50μL sample is automatically diluted with 100μL specimen diluent (phosphate buffered saline with preservative). Then assay specific diluent and magnetizable particles coated with ovine monoclonal antibody are added and incubated prior to the addition of the testosterone-acridinium tracer. Following washing, addition of pretrigger and trigger solutions the light output is measured. Results are interpolated from a calibration curve derived from 6 calibrators (0 to 30nM). 150μL of all calibrators are added to the assay without the addition of specimen diluent.
Results: Versus LC/MS/MS Gallacher et al:
2nd Gen Testosterone = 1.02 LC/MS/MS - 0.2; R^2 = 0.99; for 75 male and 120 female specimens. For female specimens R^2 = 0.90
Versus LC/MS/MS UK NEQAS ALTM:
2nd Gen Testosterone = 0.989 LC/MS/MS - 0.16; R^2 = 0.99; for 75 male and 120 female specimens. For female specimens up to 2.5nM R^2 = 0.95 (n=74)
2nd Gen Testosterone functional sensitivity < 0.06nM and LOQ < 0.07nM
Precision < 10% from 0.4 - 35nM
Dilution linearity was demonstrated for both male and female specimens.
Bilirubin up to 20mg/dL, Haemoglobin up to 100mg/dL and triglycerides up to 1000mg/dL caused less than 10% interference in male and female specimens from 0.5-25nM.
Very low cross reactivities were observed for endogenous steroids including DHEAS (0.0009%), DHT and Androstenedione. Nandrolone by contrast showed a strong cross reactivity.
Conclusion: The ARCHITECT® 2nd Generation Testosterone Assay is an automated direct immunoassay capable of measuring both male and female samples accurately and precisely. This enables laboratories to have an automated assay alongside the rest of the fertility menu thus optimising workflow without compromising accuracy.
Reference: Galagher LM, Owen LJ, Keevil BG. Ann Clin Biochem 2007; 44:48-56.

B-09

Evaluation of the DiaSorin Liaison 1-84 PTH Assay, a new automated immunoassay for the determination of the 3rd Generation PTH.

E. Cavalier, O. Rousselle, A. Bekaert, I. Carlisi, J. Chapelle, P. Delanaye. *University Hospital of Liège, University of Liège, Liège, Belgium,*

Introduction: The Liaison 1-84 PTH allows the determination of (1-84) PTH without cross-reaction to the (7-84) fragment. It is one of the first automated "3rd generation" PTH immunoassay. Unlike the "2nd generation" or "intact" PTH assays, the N-terminal antibody is specific for the first aminoacids of the peptide. The aim of that work was to evaluate the analytical performance of this new test. We also established the reference range of the parameter in a biologically well-defined healthy population.
Material and methods: On two different Liaison automates, we evaluated the precision with a modified protocol based on CLSI EP-5A2: twelve serum pools were assayed in triplicate once per day on five different days. We established the analytical and the functional sensitivity with a method adapted from CLSI EP-17. Linearity was evaluated based on CLSI EP-6A. Recovery was determined according to CLSI EP-6P. Finally, we evaluated the measurement uncertainty, accuracy and β-expectation limits. We settled the β-expectation tolerance limits with β=0.95 and considered the method as valid if each future measurements of the same level had a probability of 95% to fall in the ±20% accepted limits of accuracy. We selected 60 male and 118 female for our reference population using these variables: age >18 yo, levels of 25-OH vitamin D (25VTD) >32 ng/mL, calcium and phosphorous in the laboratory reference range and eGFR> 60 mL/min/1.73m² (estimated by the MDRD equation). All the subjects were Caucasian. We used the Kolmogornov-Smirnov test to check if the population was Gaussian.
Results: Analytical and functional sensitivity were respectively ≤0.7 and ≤2.2 pg/mL. Repeatability and intermediate precision did not exceed 8% in the validated range. The mean recovery was 94.0±3.4%. The method was found to be linear until the 1/10 dilution. Measurement uncertainty was comprised between 14 and 7.4%. The accuracy profile built with the predictive tolerance interval method shows that, on average, 95% of the future results that will be generated by this method will be included in the computed tolerance intervals of ±20% in the 4.3-1392 pg/mL studied range. Our selected population presented a mean 25VTD level of 41 ng/mL (Lowest:32; Highest: 93), a mean calcium of 2.32 mmol/L (Lowest:2.15 - Highest: 2.52) and a mean age of 56 yo (Lowest:19; Highest: 89). There was no significant difference between men and women. The reference range observed in this population was 4.6 (90% CI: 3.4-5.9) - 26.8 (90% CI: 25.6-28.0) pg/mL.
Conclusions: The Liaison 1-84 PTH is one of the first commercially available automated 3rd generation PTH assays. The analytical performances are remarkable, with an accuracy profile showing that the method is completely validated between 4.3 and 1392 pg/mL. We also established a reference range (4.6-26.8 pg/mL) on a very well biologically defined population.

B-10

The associations between metabolic syndrome and plasma fibroblast growth factor-21 levels and its genetic variation in Chinese Han Population

M. Zhang[1], l. Zeng[2], Y. Wang[2], Z. AN[1], B. Ying[3]. *[1]Division of Clinical Chemistry Laboratory, Department of laboratory Medicine,West China Hospital, Sichuan University, Chengdu ,China., Chengdu, China, [2]Department of physical examination center, West China Hospital, Sichuan University. Chengdu ,China., Chengdu, China, [3]Division of Molecular Diagnosis,Department of Lab Medicine, West China Hospital,Sichuan University, Chengdu, China., Chengdu, China,*

Objective: The purpose of our study was to investigate the associations between plasma fibroblast growth factor-21(FGF-21) and metabolic syndrome (MS) and its components and try to find out whether FGF-21 genetic variants are associated with the MS and its components in Chinese Han Population.
Methods: The subjects (n=291) were recruited in Chinese Han Population from Sichuan province. Anthropometrical measurements and serum FGF-21 in these subjects were measured. The genotypes of FGF-21 rs11665896 were determined in 291 Chinese by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genotypes were confirmed by gene sequencing analysis. A possible causal relationship between FGF-21 gene SNP rs11665896 and MS, overweight/obese and DM was explored.
Results: MS group had higher plasma FGF-21 levels than the control group (P=0.012). Plasma FGF-21 were associated with BMI and fasting blood glucose independently. Plasma FGF-21 were found to be independently associated with MS, hyperglycemia, overweight/ obese, and diabetes(DM) (all p<0.05).The results by PCR-RFLP showed that the FGF-21 gene SNP rs11665841 and rs3745706 were not polymorphisms in our population. The frequencies genotype and allele frequency of rs11665896 was not significant difference between MS, DM and overweight/obese and control. In addition, SNP rs11665896 was associated with higher risk of overweight/obese in our study, no association was found between rs11665896 polymorphism and MS, DM.
Conclusion: Plasma FGF-21 levels was found to be independently associated with MS, overweight/ obese, and diabetes. No association was found between FGF-21 gene SNP rs11665896 polymorphism and MS, DM, overweight/obese.

B-11

Salivary Cortisol in Stress Research

M. Purice[1], M. Greabu[2], E. Deliu[3], A. M. Stefanescu[1]. *[1]National Institute of Endocrinology, Bucharest, Romania, [2]Faculty of Dental Medicine, University of Medicine and Pharmacy »Carol Davila », Bucharest, Romania, [3]3Insitute of Hygiene, Public Health Services and Managemnet, Bucharest, Romania,*

Our studies were done on salivary cortisol assessment in different stress research fields: environmental, emotional and physiological stress . We also evaluated a number of psychological variables that were found to be closely associated with the individual salivary cortisol stress response.
Methods: Saliva sampling device which does not interfere with the salivary analyte (Salivette) was used. Preparation of a clear supernatant was critical.
Salivary cortisol was performed by ELISA Salivary Cortisol , DRG, GmbH (Germany)
Study groups: total number of **100 subjects** divide as:
1. Environmental stress group (n=60):
- 27 subjects (men, age 27-45) ,air traffic controllers in an area controlled (ACC) and an approached (APC) centers were investigated during the working program.
Results: there are significant differences in neuroendocrine reactivity: 18% of ACC controllers had low levels of salivary cortisol and a loose of circadian rhythm (syndrome of mal-adaptation - chronic hypo corticism of central cause) and 7% of them had very high levels of salivary cortisol, showing a pronounced reactivity under stress.
- 33 subjects (men,age 35-45) working with visual screens in a TV broadcast with high stress level (n= 16) and with medium stress level (n= 17) were investigated at 4 different moments during working program.
Results: high stress scores were associated with low salivary cortisol level. The cortisol dynamic suggests a normal reactivity of HTPA axis only in the group with medium stress score.
2. Emotional stress (n=10):
We investigated the influence of emotional stress during exams on antioxidant protection in 10 young healthy students, 20-24 years; saliva was prelevated at 9 a.m. and at 9 p.m., two weeks before a difficult exam and the day of the exam .
Results: AOX (total antioxidant capacity) under stress was significantly lower by comparison with baseline mean value (p=0.001). A positive correlation between AOX values during stress and baseline AOX values and a negative correlation between AOX and cortisol during stress were found.
We concluded that under emotional stress salivary AOX decreased by 18.43%; salivary cortisol increased by 91.66%. Soon after exams finished, antioxidant protection means normalized for both parameters.
3. Physiological stress (n=30, age 16 - 82)
Routine dental treatments or orofacial surgical procedure were applied to this group. Saliva samples were prelevated 10 min before the start of procedure and 5 min after the completion.
Results: For less anxious and optimistic patients, salivary cortisol level is significantly lower versus anxious, pessimistic patients (p< 0.001). It is significantly high on the day of surgery for patients who received general anesthesia. Periodontal diseases are associated with oxidative stress and a defect in the total antioxidant activity of saliva.
Conclusions Our results underline that the dynamic measurement of cortisol in saliva provides a reliable tool to the basic scientist as well as to the clinician for the investigation of the HPA axis activity being also a reliable marker of stress level. Several of our studies have been able to identify some of the factors responsible for the heterogeneity in salivary cortisol response patterns.

B-12

A survey of context of orders for 1,25-OH vitamin D with respect to prior orders for 25-OH vitamin D and/or parathyroid hormone (PTH)

W. Rizzo, S. J. Falasco, L. J. McCloskey, D. F. Stickle. *Jefferson University Hospital, Philadelphia, PA,*

Background: Routine evaluation of Vitamin D status is via measurement of 25-OH vitamin D (25D). Increased interest in patients' vitamin D status, unrelated to any suspected disorder in calcium homeostasis, has been fueled by numerous reports on its role in various disease processes and has resulted in a substantial increase in the number of 25D tests ordered at our institution. Correspondingly, orders for 1,25-OH vitamin D (1,25D) have also increased and now represent a high-volume test, requested at a rate of approximately 1 per 7 orders for 25D. Circumstances in which 1,25D measurement should be performed are relatively rare, and therefore the high order rate for 1,25D raises the question of whether these orders are in majority clinically appropriate. Taking the premise that clinical algorithms leading appropriately to assessment of 1,25D call for prior measurement of either 25D or PTH or both, we examined 1,25D orders with respect to the prior status of measurements for 25D and PTH.
Methods: The base dataset consisted of orders and results for 1,25D, 25D and PTH over a period of one year (2009). First-or-only orders for 1,25D were tabulated and restricted to the second six month interval of the one-year period. These orders were then assessed with respect to prior, or concurrent, orders for 25D and/or PTH within the full 1-year period.
Results: There were 1071 1,25D measurements from among 879 patients in the one-year dataset, with distribution of 7.9% low, 86% normal (15-75 pg/mL), and 6.1% high. First-or-only 1,25 D measurements in the latter 6-month period of 540 measurements were from 403 patients (Dataset A), with distribution of 9.2% low, 83.6% normal (15-75 pg/mL), and 7.2% high. In the one-year dataset, there were 7103 25D measurements from among 4677 patients, with distribution of 58.5% low (<30 ng/mL) and 41.5% adequate; for PTH, 1279 measurements (49.3% of 2594 total) were from 888 (17.8%) of the 4998 patients who had either 25D or 1,25D measurements. The following results refer to the first-or-only list of patients in Dataset A. 81.6% had no prior measurements for 25D; 18.4% had prior 25D measurements (75 total: 48 (65%) low, 27 (35%) adequate); 50.9% had concurrent 25D measurements. 94.3% had no prior measurements for PTH; 5.7% had prior PTH measurements (23 total: 1 low, 5 normal (6-40 pg/mL), 17 (74%) high); 13.9% had concurrent PTH measurements. 78.9% had no prior measurements for either 25D or PTH. 40.2% had neither prior nor concurrent measurements for either 25D or PTH.
Conclusions: The high proportion of 1,25D orders (40.2%) with neither prior nor concurrent orders for 25D or PTH suggests that likely these were misorders with respect to a clinical algorithm that would lead appropriately to 1,25D measurement. The high rate of apparent misorders suggests that the confounding issue may be clerical rather than clinical, and that greater institutional oversight of 1,25D test orders is warranted.

B-13

A new case of prohormone convertase deficiency

J. R. Delanghe, H. Segers, T. Fiers, S. E. Delanghe, Y. Taes. *University Hospital Ghent, B 9000 Gent, Belgium,*

Background: a 64 year old diabetic patient was referred for unexplained hypergastrinemia (1796 ng/ml; ref. values: 3 - 115 ng/ml), which had been known for over 20 years. Clinical history revealed a pronounced postprandial reactive hypoglycemia. Despite the hypergastrinemia, medical history and gastric endoscopy did not reveal major gastric problems. A negative octreotide scan excluded the presence of a neuroendocrine tumor. Further laboratory investigations were carried out to explain these unexpected findings.
Methods: Gel permeation chromatography of the patient's serum on a Sephadex G-50

column (phosphate buffered saline 0.1 mol/L, pH 7.4, flow rate: 0.8 mL/cm²) followed by determination of gastrin, insulin and C-pepetide in the obtained fractions using immunoassay (Siemens Immulite 2000).
Results: Gel permeation chromatography revealed that gastrin immunoreactivity mainly corresponded to preprogastrin (11 kDa) and progastrin (8.9 kDa), next to a relatively small amount (<40 %) of gastrin-34 and gastrin-17. This finding suggests a prohormone convertase 1 (EC 3. 4. 21. 93) deficiency, a proteolytic enzyme which processes inactive prohormones (eg. progastrin, procholecystokinin, proinsulin) into smaller biologically active peptides. Further analysis of the fractions obtained by chromatography revealed a high concentration of proinsulin (concentration in serum: 38.28 ng/mL, ref. values: <0.188 ng/mL), with lower amounts of C - peptide (12.99 ng/mL; ref. values 1.1- 4.4 ng/mL).
Conclusion: The patient's hypergastrinemia and diabetes could be explained by a prohormone convertase 1 (EC 3. 4. 21. 93) deficiency. This condition is extremely rare, in contrast to three other cases described, no hyperphagia was present, and no signs of hypocorticism were found (as evidenced by normal ACTH and cortisol values).

B-14

Performance evaluation of an automated direct renin assay on the Liaison® system.

D. GRUSON, D. Maisin, D. Maiter, A. Persu. *Cliniques Universitaires St Luc, Bruxelles, Belgium,*

Introduction*:* Accurate measurement of renin is relevant for the screening of primary aldosteronism through its ratio with aldosterone and for management of hypertensive patients. The reference method for the determination of circulating renin levels remains the plasma renin activity (PRA). Nevertheless, PRA is time consuming, labour intensive and lacking of standardization. Recently, direct renin assays have entered the guidelines of the Endocrine Society for the case detection and diagnosis of primary aldosteronism. Therefore, the aim of this study was to evaluate the performances and reliability of a fully automated immunoassay for direct rennin measurement which may facilitate daily laboratory and clinical practices.
Methods*:* The concentrations of renin were measured for 213 patients with the PRA isotopic method and with the Liaison® direct renin assay (Diasorin), a fully automated immunoassay with chemiluminescence based detection. The method imprecision of the Liaison® method was determined through quality control materials. Reference values for the direct renin assay and ratio with aldosterone were determined using 100 specimen from healthy and normotensive volunteers.
Results*:* Between-run imprecision study provides coefficients of variation for the Liaison® renin assay of 3.7% at 28 µUI/mL and 2.7% at 106 µUI/mL. Passing and Bablock regression analysis between the two methods provides a slope of 38.7 and an intercept of -6.22677. A positive and significant correlation was observed between the two methods (r = 0.94). Furthermore, the Kappa coefficient of agreement between the two methods was good. Using the specimen from healthy volunteers, the 95% reference interval obtained had a lower limit of 4µUI/mL and an upper limit of 50 µUI/mL. The reference values for the aldosterone to direct renin ratio were 6 to 64 pM/µUI/mL.
Conclusions: Our results demonstrate that the analytical performances characteristics of the Liaison® direct renin assay allow its use for routine laboratory practice. Furthermore, the reference values that we have established for this renin assay and its ratio with aldosterone will be helpful for accurate screening of primary aldosteronism and management of hypertensive cases.

B-15

Evaluation of HbA1c Immunoassay on TOSOH AIA® Immunoassay Analyzer.

H. Uchimura[1], Y. Shizume[1], M. Tsuura[2], Y. Aono[3]. *[1]Shizume Institute of Endocrinology and Metabolism, Tokyo, Japan, [2]Tosoh Corporation, Kanagawa, Japan, [3]Tokyo Bunka Junior College, Tokyo, Japan,*

Hemoglobin A1c (HbA1c) is known to be the most important indicator for a long-term assessment of the glycemic state for diabetic patients. According to the International Expert Committee with members appointed by ADA, EASD, and IDF, the A1C assay provides a reliable measure of chronic glycemia and correlates well with the risk of long-term diabetes complications, and the diagnosis of diabetes is made if the A1C level is >/= 6.5 %[(1)]. TOSOH developed HbA1c immunoassay for TOSOH AIA analyzers for which has provided more than 40 test items including tumor markers, cardiac markers, thyroids, reproductive hormones, etc. We had a chance to achieve performance testing and verify utility of AIA-HbA1c immunoassay using TOSOH AIA-600II.
TOSOH HbA1c immunoassay, ST AIA-PACK HbA1c*, is an enzyme immunoassay which, after pretreatment, is performed entirely in the test cups. The whole blood sample is firstly pretreated with the HbA1c Pretreatment Solution at 40°C for 20 minutes. The pretreated sample is then automatically sampled into an test cup. HbA1c present in the pretreated sample competes with hemoglobin to be captured on the magnetic beads and binds to enzyme-labeled sheep anti-HbA1c polyclonal antibody. After 10 minutes incubation at 37°C, the magnetic beads are washed to remove unbound materials and are then incubated at 37°C with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled polyclonal antibody that binds to the magnetic beads is directly proportional to the percentage of HbA1c in the sample. A standard curve is constructed using the Calibrator Set aligned to NGSP %, and unknown percentage of HbA1c is automatically calculated using this curve.
The within-run and total precisions (CV%) determined based on guidance from CLSI Protocol EP5-A2 and using 5.4%, 8.3% and 13.2% whole blood control materials were 1.7 - 1.9% and 3.3 - 3.8 %. The linearity range was demonstrated as 2.2 - 15.3 %. No clinically significant interferences were observed in samples containing elevated concentrations of bilirubin, triglycerides, albumin, labile hemoglobin A1c, carbamylated and aldehyde hemoglobins. In the correlation study, the AIA-HbA1c gave a good correlation with commercial kits (TOSOH HLC-723G8 and Siemens DCA-2000) as: AIA-HbA1c = 0.94 x (HLC-723G8) + 0.37, r = 0.96, n = 89. AIA-HbA1c = 0.95 x (DCA-2000) + 0.16, r = 0.977, n = 89. In the case of samples containing hemoglobin variants including HbC, HbS, HbD and HbE, they did not affect the results obtained by AIA-HbA1c. HbF did not interfere with the assay up to 10.0% and specimens from anemic patients did not interfere with the assay for more or equal to 7.0 g/dL total hemoglobin concentration.
In conclusion, we confirmed TOSOH HbA1c immunoassay provided reliable performances for the screening and management of diabetic patients.
(1): *Diabetes Care* 2009; 32: 1327-1334.
*This product has not been approved by the FDA yet.

B-16

Increased Plasma Levels of Methylglyoxal and Adhesion Molecules in Diabetic Ketoacidosis

J. Lu[1], Q. H. Meng[2], E. Randell[3], K. Adeli[4], J. Krahn[2]. *[1]Wenzhou Medical College, Wenzhou, China, [2]University of Saskatchewan, Saskatoon, SK, Canada, [3]Memorial University of Newfoundland, St Johns, NL, Canada, [4]University of Toronto, Toronto, ON, Canada,*

Objectives: Diabetic ketoacidosis (DKA) is a severe acute complication of diabetes. DKA is characterized by high blood glucose, acidosis and high levels of ketone bodies. Methylglyoxal (MG), a very reactive metabolite of glucose, is highly elevated in diabetes and is associated with the development of diabetic complications. Hyperglycemia is associated with increased expression of adhesion molecules including soluble intercellular adhesion molecule (sICAM) and soluble vascular cell adhesion molecule (sVCAM), indicators of vascular endothelial dysfunction. This study was to investigate the association of plasma levels of MG and adhesion molecules with diabetic ketoacidosis.
Subjects and methods: In this study, 48 diabetic patients without ketoacidosis, 47 diabetic patients with ketoacidosis and 43 healthy controls were included. Fasting blood samples were obtained. Plasma MG and MG-derived hydroimidazolone (MG-H) were measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Plasma adhesion molecules (sICAM and sVCAM) and soluble form of receptor for advanced glycation end products (sRAGE) were assayed by enzyme-linked immunosorbent assay (ELISA) using commercial ELISA kits (R&D Systems). Plasma glucose, □-hydroxybutyrate and blood hemoglobin A1c (HbA1c) were determined on the automated chemistry analyzers. Plasma ketones, a semi-quantitative method based on the nitroprusside reaction with ketone bodies were detected using Acetest® reagent tablets (Bayer Health Care, Elkhart, IN).
Results: Fasting plasma glucose, β-hydroxybutyrate and HbA1c were significantly increased in both the Diabetes and DKA groups compared to the control group. Furthermore, plasma glucose, β-hydroxybutyrate and blood HbA1c in DKA were higher than in the Diabetes without DKA ($P < 0.001$). The plasma MG and MG-H levels were highly elevated in DKA compared to the Diabetes or to the control group (325.41 ± 140.62 vs. 147.70 ± 59.26, or 169.80 ± 93.67 nmol/L for MG, $P < 0.001$). Plasma MG levels were positively correlated with fasting glucose levels ($r = 0.695$, $P < 0.001$) and HbA1c ($r = 0.323$, $P < 0.001$). Significantly elevated plasma levels of sICAM and sVCAM were found in DKA group compared to the Diabetes and to the control groups (403.98 ± 200.92 vs. 279.13 ± 95.31 and 403.98 ± 200.92 vs. 277.29 ± 79.72 ng/ml for sICAM and 892.70 ± 477.94 vs. 503.05 ± 145.03 and 892.70 ± 477.94 vs. 487.45 ± 166.71 ng/ml for sVCAM, respectively). There was no difference between the Diabetes and the control group in sICAM and sVCAM levels.
Conclusions: Fasting plasma glucose, β-hydroxybutyrate and HbA1c levels are highly elevated in DKA. Increased plasma levels of MG, MG-H, sRAGE and adhesion molecules are observed in patients with DKA compared to the Diabetes and to the control groups. These data indicate that MG is likely to be involved in the development of DKA. Moreover, in acute DKA endothelial function is damaged as indicated by high levels of adhesion molecules. Laboratory monitoring of these biochemical markers may predict the development and progression of acute diabetic complications such as DKA.
Keywords: Diabetes, Diabetic ketoacidosis, Methylglyoxal, Adhesion molecule

B-17

Increased Plasma Methylglyoxal Level, Inflammation, and Vascular Endothelial Dysfunction in Diabetic Nephropathy

Q. H. Meng[1], J. Lu[2], E. Randell[3], K. Adeli[4], J. Krahn[1]. *[1]University of Saskatchewan, Saskatoon, SK, Canada, [2]Wenzhou Medical College, Wenzhou, China, [3]Memorial University of Newfoundland, St Johns, NL, Canada, [4]University of Toronto, Toronto, ON, Canada,*

Objectives: Methylglyoxal (MG) is a highly reactive metabolite of glucose and plays an important role in the pathogenesis of diabetes and diabetic complications. Studies have shown that increased inflammatory reaction is the critical pathological step in the development of micro-vascular diabetic complications. Hyperglycemia is associated with increased expression of adhesion molecules including soluble intercellular adhesion molecule (sICAM) and soluble vascular cell adhesion molecule (sVCAM); indicators of vascular endothelial dysfunction. In this study, we investigated the association of MG, inflammation and adhesion molecules with diabetic nephropathy (DN) in type 2 diabetes (T2DM).

Materials and methods: Fasting blood samples were obtained from 42 T2DM patients without DN, 30 diabetic patients with DN and 41 age-matched healthy controls. Plasma MG and MG-derived hydroimidazolone (MG-H) were measured by liquid chromatography/ tandem mass spectrometry (LC-MS/MS). Plasma cytokines (IL6 and TNF-α), adhesion molecules (sICAM and sVCAM) and soluble form of receptor for advanced glycation end products (sRAGE) were assayed by enzyme-linked immunosorbent assay (ELISA) (R&D Systems). Other biochemical assays were performed in the chemistry laboratories using automated instruments.

Results: The plasma levels of fasting glucose in DN were significantly higher than those in T2DM and non-diabetic controls (13.06 ± 4.35 vs. 10.41 ± 3.71, $P < 0.05$ and 13.06 ± 4.35 vs. 5.18 ± 0.43 mmol/L, $P < 0.001$, respectively). The blood hemoglobin A1c (HbA1c) levels were significantly elevated in DN (10.45 ± 2.32%) compared to those in T2DM (9.18 ± 2.24%) and non-diabetic controls (5.33 ± 0.37%). The plasma MG levels in DN patients were significantly higher that in T2DM patients and the controls (311.64 ± 134.67 vs. 202.16 ± 73.44 and 311.64 ± 134.67 vs. 147.82 ± 77.93 nmol/L, respectively, $P < 0.001$). The plasma MG levels in the T2DM were elevated but did not reach statistical significance compared to the controls. The plasma levels of MG were positively correlated with the fasting glucose levels ($r = 0.695$, $P < 0.001$), HbA1c ($r = 0.323$, $P < 0.001$), and urinary albumin/creatinine ratio ($r = 0.754$, $P < 0.05$). Significantly elevated plasma levels of MG-H and sRAGE were also found in DN groups as compared to controls. Plasma IL-6 and TNF-α levels were markedly increased in DM and DN compared to the controls with the DN group having a significantly higher value than the DM group ($P < 0.05$). Highly elevated plasma levels of sICAM and sVCAM were observed in DN (400.80 ± 195.93 and 889.42 ± 495.55 ng/ml) compared to the T2DM (305.27 ± 131.77 and 576.91 ± 251.74 ng/ml) and to the controls (278.33 ± 81.19 and 486.31 ± 170.04 ng/ml, respectively).

Conclusions: Increased plasma levels of MG, cytokines and adhesion molecules are observed in patients with DN. The levels of these biochemical markers are well associated with development of DN. Our data indicate that MG, cytokines and adhesion molecules are likely to be involved in the development of DN in T2DM. Monitoring of these biochemical markers may predict the development and progression of DN.

Keywords: Methylglyoxal; Inflammation; Adehesion Molecules; Diabetes; Diabetic Nephropathy

B-18

Quantification of active Renin from EDTA plasma with a sandwich ELISA and chromogenic substrate

M. Herkert. *DRG International, Inc., Mountainside, NJ,*

Introduction: Renin is an aspartic acid protease that belongs to the Renin-Angiotensin-Aldosterone System (RAAS) that controls blood pressure, renal blood flux, glomerular filtration, and sodium/potassium homeostasis. Renin can be quantified either as plasma renin activity (PRA) or as concentration of active plasma renin protein. Until now, renin concentration was determined either with a closed system based on a direct, two site, sandwich type immunoluminometric assay using magnetic particles, or with open systems based on a two-site immunoradiometric assay (IRMA). Here we present a non-radioactive direct sandwich ELISA for the quantification of active renin in human EDTA plasma. Procedure: Microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site of human Renin. 75 µl of EDTA plasma are incubated for 2 hours in the coated well together with 25 µl Assay Buffer. Unbound components are washed off with 4x300 µl washing buffer, and 100 µl Enzyme Conjugate, which is an anti-Renin antibody conjugated with horseradish peroxidase, is added for 30 min. After 4x300 µl washing steps, 100 µl TMB substrate is incubated for 15 min, and the reaction is stopped with 100 µl Stop Solution. OD values are measured at 450 nm, and quantification is done based on the standard curve. Specimen exceeding the highest standard can be diluted with Assay Buffer and analyzed again. Only EDTA plasma can be used in this assay. Specimens should be capped and stored at room temperature. If samples cannot be tested within 4 hours of primary collection, store frozen at -20°C or below. Renin standard curve comprises 7 standards that have been calibrated to the 1st WHO International Reference Preparation (NIBSC Code 68/356). Best fit is obtained using a non-weighted 4-parameter equation. Two controls containing low and high concentrations of renin are included in the ELISA Kit for performance control. Results: The dynamic range of the assay is between 1.13 - 128 pg/mL. The analytical sensitivity of the DRG ELISA was found to be 1.13 pg/mL. The mean intra-assay precision (determined with 3 samples covering the measuring range of the assay) was 6.33 % (n=3; range from 5.5-7.3%). The mean inter-assay precision was 6.37% (n=3; range from 4.2-7.6). The mean recovery was 101.13 % (n=3; range from 89.5-108.8). The mean linearity was 93.87 % (n=3; range from 85.3-101.8%). No high dose hook effect was observed up to 8,192 pg/mL of Renin. Cross reactivity with Prorenin was 0.69 % (mean value when prorenin was spiked in human plasma without analyte in a concentration range from 256 - 4096 pg/mL. Hemoglobin (up to 1 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) had no influence on the assay results. The accuracy of the active Renin ELISA was determined by comparison with Renin III Generation IRMA (Cisbio). Correlation coefficient was 0.943 (n=100); $y=0.945x + 3.096$; sample concentration ranged from 1.7-240 pg/mL. Conclusion: In case of dysfunction of the RAAS, quantification of plasma Renin with DRG´s non-radioactive sandwich ELISA will provide standardized and reliable results in less than 3 hours.

B-19

HbA_{1c} is not a good screening test for diabetes

P. Steele[1], T. Higgins[2], G. Cembrowski[3], D. Tran[3], C. Shalopay[3]. *[1]Marshfield Laboratory, Marshfield, WI, [2]DynaLIFE Diagnostics, Edmonton, AB, Canada, [3]University of Alberta Hospital, Edmonton, AB, Canada,*

Introduction: Following recommendations from a 2009 International Expert Committee regarding the clinical utility of $HbA1_c$, the American Diabetes Association (ADA) adopted a threshold of ≥6.5% as criteria for the diagnosis of diabetes. The objective of this study was to determine the positive and negative predictive values of using $HbA1_c$ as a screening test compared to the long standing criteria based upon plasma glucose.

Methods: A retrospective study on plasma glucose (random, fasting, or following a 2 hour oral glucose tolerance test) together with $Hb1A_c$ results over a 3 year period were extracted from a laboratory located in central Wisconsin. In total, 271 data pairs were collected; 78 met the ADA criteria for the diagnosis of diabetes (fasting glucose ≥126 mg/dL or random glucose of ≥ 200 mg/dL or a 2 hour glucose of ≥ 200 mg/dL following an oral glucose tolerance test). ROC curves were plotted to determine the $HbA1_c$ value with optimal sensitivity and specificity using the glucose values as the reference standard. Positive (PPV) and negative predictive values (NPV) were calculated.

Results:

HbA1c result (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value	Negative Predictive Value
5.7	96.2	38.3	0.147	0.981
6.0	78.2	64.2	0.195	0.964
6.5	39.7	93.8	0.416	0.933
7.0	16.7	99.0	0.645	0.085

Conclusion: These values are comparable to results published earlier by the latter authors derived in a large metropolitan area in Canada. The current $HbA1_c$ cut point recommended by the ADA did not yield an optimal balance between sensitivity and specificity. While the PPV below the current threshold were low, the NPV were quite high. While these data suggest that the current cut point is suboptimal in the diagnosis of diabetes, the NPV of $HbA1_c$ may be useful to rule out this disease

B-20

C-peptide isolation from human urine and plasma and quantitative analysis by LC/MS.

A. Stoyanov, C. Rohlfing, R. Little. *University of Missouri, Columbia, MO,*

Background: C-peptide results from the proteolytic cleavage of pro-insulin; it serves is an important indicator of endogenous insulin production in patients with diabetes. There are a number of commercial C-peptide assay methods currently in use; it has been shown that results from these methods are not always comparable to each other, and that harmonization to a reference method greatly improves the comparability of results. Here, we demonstrate a potential reference method for measuring C-peptide in urine and plasma utilizing LC/MS.

Methods: Serum and urine samples were initially mixed with two volumes of methanol

followed by centrifugation for 5 minutes at 10g. The samples were then prepared using either sequential ion-exchange chromatography in combination with SPE or ultrafiltration. Isotope labeled C-peptide (M+30) was used as the internal standard. A API-4000 MS/MS mass spectrometry system (AB SCIEX, Foster City, CA) with a Shimadzu Prominence LC (Shimadzu Scientific Instruments, Columbia, MD) was used for quantitation. Two-dimensional LC utilizing ion-exchange and reverse phase chromatography was used to for the initial separation.

Results: The reference method allowed high throughput and detection of very low (less than 10pg/mL) C-peptide concentrations in biological fluids due to the fact that either IE/SPE or ultrafiltration achieves 10x or greater concentration of C-peptide prior to analyses.

Conclusion: The proposed reference method for C-peptide using 2D ion-exchange/reverse phase LC followed by MS achieves both high throughput and high sensitivity. This technique has potential for wide-ranging application in the analyses of biological fluids and multi-compound mixtures.

B-21

Performance of HbA1c as a Diagnostic Test for Diabetes and Pre-Diabetes

G. Ogbonna[1], S. Stearns[1], R. White[1], D. Hawks[1], L. Fox[1], P. B. Musholt[2], T. A. Mohr[2], T. Forst[2], A. Pfutzner[3]. *[1]Ortho-Clinical Diagnostics, Rochester, NY, [2]IKFE, Institute of Clinical Research, Mainz, Germany, [3]IKFE, Insitute of Clinical Research, Mainz, Germany,*

Despite the wide availability of tests to diagnose type 2 diabetes (T2D), the disease remains under-diagnosed as up to 25% of patients with a new diagnosis already have micro-vascular complications. To identify more diabetics and pre-diabetics, the American Diabetes Association has included the HbA1c test in their clinical practice recommendations for diabetes and prediabetes diagnosis. The recommendation called for an HbA1c cutoff of 6.5% for diabetes diagnosis and values from 5.7% to 6.4% for the diagnosis of pre-diabetes. We have assessed the performance of the HbA1c test to diagnose diabetes and prediabetes in comparison to the oral glucose tolerance test (OGTT) and fasting plasma glucose (FPG). In the study, we recruited subjects at high risk of metabolic diseases without a prior diagnosis of diabetes. The subjects were within the age range of 18 to 75 years and met at least two risk factors for the development of metabolic diseases as defined by the National Cholesterol Education Program's Adult Treatment Panel III report. The subjects were pre-screened two weeks before the OGTT procedure based on FPG cutoff of 126 mg/dL to include mostly OGTT diabetic and impaired glucose tolerance (IGT) subjects. The subjects were prepared by a physician for the OGTT procedure in order to create uniform instructions for participants. Glucose was measured using fasting plasma samples collected in sodium fluoride phlebotomy tubes. HbA1c was measured using EDTA whole blood collected before the OGTT procedure. The subjects included 124 normal glucose tolerant (NGT), 175 pre-diabetics and 32 type 2 diabetics. The pre-diabetics included 94 isolated impaired fasting glucose (IFG), 24 isolated IGT and 57 combined IFG and IGT subjects. The type 2 diabetes subjects included 6 by FPG only, 13 by OGTT only and 13 by both FPG and OGTT. We estimated the ability of HbA1c with a cutoff of 6.5% to detect type 2 diabetes as defined by either OGTT ($\geq$ 200 mg/dL) or FPG ($\geq$126 mg/dL) in the whole population to have sensitivity of 28.1% and specificity of 100%. We also estimated the ability of the range 5.7% $\leq$ HbA1c < 6.5% to detect pre-diabetics as defined by 140 mg/dL $\leq$ OGTT 2 hour glucose < 200 mg/dL or 100 mg/dL $\leq$ FPG < 125 mg/dL in the whole population to have sensitivity of 28.6% and specificity of 89.7%. Our study results, in a high risk population, support an HbA1c cutoff of 6.5% to rule in patients with type 2 diabetes. The results also support the use of an HbA1c range from 5.7% to 6.4% to rule in patients with pre-diabetes. However, in this at risk population, HbA1c alone and at these cutoffs fails to detect more than 70% of diabetic and pre-diabetic subjects in a population previously screened with fasting plasma glucose.

B-22

Development and validation of an isotope dilution tandem mass spectrometry method for simultaneous quantification of 3-iodothyronamine, thyroxine, triiodothyronine, and 3,3'-diiodo-L-thyronine

J. Gu[1], O. P. Soldin[2], S. J. Soldin[1]. *[1]Bioanalytical Core Laboratory, GCRC, Georgetown University, Washington, DC, [2]Department of Oncology, Lombardi Cancer Center, Georgetown University, Washington, DC,*

Introduction: The thyroid hormones, thyroxine (T4), triiodothyronine (T3), and diiodothyronines (T2), are important in regulating a number of biological processes. 3-Iodothyronamine (T1AM), the other endogenous iodine-containing signaling molecule, is a newly discovered derivative of T4 with opposite biological effects to those of T3. To fully understand the physiology and pharmacology of 3-iodothyronamine, a sensitive and selective analytical method is required. The goal of the study was to develop an isotope dilution tandem mass spectrometry method to simultaneously measure T1AM , T4, T3, and 3,3'-diiodo-L-thyronine (3,3'-T2) in human plasma/serum, which is the first to enable the quantification of endogenous T1AM and 3,3'-T2 in human plasma/serum.

Methods: An API-5000 tandem mass spectrometer equipped with TurboIonSpray source and Shimadzu HPLC system was employed to perform the analysis using isotope dilution with internal standard for each analyte. 200 uL of human plasma/serum was deproteinized by adding 200 uL of acetonitrile containing internal standards. After centrifugation, 250 µL of supernatant was diluted with 250 µL of distilled de-ionized water and a 250 µL aliquot was injected onto an Agilent Zorbox SB-C18 (2.1 x 30 mm, 1.8-micron) chromatographic column, where it underwent cleaning with 2% (v/v) methanol in 0.01% formic acid at a flow rate of 0.25 mL/min. After a 5 min wash, the switching valve was activated and the analytes of interest were eluted from the column with a water/methanol gradient at a flow rate of 0.25 mL/min and then introduced into the mass spectrometer. Quantification by multiple reaction-monitoring (MRM) analysis was performed in the positive mode. The transitions to monitor were selected at mass to-charge (m/z) 356.1->212.0 for T1AM, 360.1->216.1 for T1AM-d4, 777.7->731.5 for T4, 782.7->736.5 for T4-d5, 651.8->605.8 for T3, 657.8->611.7 for T3-13C6, 525.9 ->480.0 for 3,3'-T2, 531.9->485.9 for 3,3'-T2-13C6 . Nitrogen served as auxiliary, curtain, and collision gas. The main working parameters of the mass spectrometer were: collision gas 9, curtain gas 35, nebulizer gas 50, turbo gas 50, ionspray voltage 5500 V, probe temperature 650 °C, and dwell time 200 msec. Accuracy and precision were evaluated by analyzing three concentration levels of in-house quality controls on 10 different days.

Results: The within-day and between-day coefficients of variation (CVs) were between 2%-9 % for all analytes at all concentration tested. Accuracy ranged between 95% and 105%. Good linearity was also obtained within the concentration range of 2.5-1000 pg/mL for T1AM, 2.5-1000 pg/mL for 3,3'-T2, 0.025-10 ng/mL for T3 and 0.5-200 ng/mL for T4 (r > 0.999). Concentrations of T1AM found in controls ranged between 0-30 pg/mL.

Conclusions: A sensitive, simple, accurate, and specific isotope dilution tandem mass spectrometry method was developed for the simultaneous determination of T1AM , T4, T3, and 3,3'-T2 in human plasma/serum samples by employing the API-5000 tandem mass spectrometer.

B-23

Performance of LOCI® Assays for FSH*, LH*, and Prolactin* on the Dimension Vista® System

N. Zolotarjova, L. Amsel, E. Garcia, C. Moran, M. Wasson, J. Pierson-Perry. *Siemens Healthcare Diagnostics Inc., Newark, DE,*

We describe the design and analytical performance of fully automated homogeneous sandwich immunoassays for follicular stimulating hormone (FSH*), luteinizing hormone (LH*), and prolactin (PRL*) on the Dimension Vista® system. The methods are based on LOCI® technology. Each method uses three reagents: two synthetic bead reagents and a biotinylated analyte-specific tag antibody fragment. The first bead reagent (Sensibeads) is coated with streptavidin and contains a photosensitizer dye. The second bead reagent (Chemibeads) is coated with the respective method's capture antibody and contains chemiluminescent dye. Test sample is incubated with Chemibeads and biotinylated antibody to form bead-analyte-biotinylated antibody sandwiches. Sensibeads are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of the complexes at 680 nm generates singlet oxygen from Sensibeads which diffuses into the Chemibeads, triggering a chemiluminescent reaction. The resulting signal (measured at 612 nm) is directly related to the analyte concentration. Time to first result is 10 minutes for each method.

Sample volumes are 2 µL (LH, PRL) or 4 µL (FSH) of serum or plasma. Analytical measurement ranges are 0.2-200 mIU/mL (FSH), 0.2-150 mIU/mL (LH), and 0.2-250 ng/mL (PRL), standardized respectively to WHO 92/510, 80/552, and 84/500. No high-dose hook effect was observed to at least 2,000 mIU/mL (FSH, LH) or 30,000 ng/mL (PRL). Precision was assessed per CLSI EP5-A2 using patient pools and commercial QC materials. Repeatability < 2.4 %CV and with-lab precision < 4.5 %CV were observed across the assay ranges for the respective methods. No significant interference (<10 % bias) was seen from lipemia (1000 mg/dL triglycerides), hemolysis (1000 mg/dL hemoglobin), or icterus (40 mg/dL conjugated bilirubin or 60 mg/dL unconjugated bilirubin). Comparison of results from patient samples processed by the three methods (Y) to their respective predicate methods on the ADVIA Centaur® system (X) showed good agreement by linear regression across the respective assay ranges: Y = 1.05X + 0.20, r = 0.99 for FSH;. Y = 0.84X + 1.9, r = 0.99 for LH; and Y = 1.14X + 0.37, r = 0.99 for PRL. We conclude that the LOCI methods for FSH, LH, and prolactin on the Dimension Vista system provide acceptable accuracy, precision, turnaround time, and assay range for quantitation of the analytes in serum and plasma.

* Product under development_Not available for sale

B-24

Development and Performance of an Estradiol Assay* on the Dimension Vista® System.

L. Geng, J. E. Thomas, Z. Teng, M. Drinan. *Siemens Healthcare Diagnostics, Newark, DE,*

We describe the design and analytical performance of a fully automated homogeneous competitive immunoassay for estradiol (E2) on the Dimension Vista® System. The E2* method utilizes LOCI® technology and three reagents: two synthetic bead reagents and a biotinylated monoclonal antibody specific for estradiol. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibeads) is coated with estriol (E3), an estradiol analog, and contains chemiluminescent dye. In the first step, sample is incubated with biotinylated antibody which allows E2 from the sample to saturate a fraction of the biotinylated antibody that is directly related to the E2 concentration. In a second step, E3 chemibeads are added and form bead/antibody immunocomplexes with the non-saturated fraction of the biotinylated antibody. Sensibeads are then added and bind to the biotin to form bead pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from sensibeads, which diffuses into chemibeads, triggering a chemiluminescent reaction. The resulting signal (measured at 612 nm) is an inverse function of the E2 concentration in the sample.

The method uses a 10 µL sample volume of serum or plasma and has an analytical range of 10-1500 pg/mL, with results traceable to an ID/GC/MS reference method. The time to first result is 10 minutes. Precision was evaluated per CLSI EP5 using serum pools and commercial quality control materials. Repeatability and within-lab precision were ≤ 3.6 %CV and ≤ 7.1 %CV, respectively, across the assay range. Good agreement was observed in patient sample method comparison studies versus three different systems by Ordinary Least Squares regression analysis : Dimension Vista E2 = 1.01 * ID/GC/MS Estradiol + 0.4 pg/mL (r = 0.999, n = 54), Dimension Vista E2 = 0.99 * IMMULITE® 2000 E2 + 5.4 pg/mL (r = 0.986, n = 59), and Dimension Vista E2 = 0.91 * Roche ELECSYS® 2010 E2 - 2.6 pg/mL (r = 0.999, n = 51). Minimal cross reactivity (<0.1%) was observed with key compounds including: 17α-estradiol, estrone, estrone-3-sulfate, estriol, estriol-3-sulfate, DHEA, DHEA-sulfate, androstenedione, androsterone, testosterone, 5 α-dihydrotestosterone, progesterone, 17α-hydoxyprogesterone, cortisol, 11-deoxycortisol, corticosterone, ethinyl estradiol, clomiphene and prednisolone.

Conclusions. We conclude that use of LOCI technology provides acceptable sensitivity, precision, accuracy, turnaround time, and dynamic range suitable for measurement of estradiol.

*product under development_not available for sale

B-25

Development and Performance of a Progesterone Assay* on the Dimension Vista® System

L. Geng, J. E. Thomas, S. Hopkins, Z. Teng, M. Drinan. *Siemens Healthcare Diagnostics, Newark, DE,*

We describe the design and analytical performance of a fully automated homogeneous competitive immunoassay for progesterone (PROG) on the Dimension Vista® System based upon LOCI® technology. The PROG* method utilizes three reagents: two synthetic bead reagents and a biotinylated monoclonal antibody specific for progesterone. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibeads) is coated with progesterone and contains chemiluminescent dye. In the first step, sample is incubated with biotinylated antibody which allows progesterone from the sample to saturate a fraction of the biotinylated antibody that is directly related to the progesterone concentration. In a second step, progesterone Chemibeads are added and form bead/antibody immunocomplexes with the non-saturated fraction of the biotinylated antibody. Sensibeads are then added and bind to the biotin to form bead pair immunocomplexes. Illumination of the complex at 680 nm generates singlet oxygen from Sensibeads, which diffuses into Chemibeads, triggering a chemiluminescent reaction. The resulting signal (measured at 612 nm) is an inverse function of the progesterone concentration in the sample.

The method uses a 10 µL sample volume of serum or plasma and has an analytical range of 0.2-40 ng/mL, with results traceable to an ID/GC/MS reference method. The time to first result is 21 minutes. Precision was evaluated per CLSI EP5 using serum pools and commercial quality control materials. Repeatability and within-lab precision were ≤ 5.7 %CV and ≤ 6.3 %CV, respectively, across the assay range. Good agreement was observed in patient sample method comparison studies versus three different systems by Passing and Bablok linear regression analysis: Dimension Vista PROG = 0.90 * ID/GC/MS Progesterone + 0.52 ng/mL (r = 0.985, n = 38), Dimension Vista PROG = 0.98 * IMMULITE® 2000 PRG + 0.15 ng/mL (r = 0.975, n = 34), and Dimension Vista E2 = 0.78 * Roche ELECSYS® 2010 PROG + 0.52 ng/mL (r = 0.977, n = 34). Minimal cross reactivity (<1%) was observed with key compounds including: 17α-hydoxyprogesterone, pregnenolone, 17β-estradiol, DHEA-sulfate, androsteindiol, testosterone, cortisol, 11-deoxycortisol, corticosterone, 11-deoxy corticosterone.

Conclusions. We conclude that use of LOCI technology provides acceptable sensitivity, precision, accuracy, turnaround time, and dynamic range suitable for measurement of progesterone in serum and plasma.

* Product under development_Not available for sale

B-26

Evaluation of a radioimmunoassay specific for 1-25 Dihydroxy Vitamin D2 and D3 measurement: Comparison with LC/MS/MS.

S. Hsu, T. Daly, M. K. Gupta. *Cleveland Clinic Foundation, Cleveland, OH,*

Introduction: Vitamin D3 is hydroxylated at 25 position in the liver to form 25[OH]D, the major reservoir for circulating vitamin D in the body. A second hydroxylation occurs at the 1α position primarily in the kidney to produce 1,25-$[OH]_2$-D. In renal disease this hydroxylation step is compromised leading to reduced circulating levels which lowers serum calcium and increase parathyroid hormone production thus causing the secondary hyperparathyroidism.

Methods: We evaluated the Diasorin 1,25$[OH]_2$D radioimmunoassay [RIA] that is specific for both 1,25 $[OH]_2$ D2 and D3. The assay required acetonitrile extraction of samples followed by further purification on C_{18}OH cartridges prior to RIA analysis. The analytical parameters were assessed. The clinical validation included analysis of 111 samples from healthy subjects and 114 from clinical patient samples sent to outside reference lab [86 by RIA from another lab and 28 by LC-MS/MS].

Results: The diagnostic sensitivity of the assay was 7 pg/mL and both within and between assay precisions ranged between 6.4-14%. The mean ± SD for 1-25$[OH]_2$ D level in 111 healthy subjects was 52.8 ±17 pg/mL and the central 95% range was19.6-83.6 pg/mL. The correlation coefficient for RIA was 0.87 [slope 0.89; intercept 8.3] and for LC-MS/MS was also 0.87 [slope 1.29 intercept -5.3]. There was 99% concordance between our results and RIA performed at another lab and 100% concordance between RIA and LC-MS/MS assays for the detection of abnormal low levels.

Conclusions: Our results demonstrate that RIA correlates well with LC-MS/MS even though the levels generated by LC-MS/MS were slightly lower. It is a reasonably precise and accurate method for assessment of 1,25[OH]2 D levels to detect renal disease associated secondary hyperparathyroidism.

B-27

Free Testosterone By Equilibrium Dialysis-LC-MS/MS

E. Erdogan[1], C. J. Haglock[2], J. C. Sawyer[2], M. M. Kushnir[2], B. Yue[2], W. L. Roberts[1], W. A. Meikle[1], A. L. Rockwood[1]. *[1]Department of Pathology, University of Utah, Salt Lake City, UT, [2]ARUP Institute for Clinical & Experimental Pathology, Salt Lake City, UT,*

Introduction: Testosterone (Te), a major androgen in males, is involved in the development and maintenance of the male phenotype. Te, also the predominant bioactive androgen in women, circulating at lower concentrations compared those in men. Te is important for muscle mass, bone metabolism, cognitive function, and libido. In men, testosterone concentrations are usually measured to evaluate Leydig cell function. In women, Te concentration is measured as part of the investigation of hyperandrogenism. In children, Te concentration is measured for gender assignment of newborns or infants with ambiguous genitalia, for disorders of puberty, inborn errors of sex-steroid metabolism, and in the follow-up of patients with congenital adrenal hyperplasia. Te circulates bound to sex hormone binding globulin (SHBG), and albumin as well as free Te. Free and albumin bound forms are biologically active. Medical conditions that alter the serum concentrations of SHBG or albumin may affect the total testosterone level, and measuring free Te (FTe) would better reflect a patient's testosterone status. In this work we report a direct free testosterone test which will include; physical separation of free testosterone by equilibrium dialysis followed mass spectrometry for quantitative analysis.

Methods: Aliquots of 200 µL serum samples or quality controls were dialyzed against 200 µL dialysis buffer (PBS) at 37 °C and pH 7.2 for approximately 20 h. The dialysate was spiked with stable isotope-labeled internal Te standards and extracted into methyl-tert-butyl ether. The solvent was evaporated and steroids derivatized with hydroxylamine. Instrumental analysis was performed on an API 4000 (Applied Biosystems/ MDS SCIEX) using multiple reaction monitoring (MRM) mode with MRM transitions of 304 to 124, and 304 to 112 (m/z). Two-dimensional chromatographic separation was performed using a C6 cartridge as the first dimension, and a Gemini C18 column as the second dimension.

Results: LOQ and ULOL were 5 and 1000 pg/mL, respectively. Total imprecision of the method, at concentrations 5-200 pg/mL, was less than 15% for free testosterone. We analyzed 45 male samples (0.2-193 pg/mL), whose total testosterone concentrations were determined by the LC-MS/MS method, and FTe concentrations were calculated

from total Te, SHBG, and albumin concentrations using a mathematical algorithm. The same samples were dialyzed and analyzed by LC-MS/MS (ED-LC-MS/MS). The method comparison yielded the linear regression equation LC/MS-MS (mathematical algorithm) = 0.95*ED-LC-MS/MS - 3.37 (r=0.98).
Conclusion: The results obtained from dialysis validate the mathematical modeling we use routinely to determine FTe concentrations. The method has sufficient sensitivity to determine free testosterone concentrations in pubertal and post-pubertal males.

B-28

Development of a New Generation Hemoglobin A1c Assay on Synchron UniCel® DxC Clinical Chemistry Systems from Beckman Coulter.*
M. NECULA, P. Kraght, L. Eguchi, M. Herrera, T. Houts, M. Metzler, L. Wong. *Beckman Coulter, Brea, CA,*

Objective: This study was aimed at assessing the feasibility of a new Hemoglobin A1c (HbA1c) assay on Synchron UniCel DxC® systems for the quantitative determination of HbA1c in human whole blood (hWB).
Relevance: HbA1c is an increasingly widely used marker in the management of diabetes.
Methodology: Hb and HbA1c assays are used to determine HbA1c concentration as a percentage of total hemoglobin (Hb). Concentration is measured by colorimetric and immunoturbidimetric methods for Hb and HbA1c, respectively. System parameters (volumes, injection times, wavelengths, extinction coefficients, volume correction, interference correction, calibrator levels, and dose-response math model) were optimized by combining design of experiments (DOE) and classic experimentation. Anchoring to the reference method was achieved by assigning assay calibrators directly from IFCC (International Federation of Clinical Chemistry) calibrators. Accuracy was determined by assaying hWB samples with known NGSP (National Glycohemoglobin Standardization Program) target values, following NGSP protocol. The results were evaluated using NGSP calculation (Avg. Bias ± 1.96xSD of the Bias) and linear regression analysis. Performance in College of American Pathologists (CAP) proficiency testing (PT) was estimated by determining the percentage of replicates within ± 6% of NGSP target. These calculations were also performed using IFCC calibrators assayed in multiple runs and data expressed in NGSP units. Total error (TE) was calculated using IFCC calibrators, as %Avg.Bias + 1.96xCV, where average bias and CV were determined across multiple runs. On-board reagent stability and calibration frequency were assessed by monitoring the recovery of calibrators and controls over time. Reagent stability was evaluated using an accelerated stability method.
Results: Bias per NGSP calculation was within ± 0.5 %HbA1c when using hWB samples and within ± 0.3 %HbA1c when using IFCC calibrators. Correlation to NGSP method was characterized by slopes of 1±0.03, intercepts of ±0.3 %HbA1c, and correlation coefficients of 0.99. Slopes, intercepts, and correlation coefficients were 1±0.01, ±0.05 %HbA1c, and 0.99, respectively, when data was correlated to IFCC method. 98.3% of hWB replicates and 100% of IFCC replicates were within ± 6% of NGSP and IFCC targets, respectively. TE was 2-5.3%. On-board reagent stability and calibration stability were 17 and 13 days, respectively. Reagent shelf life of 14 months was demonstrated.
Conclusions: The new HbA1c assay on UniCel DxC systems demonstrates acceptable performance. The improvements in precision and bias relative to the IFCC reference method support performance consistent with satisfying NGSP and CAP PT requirements.
*Pending submission and clearance by the United States Food and Drug Administration; not yet available for in-vitro diagnostic use. Product is For Investigational Use Only. The performance characteristics of this product have not been established.

B-29

Follistatin related gene (FLRG) protein levels in second trimester pregnancy serum to predict the development of preeclampsia
G. Lambert-Messerlian[1], E. Eklund[1], L. Neveux[1], J. Canick[1], A. Schneyer[2]. [1]*Alpert Medical School of Brown University and Women & Infants Hospital, Providence, RI,* [2]*Pioneer Valley Life Science Institute, Springfield, MA,*

Activin is a peptide hormone initially identified in the reproductive system as a regulator of pituitary follicle-stimulating hormone. More recently, activin has been found in multiple tissues and shown to have numerous biological roles (Xia and Schneyer, J Endocrinol 202:1, 2009). Activin is expressed by placenta and levels are increased in pregnant animals with experimentally induced hypoxia (Jenkin *et al*., Endocrinology 142:963, 2001) and in women with preeclampsia (Muttukrishna *et al*. Hum Reprod 15:1640, 2000). Activin is regulated in part by follistatin related gene (FLRG) protein; a binding protein that neutralizes its bioactivity. The placenta demonstrates the highest expression of FLRG and levels were further increased in samples from women at the time of preeclampsia (Pryor-Koishi *et al.,* BJOG 114:1128, 2007). Our objectives were to validate an ELISA for FLRG measurement in pregnancy serum, and to explore whether second trimester levels were predictive of preeclampsia.
METHODS: Residual maternal serum samples were selected randomly across the range of gestational ages available (11 -20 weeks) in the Division of Medical Screening and Special Testing at Women & Infants Hospital (n = 79). In addition, second trimester samples from women who developed preeclampsia later in pregnancy (n = 10) were retrieved from freezer storage. Preeclampsia was defined as newly diagnosed hypertension (blood pressure > 140 mmHg systolic and 90 mm Hg diastolic on two occasions) with proteinuria (2+ or greater on dipstick or > 300 mg/ 24 h) and the diagnosis was confirmed by medical record review. Five control serum samples were selected from freezer storage to match each case (n = 60) for gestational age at collection (same completed week), duration of freezer storage (within 3 months) and maternal race (Black versus non-Black). Assay reagents for ELISA were purchased from R&D Systems (Minneapolis, MN).
RESULTS: FLRG assay results were linear only upon dilution of serum (88% -102%, n = 4), therefore, subsequent experiments were performed using serum after 1:2 dilution. Recovery of exogenous FLRG (500-1000 pg/mL) was 106-114%. The inter-assay coefficient of variation for a second trimester serum pool with a mean concentration of approximately 4500 pg/mL was 6.2%. Analytic sensitivity was < 200 pg/mL. Levels of FLRG were significantly higher in the first (11-13 weeks, median = 3808 pg/mL) than the second (2677 pg/mL) trimester (p=0.03). Using matched rank analysis, second trimester levels of FLRG were higher in women who later developed preeclampsia relative to matched controls (mean rank = 5.2, x^2=9.91, P < 0.01).
CONCLUSIONS: These data suggest that the FLRG ELISA is suitable for use in maternal serum and that increased levels in the second trimester precede the onset of preeclampsia. Further work is underway to explore the clinical utility of FLRG, in conjunction with other markers, to predict preeclampsia.

B-30

Development of a Simple and Fast Method for the Measurement by LC-MSMS of Salivary Cortisol, Androstenedione and Testosterone.
B. Casetta. *AB SCIEX, Monza, Italy,*

Background: An increasing number of studies are utilizing saliva sampling as a method of assessing steroid secretion. Saliva samples have certain advantages over plasma, being non-invasive and easily collected. The measurement of steroid hormones in saliva has been proposed as an alternative to the assessment of the concentration of free steroids in blood plasma since it is widely accepted that the concentration in saliva is related to their free non-protein-bound fraction in plasma.
Here we describe an analytical strategy that incorporates an on-line sample cleanup step running coupled to tandem mass spectrometry that minimize the sample preparation and guaranteeing low level detections.
Methods: Sample preparation is limited to a protein precipitation step. Supernatant is directly injected in a chromatographic system centered on a perfusion chromatographic column as for the first dimension. Plug carrying the analytes is trapped and introduced in the second chromatographic dimension coupled to the API 4000 tandem mass spectrometer for the quantitative measurement. Results are generated through calibration with standard solutions of the investigated steroids and by using d4-cortisol as internal standard.
Results: With the proposed configuration, total analysis time per sample results in less than 7 minutes with a sample consumption of 50 µL and with a LOQ of 50 picomoles/L for Cortisol, Testosterone and 15 picomoles/L for Androstenedione. Linearity has been tested from LOQ up to over 20 nanomoles/L
The range of the total assay precision is 5-10%, and the average accuracy, is better than 15% for measurements above 60, 250, 200 picomoles/L for Cortisol, Androstenedione and Testosterone respectively.
Conclusion: The proposed methodology enables reliable quantitation of cortisol, androstenedione and testosterone in saliva samples after performing just a simple protein precipitation. The resulting measurement time necessary for performing the automatic on-line cleaning is compensated for by the freedom from any sample manipulation and the associated risks of loosing and/or degrading the analytes as it should be in case of labor-intensive purification-concentration steps.

B-31

Multivariate analysis of laboratory data for classification of patients with disorders of calcium homeostasis
S. S. O'Neill, C. J. Gordon, C. R. McCudden. *University of North Carolina, Chapel Hill, NC,*

Background: Employed by some laboratories, PTH nomograms use measurements of total calcium and intact parathyroid hormone (iPTH) to classify patients with disorders of calcium homeostasis. Patients with abnormal values are categorized based on graphical clustering of their results; categories typically include primary hyperparathyroidism, secondary hyperparathyroidism, PTH-independent hypercalcemia, hypoparathyroidism, and indeterminate. While there is little published data describing the performance and

accuracy of PTH nomograms, we hypothesized that we could improve the accuracy of classification using multiple additional laboratory values.

Objective: To determine if using a combination of multiple laboratory values and clinical data improves the classification accuracy of the PTH nomogram for calcium homeostasis disorders.

Methods: Laboratory and medical record data were collected for 236 patients with physician ordered iPTH and total calcium tests (139 patients were included; 97 were excluded due to insufficient laboratory data or clinical notes). Patients were classified using three different approaches as follows: 1) using a PTH nomogram plotting total calcium and iPTH results against known cases; 2) classification based on all available laboratory data and chart review (gold standard); 3) classification using a multivariate model (classification and regression tree, CART) including 32 laboratory and clinical variables (e.g. gender, BUN, creatinine, calcium, iPTH, and renal transplantation). The multivariate model was developed using the gold standard patient classification and validated using leave-one-out cross-validation ('R' software version 2.10.1). The performance of the PTH nomogram was compared to the multivariate model based on the accuracy of each against the gold standard classification (chart review and all laboratory data).

Results: The multivariate model was more accurate (81%) than the PTH nomogram (65%) at classifying patients with calcium homeostasis disorders; this difference was statistically significant (p=0.0028). The multivariate classification model utilized 6 of the 33 included variables (iPTH, calcium, creatinine, renal transplant, gender, and BUN) and had a misclassification error rate of 0.0863 (12/139). While both classification methods appropriately identified all cases of PTH-independent hypercalcemia, the multivariate model was more effective at identifying cases of secondary hyperparathyroidism (p=0.0057). In this study, 25 patients (18%) had tertiary hyperparathyroidism, defined as a high calcium and iPTH after correction of a long standing secondary cause (e.g. transplant for renal failure). The multivariate model appropriately allocated 60% of these patients whereas the PTH nomogram can not differentiate this as a category, and thus failed to accurately classify these patients. The PTH nomogram was slightly, but not statistically, better at predicting primary hyperparathyroidism and hypoparathyroidism.

Conclusions: The classification of patients with disorders of calcium homeostasis based on the PTH nomogram can be improved by utilizing the multivariate (CART) model developed in this study. This improvement is a result of several factors, such as the inclusion of indicators of renal function (creatinine and BUN), which facilitate differentiation of primary and secondary hyperparathyroidism. In addition, this model can identify tertiary hyperparathyroidism by accounting for renal transplantation. These findings support the use of a multivariate model for classification patients with disorders of calcium homeostasis. Future studies are aimed at using the model to predict disease progression.

B-32

Performance Characteristics of a New IMMULITE 2000 Erythropoietin Assay

W. E. Owen[1], W. L. Roberts[2]. [1]*ARUP Laboratories Inc., Salt Lake City, UT,* [2]*Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT,*

Erythropoietin (EPO) is a glycoprotein hormone produced by specialized cells in the kidney that act as the primary regulators of erythropoiesis. Serum EPO measurements are useful for diagnostic evaluations of anemia, polycythemia, and other erythroid disorders. We obtained a new formulation of the chemiluminescent immunoassay for EPO* on the IMMULITE 2000 analyzer (Siemens Healthcare Diagnostics). We evaluated this method for imprecision, comparison to another commercially available assay and cross-reactivity with two recombinant EPO preparations. Imprecision was evaluated over 10 days of testing using control material at 3 concentrations and serum pools at 7 concentrations using two lots of reagent. A method comparison study was performed with the Access 2 (Beckman Coulter) method using 67 patient samples. Six samples were above the measurement range of one or both of the assays and were eliminated from the analysis. Cross-reactivity was studied using epoetin alfa (Procrit, 2000 kIU/L) and darbopoetin alfa (Aranesp, 25 mg/L). These were each manually diluted using the diluents recommended for the IMMULITE 2000 and Access 2 assays and assayed in triplicate. The total imprecision results are summarized in Table 1. Deming regression analysis of data from the method comparison study gave a slope of 1.09 ± 0.03, an intercept of 0.44 ± 6.09, and a $S_{y/x}$ of 19.5 (r = 0.99) over the range tested (1.6 to 708 IU/L). Epoetin alfa at 1:10,000 dilutions gave mean results of 99 IU/L and 128 IU/L for IMMULITE 2000 and Access 2, respectively. Darbopoetin alfa at 1:10,000 dilutions gave mean results of 62 IU/L and 102 IU/L for the IMMULITE 2000 and Access 2, respectively, demonstrating that the two assays have different cross-reactivities with recombinant EPO preparations. The new IMMULITE 2000 EPO assay shows acceptable performance and is suitable for routine clinical use.

*This assay has not been cleared by the FDA.

Table 1. Summary of IMMULITE 2000 EPO Imprecision Study Results

Sample	Mean Concentration	Within-Run	Total
	IU/L	CV(%)	CV(%)
PP1	3.6	10.7	12.1
PP2	10.7	4.8	6.4
QC1	12.0	4.9	5.6
PP3	27.6	3.6	5.1
QC2	45.2	5.0	5.7
PP4	66.3	3.3	4.2
PP5	109.7	3.0	4.5
QC3	123.4	4.5	5.5
PP6	187.1	4.0	5.1
PP7	598.1	3.4	4.8
PP = patient pool QC = quality control material			

B-33

Performance Evaluation of the ADVIA Centaur® Enhanced Estradiol Assay

J. Gray[1], S. Tan[2], T. Thorne[2], W. J. Canfield[2], S. P. Volkir[3], S. St-Onge[2], R. H. Christenson[1]. [1]*University of Maryland School of Medicine, Baltimore, MD,* [2]*Siemens Healthcare Diagnostics, Inc., Tarrytown, NY,* [3]*Siemens Healthcare Diagnostics, INc., Tarrytown, NY,*

Objective: To evaluate the ADVIA Centaur® Enhanced Estradiol (eE2) immunoassay* in terms of imprecision, functional sensitivity, and correlation with an existing commercial method.

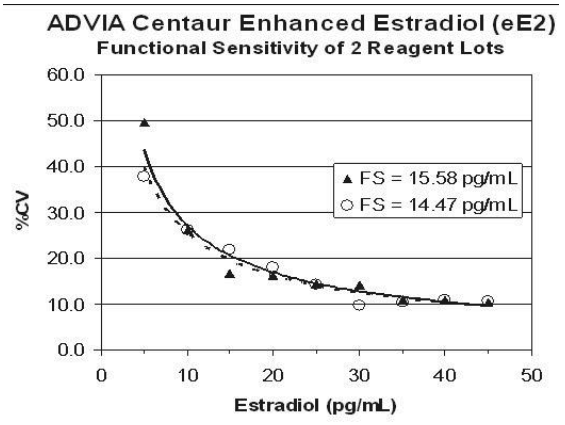
Relevance: Serum estradiol measurement is useful for clinical assessment of menstrual dysfunction, hypoestrogenicity, menopause, precocious puberty, and monitoring of fertility therapy. Characterization of this new method has not been determined.

Methodology: The ADVIA Centaur Enhanced Estradiol method is a monoclonal competitive immunoassay with chemiluminescent detection, standardized to ID-GC/MS. Two ADVIA Centaur eE2 reagent lots were evaluated. Within-run and total Imprecision was assessed by ANOVA using a 10-day CLSI EP15-A2 protocol. Method correlation was performed over five days with the Roche Elecsys 2010 using 188 serum specimens. Functional sensitivity, defined as the lowest measured concentration with 20% CV, was determined over 5 days using a panel of serum samples ranging from 5 - 45 pg/mL.

Results: Method correlation over the range of 0-3000 pg/mL (undiluted serum samples) resulted in the following linear regression equations when compared to the Roche Elecsys 2010: Centaur eE2 (Lot 98) = 0.823*Roche + 13.79, R=0.984; Centaur eE2 (Lot 99) = 0.853*Roche + 12.80, R=0.984. ADVIA Centaur eE2 total and (within run) %CV estimates ranged from 2.6% - 10.2% (1.8% - 8.9%) using four human serum pools and 4.7% - 14.5% (3.2% - 12.7%) with commercial controls. For two lots, ADVIA Centaur eE2 functional sensitivity was determined to be 14.47 and 15.58 pg/mL (see Figure).

Conclusions: The ADVIA Centaur Enhanced Estradiol method is a precise assay with functional sensitivity at low concentrations and a wide reportable range. This new ID-GC/MS standardized method demonstrates expected agreement with an accepted method and is an attractive alternative for estradiol measurement.

* For investigational use only. The performance characteristics of this product have not been established.



B-34

The Performance of Direct % Glycated Haemoglobin Assay on VITROS® 5600 Integrated System

S. SAW, C. WANG, C. YIN, S. SETHI. *NATIONAL UNIVERSITY HOSPITAL, SINGAPORE, Singapore,*

Background: The ability to turn around a glycated haemoglobin result rapidly on the same platform as the basic chemistry and immunoassay tests is an attractive option for laboratories who want to operate a single workflow for all their tests. Glycated haemoglobin is a frequently requested test from outpatient clinics where the patients often come for blood tests and resulting prior to seeing their doctor. VITROS® 5600 Integrated System (Ortho-Clinical Diagnostics, USA) provides laboratories this one-stop option in easily and rapidly serving their patient.

Methods: We performed the technical evaluation of the glycated haemoblobin assay including sample correlation with our current method, Variant II (Bio-Rad Laboratories, USA) using routine requested patient samples (n=103). Precision studies using replicates of two levels patient pools over a twenty day period. Linearity was determined using selected dilution ratios of a high and a low patient sample.

The d%A1c assay uses an anti-HbA1c to form a soluble antigen-antibody complex. The unbound anti-HbA1c is reacted with a polyhapten to form an insoluble complex which is measured turbidimetrically at 340nm. The haemoglobin in the haemolysed whole blood is converted in an alkaline solution to a hematin derivative, then measured at 575 and 700nm. The d%A1c is calculated from the quantitative measurement of both haemoglobin and haemoglobin A1c.

Results: The correlation between the VITROS d%A1c assay 5600 and the Variant II %A1c assay was VITROS 5600 =0.9112(Variant II) + 0.756, r = 0.983. There were no haemoglobin variants in the cohort studied. A slight negative bias is seen in the results from VITROS® 5600 Integrated System when compared to Variant II. Total precision for the 5.9% HbA1c pool was 1.31% and for the 9.7% HbA1c pool was 1.51%. Linearity 5600 = 1.008x - 0.403, r = 0.996 over a concentration range of 5.3 to 12.8%.

Conclusion: An excellent performance was obtained for the precision pools, going exceeding our own laboratory requirements of <2.0% which is set to surpass the precision requirements of NGSP Level I laboratory certification. Results are comparable to our current analyzer with our next step to determine VITROS 5600 d%A1c results compared to samples from an NGSP secondary reference laboratory to achieve a 95% CI of the differences between methods and fall within ±0.70%. Overall, a good performance of the d%A1c assay and an excellent instrument option for integrating a diverse menu of tests for a busy laboratory.

B-35

Validation of a New Automated Intact Parathyroid Hormone Assay on the IDS-iSYS Automated System

C. Dixon, C. E. Wynn, D. Laurie, C. J. Fox, M. J. Gardner, A. K. Barnes, M. L. Garrity. *Immunodiagnostic Systems Ltd, Boldon, United Kingdom,*

The objective of this study is to validate a new automated intact parathyroid hormone (PTH) assay on the IDS-iSYS Automated System. The IDS-iSYS is a closed, fully automated random access system that will enable clinical laboratories to determine a number of analytes from a single specimen tube.

Regular monitoring of PTH levels in patients with chronic kidney disease (CKD) is recommended. The relationship between elevated serum PTH and high-turnover bone disease is well established and, as renal function in CKD patients declines, PTH levels increase. This increase in PTH can in turn cause pain and disability for patients through stimulating bone resorption. Other recognised complications of elevated PTH in CKD patients are coronary artery disease, congestive heart failure and dyslipidemia.

The measurement of Intact PTH on the IDS-iSYS analyser is performed as a two-site chemiluminescent immunoassay. Sample, biotinylated anti-PTH and an acridinium labelled anti-PTH antibodies are mixed, forming a complex where the PTH is bound between the two antibodies. Streptavidin coated magnetic particles are then added to the mix where the biotinylated anti-PTH facilitates binding of the complex to the magnetic particle. The complexes are captured by a magnet during a wash step. After which the bound PTH is ready to be measured using a high sensitivity luminometer, where signal generated by the acridinium conjugate is directly proportional to the concentration of PTH in the sample.

The Intact PTH assay has a range of 5-5000pg/ml. Time to first result is 38 minutes. Assay precision measured by NCCLS method is 5.8% within run, 4.3% within day and 7.3% within device. Recovery was measured at 92-98%. LoB was measured at 1.35pg/ml, LoD at 1.61pg/ml and LoQ at 2.25pg/ml. Linearity across the full assay range is 92-99%. No significant interference was observed with clinically relevant levels of haemoglobin, biotin, bilirubin, red blood cells, protein, lipid, human anti-mouse antibody and rheumatoid factor. No evidence of hook was observed at 50,000pg/ml PTH (1-84). No evidence of assay drift was observed over runs of 300 replicates of patient sample. The Intact PTH assay is specific for full length (1-84) PTH and the (7-84) N-terminal fragment. The assay is specifically formulated to handle PTH (39-84) fragments at high levels expected in CKD patients without significant interference. Recombinant PTH (1-34) (Forsteo®) does not cross-react at clinically relevant levels. This assay has potential to provide rapid, accurate, automated data for monitoring full length and large N-terminal fragment PTH across the full clinical range. The Intact PTH assay displays excellent correlation with existing assays. This assay will be part of a comprehensive bone panel on the IDS-iSYS analyser that will be essential for the clinical laboratory.

B-36

A First Fully Automated Parathyroid Hormone (1-34) Assay on the IDS-iSYS Automated System

J. N. Tran, C. Dixon, Q. H. Tran, C. E. Wynn, D. Laurie, A. K. Barnes, M. L. Garrity. *Immunodiagnostic Systems Ltd, Boldon, United Kingdom,*

Osteoporosis is a major health concern for both women and men. Both genders over 50 years old are at higher risk of osteoporosis-related fractures. Parathyroid hormone (PTH) 1-34 is used to treat severe osteoporosis by increasing skeletal mass and bone strength. PTH (1-34) is the biological active N-terminal portion of human parathyroid hormone (1-84). Measurement of circulating PTH 1-34 may be useful in assessing the pharmacological properties of this peptide, including dosing and interval of treatment. The first fully automated PTH (1-34) assay is part of a comprehensive panel of bone tests on the IDS-iSYS Automated System™. The PTH (1-34) assay is a two-site chemiluminescent immunoassay standardized to recombinant human PTH (1-34) (Forteo®). PTH (1-34) is treated and bound to highly specific Biotinylated C-terminal and Acridinium labelled N-terminal antibodies. The complexes are captured by streptavidin-coated magnetic particles. After a wash step, bound PTH (1-34) is measured in a luminometer where the signal generated by the acridinium conjugate is directly proportional to the concentration of PTH (1-34) in the sample. The PTH (1-34) assay range is 10-750pg/mL. All samples used for evaluation were spiked with Forteo® at various concentrations. Analytical sensitivity was <2pg/mL. Lower limit of quantitation observed at <6.8pg/mL. Within run precision at 6.9pg/mL, 33.4pg/mL, 203pg/mL, and at 600pg/mL is 6.6%, 2.2%, 3.8%, and 3.5% respectively. Within device precision at 6.9pg/mL, 33.4pg/mL, 203pg/mL, and 600pg/mL is 12.9%, 3.8%, 8.5%, and 9.4% respectively. Excellent linearity of 99%-106% was observed across the assay range. Recovery is measured at 88% - 94%. Sample dilution showed 92%-98% recovery. Interference of bilirubin up to 20mg/dL, lipid up to 3000mg/dL, red blood cells up to 0.4%, haemoglobin up to 250mg/dL, and biotin up to 300nM was within acceptable limits. Excellent correlation of ninety five PTH (1-34) spiked samples with a commercially available ELISA assay was seen with a slope of 1.8, intercept of -4.1, and correlation coefficient (r=0.97). The assay showed no evidence of hook up to 39,000pg/mL. The assay is highly specific for PTH (1-34) with <0.01% cross reactivity with PTH (7-84), and 3.6% cross reactivity with PTH (1-84). Time to first result is 64 minutes, with a throughput of 58 tests per hour. The IDS-iSYS PTH (1-34) assay provides a short turn around time, excellent precision, and excellent correlation with an existing method.

B-37

Development of a new Insulin Like Growth Factor-I immunoassay on the IDS-iSYS Automated Analyser

M. Bougoussa[1], J. Manolopoulou[2], A. K. Barnes[3], S. Lecane[1], M. L. Garrity[3], M. Bidlingmaier[2]. *[1]Immunodiagnostic Systems Ltd, Liege, Belgium, [2]Medizinische Klinik - Innenstadt, Ludwig-Maximilians University, Munich, Germany, [3]Immunodiagnostic Systems Ltd, Boldon, United Kingdom,*

Insulin Like Growth Factor-I (IGF-1) consists of 70 amino acids in a single chain. Production mainly occurs in the liver in response to growth hormone (GH) stimulation and can be retarded by undernutrition. IGF-1 is used by physicians as a screening test and a marker to monitor therapy of GH related diseases like GH deficiency and acromegaly.

The techniques for measuring IGF-I have evolved over the decades, immunoassays are still the primary tool used in routine laboratories. There is a large variability among commercial IGF-I immunoassays due to interferences from IGF binding proteins (IGFBPs) and differences in the reference preparations used. There is a need for IGF-I immunoassay harmonization and for the establishment of adequate reference values.

The IDS Automated Analyser IDS-iSYS IGF-I assay uses two highly specific monoclonal antibodies. The biotinylated antibody is directed against the N-terminal fragment. The second antibody is coupled to an acridinium ester derivative. After a sample pre-treatment which dissociates IGF-I from the binding proteins, a 50µl portion of this is incubated with the biotinylated and second antibody in the presence of excess IGF-II**.** 20 µl of streptavidin magnetic particles are added to the reaction. After a second incubation, followed by a washing step, triggers are added and the measured luminescence is directly proportional to

the IGF-I concentration present in the sample.
First result is available after 32min. The analytical range of the assay is 10 to 1200 ng/ml. The performances were established using CLSI protocols. The analytical sensitivity is ≤ 5 ng/ml and the functional sensitivity is ≤10ng/ml. Intra-assay precision is < 3.9% and the total CV is <9.7 %. The IGF-I recovery and linearity averages 102% and 98% respectively, expressed as Observed/Expected ratios. No interference or cross reactivity was observed with IGF-II, Insulin, Proinsulin and the six high affinity IGFBPs. A correlation study was performed using 97 serum samples. The assay demonstrates good correlation with the Siemens DPC Immulite kit (r=0.95) and yields the following regression equation: IDS-iSYS Automated Analyser = 0.81 x Immulite -0.51 ng/ml. The IDS-iSYS IGF-I is calibrated against the new WHO standard 02/254.The results indicate that the IDS-iSYS Automated Analyser IGF-I assay provides a sensitive, specific and reproducible assay for the measurement of IGF-I. Normal ranges have been established.

B-38

Age Related Reference Ranges for Anti- Mullerian Hormone (AMH) in Healthy Women

S. A. Faye[1], S. Pfeiffer[2], M. Scholtes[3]. *[1]Beckman Coulter, Nyon, Switzerland, [2]Labor Benrath, Dusseldorf, Germany, [3]Interdisciplinary Centre for Human Reproduction,, Dusseldorf, Germany,*

Background: Anti-Mullerian Hormone (AMH) is a glycoprotein dimer composed of two 72kDa monomers linked by disulfide bridges. It belongs to the transforming growth factor-ß family. AMH performs various physiological functions. In females, AMH is produced in small amounts by ovarian granulosa cells and is an important regulator of folliculogenesis. Values are maximal from birth until puberty and decline thereafter until the menopause when levels become undetectable. Therefore age-related reference intervals are required to facilitate the correct interpretation of AMH levels.
Objective: To establish age related reference ranges for AMH using a commercially available ** ELISA assay.
Methods: 1642 healthy women, aged 15-62 (mean 35.8), recruited in a fertility centre were divided into age-based groups using a three year interval. Blood was taken prior to any in vitro fertilisation treatment. AMH was analysed using a double antibody ELISA assay from Beckman Coulter.
Results: Assay precision was as follows; intra-assay CV was 2.4 - 4.6%, inter-assay CV was4.8 - 8.0%. The median values were different in each group. Group I (age 18-20) had a median AMH value of 6.79 ng/mL, group II (age 21-23) had a median AMH value of 3.16 ng/mL, group III (age 24-26) had a median AMH value of 2.05 ng/mL, group IV (age 27-29) had a median AMH value of 1.96 ng/mL, group V (age 30-32) had a median AMH value of 2.23 ng/mL, group VI (age 33-35) had a median AMH value of 1.50 ng/ML, group VII (age 36-38) had a median value of 0.96 ng/mL, group VIII (age 39-41) had a median AMH value of 0.70 ng/mL, group IX (age 42-44) had a median AMH value of 0.40 ng/mL and group X (age 45-47) had a median AMH value of 0.15 ng/mL
Conclusions: AMH values declined with age as expected form the physiology of this hormone. Age related reference intervals were estimated for the Beckman Coulter AMH ELISA assay.
* Not intended as off-label promotion of any Beckman Coulter product.
** Available in the European Union. Not available in the US.

B-39

Sandwich immunoassay prototypes for human LMW adiponectin and for complex of adiponectin with serum albumin.

A. Kogan[1], V. Filatov[1], O. Kolosova[2], I. Katrukha[3], O. Nagibin[4], A. Katrukha[1]. *[1]HyTest Ltd, Turku, Finland, [2]Moscow Institute of Medical Ecology, Moscow, Russian Federation, [3]Moscow State University, Moscow, Russian Federation, [4]State Hospital #2, Ryazan, Russian Federation,*

Human adiponectin (Adn) is a protein hormone regulating glucose and lipid metabolism. For last ten years it is intensively exploited in clinical studies as a candidate predictor of type 2 diabetes mellitus (T2DM) in apparently healthy adults and as a prognostic marker of cardiovascular complications in T2DM patients.
Adn is a complex molecule known to exist in the bloodstream as a mixture of three homooligomeric forms: trimer - low molecular weight form (LMW), hexamer - medium molecular weight form (MMW), and high molecular weight form (HMW) - 12-32-mer. The information about a ratio of oligomeric forms in human blood, about their involvement in Adn hormonal activity and about their possible utilization as markers is contradictive and incomplete. The aim of the current study was to obtain a big panel of Adn-specific MAbs including form-specific antibodies, and by using such MAbs to study Adn forms, Adn complexes with other proteins and possible clinical application of Adn as T2DM marker.
A panel of Adn-specific MAbs was obtained after mice immunization with native human Adn (HyTest, Turku, Finland). All MAbs were tested in direct ELISA and in sandwich immunoassays with three major Adn oligomeric forms - HMW, MMW, LMW, and total Adn fraction. MAbs Adn27, Adn94 and Adn63 were shown to recognize all three Adn forms, thus assay utilizing MAb Adn94 as capture and MAb Adn63 as detection was utilized for total Adn measurements. MAb Adn214 was specific only to LMW form of the protein, so immunoassay Adn214-Adn27 utilizing this MAb as capture antibody was used for LMW quantification.
It was suggested earlier that some portion of LMW adiponectin is complexed with HSA. To prove the existence of Adn-HSA complex in human blood, we have designed mixed sandwich immunoassay where LMW-specific MAb Adn214 was used as a capture and HSA-specific MAb HSA20 (HyTest, Turku, Finland) was used as a detection antibody. Such mixed sandwich assay was applied for immunoreactivity measurements in fractions after human serum proteins separation by gel filtration method. It was shown that Adn-HSA complex exists in human blood.
LMW Adn and Adn-HSA complex measurements in serum samples of T2DM patients (n=69) and in healthy donors (n=37) have revealed that LMW and Adn-HSA complex levels are decreased in T2DM (2.9±0.8 µg/ml v.s. 3.3±0.7 µg/ml for LMW and 6.9±1.0 artificial units (a.u.), v.s. 9.1±2.6 a.u., for Adn-HSA complex respectively). Total Adn levels (assay Adn94-Adn63) were also lower in T2DM patients than in healthy donors (9.3±8.5 µg/ml and 13±8.6 µg/ml, respectively).
In summary, 1) we have demonstrated existence of LMW Adn-HSA complex in human serum; 2) preliminary clinical studies have shown reduction of LMW and Adn-HSA complex levels in T2DM patients in comparison with healthy donor.

B-40

The Measurement of Salivary Testosterone by Mass Spectrometry and Comparison to Paired Free and Bio-Available Testosterone Samples

J. Orrock, R. Taylor, S. Grebe, R. Singh. *Mayo Clinic, Rochester, MN,*

Background: Testosterone is an anabolic steroid and is the major androgenic hormone in both males and females. In the serum, the majority of testosterone is protein bound to sex hormone-binding globulin (SHBG) and to a lesser extent albumin. Until recently, it was thought that only the fraction of non-protein bound or free testosterone was considered biologically active. However, because testosterone binds weakly to albumin, the albumin bound testosterone can disassociate freely in the capillary bed and therefore may be also considered "bio-available". The most common assays for the determination of testosterone are serum total, free and bio-available testosterone. Additionally, salivary testosterone, which may have several advantages over the serum testosterone assays, is also available in several laboratories. Although the measurement of testosterone by LC-MS/MS is considered the "gold standard", to our knowledge there is no commercial laboratory that offers salivary testosterone by LC-MS/MS. Historically, because the testosterone concentration in the saliva is only a fraction of what is in the serum, the sensitivity of LC-MS/MS was insufficient to reliably measure testosterone by this method. The purpose of this project was to develop a LC-MS/MS salivary testosterone assay and compare the values obtained from paired salivary and serum samples assayed for total, free and bio-available testosterone.
Methods: The salivary testosterone LC-MS/MS assay was developed by subjecting saliva samples to a solid phase extraction and then running them on a LC-MS/MS system with a modified version of the total testosterone method. Inter assay precision was determined by running replicates of low, medium and high control samples on 5 separate days. Intra assay precision was determined by running low, medium and a high controls 20 times each on the same run. Recovery was determined by spiking known low, medium and high concentrations into diluent and determining the percent recovery. Linearity was determined by diluting known concentrations, low, medium and high, 1:2, 1:4, 1:8, and 1:16 and calculating the percent of measured vs. expected. To compare the LC-MS/MS salivary testosterone method to the various serum testosterone methods, 53 healthy volunteers, 16 males and 37 females had paired serum and salivary samples drawn for comparison. Salivary and serum samples were obtained in the AM (7-8 AM) and an additional salivary sample was collected in the afternoon (3-4 PM). The serum results were obtained following routine protocols.
Results: Inter-assay precision for the low (1.3 ng/dL), medium (4.0 ng/dL) and high (46.8 ng/dL) samples was 9.4, 4.5 and 11.2% respectively. Intra-assay precision for the low, medium and high samples was 6.0, 3.6 and 3.8% respectively. The samples diluted linearly and recovery was 99%. The salivary testosterone in males showed correlation (r^2=0.64) with the bio-available testosterone assay and no significant correlation was observed in females. The salivary testosterone values did not show correlation with the total and free testosterone in both males and females.
Conclusions: Salivary testosterone can reliably be measured on the LC-MS/MS platform. Salivary testosterone by LC-MS/MS correlates only with the serum bio-available testosterone. Further correlation studies are needed.

B-41

A Multi Centre Evaluation of the new Beckman Coulter Anti-Müllerian Immunoassay (Active AMH Gen II)

A. M. Wallace[1], S. M. Nelson[2], R. Fleming[3]. *[1]Dept Clinical Biochemistry, Glagow Royal Infirmary, Glasgow, United Kingdom, [2]Reproductive and Maternal Medicine, University of Glasgow, Glasgow, United Kingdom, [3]Glasgow Centre for Reproductive Medicine, Glasgow, United Kingdom,*

Introduction: Anti-Müllerian Hormone (AMH) is produced by human ovarian granulosa cells from early stages of development until the early antral stage. Circulating AMH concentrations in females are low compared to males but increase after puberty to maximal concentrations. Thereafter, a steady and unrelenting decline is seen until AMH becomes immeasurable in the circulation prior to the menopause. The decline in AMH through reproductive life appears to mirror the decay in the number of non-growing primordial follicles - the ovarian reserve. Correspondingly, one of the main clinical utilities of the assay is in the assessment of ovarian reserve, which allows prediction of likely response to controlled ovarian stimulation.

Until recently two enzyme linked immunosorbent (ELISA) assays have been available; from DSL and Immunotech. These have been standardized differently and give different values for quantitation of AMH. This has created some confusion for investigators. Following the acquisition of DSL and Immunotech by Beckman Coulter the two assays have been harmonized by replacing the DSL ELISA with the Active AMH Gen II assay incorporating DSL antibodies but calibrated using the same standard as used in the Immunotech assay.

Objective: The objective of this study was to assess the performance of the new Active AMH Gen II assay versus the original Diagnostics Systems Laboratories (DSL) ELISA assay and make recommendations regarding appropriate clinical cut off values for use in controlled ovarian stimulation programmes.

Methods: An evaluation of the Active AMH Gen II assay was performed at three sites, each with extensive experience in measuring circulating AMH in the adult human female. Results were compared with the original DSL ELISA assay. A total of 300 patient samples were analysed in duplicate (100 at each of the three sites) to provide comparative data, with additional comparison of a series of common pooled samples at critical clinical levels. Linearity, precision and sensitivity of the new assay were also assessed.

Results: The values obtained demonstrated good agreement with a regression equation AMH Gen II = 1.41DSL - 0.78 pmol/L, r= 0.97, n= 300. Linearity was good with observed values close to expected (mean recovery 106.3%). Within and between batch precision was assessed at various levels on a range of control materials, between batch CVs were (11.1- 14.8% at 1.5 pmol/L,10.1 to 17.7% at 5 pmol/L,3.4 to 7.9% at 15 pmol/L and 2.9 to 6.8% at 70 pmol/L). The analytical sensitivity of the assay was 0.6 pmol/L.

Conclusions: This study effectively demonstrates that the results from Active AMH Gen II assay correlate well with the original DSL ELISA assay. Laboratories transitioning between methods should expect similar precision and excellent correlation compared to the DSL assay. Values are, however, approximately 40% higher by Gen II so laboratories should adjust their cut points accordingly.

B-42

Hormonal status of women with a novel non-synonymous polymorphism in the luteinizing hormone beta gene (*LHB)*

G. Barra[1], A. Lofrano-Porto[2], M. Borba[3], J. Gomes[3], P. Godoy[1], B. Luitgards[3], V. Caldas[3], A. Queiróz[3], L. da Motta[4], L. Casulari[3], F. Neves[5]. *[1]Laboratorio Sabin, Brasilia, Brazil, [2]Unidade de Endocrinologia, Ambulatório de Gônadas e Adrenais, Hospital Universitário de Brasília., Brasilia, Brazil, [3]Unidade de Endocrinologia, Ambulatório de Gônadas e Adrenais, Hospital Universitário de Brasília, Brasilia, Brazil, [4]Departamento de Ginecologia e Obstetrícia, Faculdade de Medicina, Universidade de Brasília, Brasilia, Brazil, [5]Laboratório de Farmacologia Molecular, Faculdade de Ciências da Saúde, Universidade de Brasília., Brasilia, Brazil,*

Background: We recently described a new non-synonymous polymorphism in the *LHB* gene (C1430A). This polymorphism was found only in heterozygosity and would result in the amino acid change Thr78Asp in luteinizing hormone (LH), a variant not previously described in humans. Interestingly, chorionic gonadotropin beta (CGB) of humans and others primates as well as LH of other primates all have an asparagine in the corresponding position. As the *CGB* genes originated from duplication of the *LHB*, we can not rule out the hyphotesis of an ancestral variant. In this study, we evaluated the LH dependent hormonal profile by measuring serum levels of LH, total testosterone (TT), estradiol (E2), androstenedione (AD), and dehydroepiandrosterone sulfate (DHEA-S) in patients with this mutation, in order to observe LH-dependent *in vivo* functional changes resulting from the Thr78Asp.

Methods: four volunteers agreed with the hormone measurements, all female in the folicular phase, and one with polycystic ovary syndrome (PCOS). Genomic DNA was extracted from blood leucocytes and the region spanning exon 2, intron 2 and exon 3 of *LHB* was sequenced. Multisite variation polymorphism (MSV), which is the sum of signals of highly similar genes that vary in sequence (such as *LHB* and *CGB*), was excluded by inspection of LH speficific nucleotides. Hormone levels were measured by a chemiluminescence immunoassay.

Results: Hormone levels can be viewed on table 1; no significant change was observed. Several LH specific sequences other than C1430A in homozygous state indicate that this mutation is not a MSV.

Conclusion: There were no functional changes resulting from the mutation Thr78Asp in these volunteers. It is important to emphasize that only heterozygous carriers were tested, and the results could have been masked by the wildtype allele. Thus, identification of homozygous carriers is essential for characterization of Thr78Asp effects on LH dependent hormonal profile *in vivo*.

Table 1: Hormonal status of women heterozygous for T78N LH Variant

Patient	Gender	Age	PCOS	LH (mUI/mL))	TT (ng/dL)	E2 (pg/mL)	DHEA-S (ug/dL)	AD (ng/mL)
1	Female	22	Positive	4,8	37,5	106,07	90,4	2
2	Female	22	Negative	4,1	48,9	46,28	220	3
3	Female	18	Negative	3,9	44,4	116,37	226	3,3
4	Female	44	Negative	7,5	36,4	151,12	32,6	1,1
Average	-	-	-	5,075	41,8	104,96	142,25	2,35
SD	-	-	-	1,66	5,91	43,61	96,21	1,00
Normal Range	-	-	-	1,9-12,5	14-76	9-221	34-430	0,8-4,4

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Comparison of intact parathyroid hormone assays between automated immunochemiluminescent analyzers and immunoradiometric assay for end-stage renal disease patients

T. Yang[1], H. Chuang[1], C. Lai[1], M. Tsai[2], P. Lin[1], H. Chang[3]. *[1]Department of Laboratory Medicine, Chang Gung Memorial Hospital at Chiayi, Putz, Taiwan, [2]Department of Nuclear Medicine, Chang Gung Memorial Hospital at Chiayi, Putz, Taiwan, [3]Department of Nephrology, Chang Gung Memorial Hospital at Chiayi, Putz, Taiwan,*

Background: Intact parathyroid hormone (iPTH) is measured in serum or plasma for management of osteodystrophy and calcium metabolism in patients with end-stage renal disease (ESRD). The aim of this study was to determine the extent of how the results from two automated immunochemiluminescent iPTH assays (ICMA) diverge from results with the Nichols Allegro iPTH immunoradiometric assay (IRMA).

Methods: The iPTH values of K_2-EDTA plasma samples from 93 patients under hemodialysis with ESRD were measured with the following iPTH assays: Roche Modular E170 analyzer, Advia Centaur® XP analyzer, and Nichols Allegro iPTH IRMA.

Results: The average percentage recoveries between iPTH values assessed by the two analyzers and the Nichols Allegro IRMA method were 102% for Roche Modular E170 and 110% for Advia Centaur® XP, respectively. The correlation coefficient showed good association between the two analyzers and the Nichols Allegro iPTH IRMA (r = 0.994 for Roche Modular E170 and r = 0.987 for Advia Centaur® XP, respectively). The iPTH values form the Roche Modular E170 have no statistical difference with those from Nichols Allegro iPTH IRMA ($P = 0.062$, Wilcoxon signed-ranks test). However, the Bland and Altman difference graph demonstrated a significant increase in mean difference in higher iPTH concentrations with the Advia Centaur® XP compared to the Nichols Allegro iPTH IRMA ($P < 0.001$).

Conclusions: In our study, iPTH assays on the Advia Centaur® XP analyzer were higher as comparing to the Nichols Allegro iPTH IRMA.

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Validation of Roche Elecsys free T_4 assay by comparison to a Resin-Uptake free T_4 Index Assay.

J. Song, N. Babic, S. J. Zibrat, N. Scherberg, K. T. J. Yeo. *University of Chicago, Chicago, IL,*

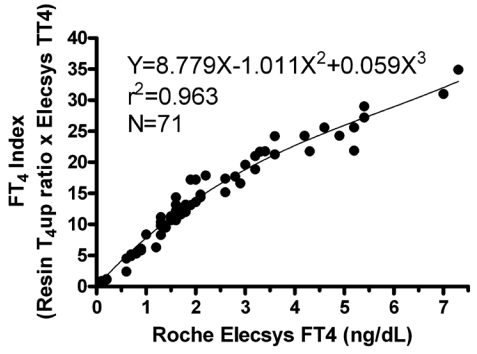
Objective: Our aim was to validate a direct free T_4 (FT_4) assay on Roche Elecsys® platform by comparing it with our classical in-house resin uptake-derived free T_4 index (FT_4I) assay.

Methods: Seventy-one patients who underwent thyroid function testing at our laboratories were selected and designated as the *training set* to define the correlation between FT_4I and FT_4I. Serum samples were tested for FT_4I (resin-uptake ratio x Elecsys TT_4), Elecsys FT_4 and Elecsys TSH (Roche Diagnostics, Indianapolis, IN) in parallel. Regression analyses

were performed using Prism software (GraphPad Software Inc., La Jolla, CA). A separate validation set of 87 randomly selected patients were also tested for FT_4I, FT_4 and TSH. The regression equation from the training set was then applied to FT_4 results of the validation set to calculate *estimated FT_4I (eFT_4I)*, which was then compared with resin-uptake derived FT_4I by linear regression analysis.

Results: The training set showed a third-order polynomial correlation equation: $[FT_4I] = 8.779[FT_4] - 1.011[FT_4]^2 + 0.059[FT_4]^3$, with $r^2 = 0.963$. Comparison of eFT_4I and FT_4I in the validation set showed a very strong linear correlation, $[FT_4I]=1.06\ [eFT_4I]-1.22$, $r^2 = 0.98$, n=87). There were 17 cases (11%) in both groups that had apparent discordance, namely normal FT_4 (reference range 0.90-1.70 ng/dL) and slightly elevated FT_4I (reference range 6.0-10.5). Fourteen (82%) of the discordant results were derived from patients on synthroid therapy. Of the 17 discrepancies, 5 had low TSH, while 9 had normal and 3 had high TSH (reference range 0.30-4.00 mIU/L).

Conclusion: The Roche Elecsys direct FT_4 immunoassay showed a very strong correlation with the classical resin-uptake FT_4I assay and can be used to replace the manually intensive FT_4I assay. FT_4 may be used to calculate eFT_4I if needed to assist in the crossover of patients being monitored by the resin-uptake FT_4I.



B-45

Urinary C-peptide creatinine ratio is a novel non invasive alternative to the inpatient mixed meal tolerance test in young onset Type 1 diabetes

T. J. MCDONALD[1], R. Besser[2], A. Jones[1], B. Shields[2], K. Wensley[1], B. Knight[2], G. Githens-Mazer[2], A. Hattersley[2]. *[1]Royal Devon and Exeter Hospital, Exeter, United Kingdom, [2]Peninsula Clinical Research Facility, Peninsula Medical School, University of Exeter, Exeter, United Kingdom,*

Introduction Stimulated serum C-peptide (sCP) during a mixed meal tolerance test (MMTT) is the gold standard measure of beta cell function in Type 1 diabetes (T1D). However invasiveness of sampling, the need to discontinue insulin prior to the test and processing of samples limit its widespread use, particularly in children. Urinary C-peptide creatinine ratio (UCPCR), a stable measure of endogenous insulin production, has the potential to be a non invasive alternative.

Aims/Objectives:

To determine if UCPCR can replace stimulated sCP following: (1) a standard MMTT; (2) a MMTT with continuation of exogenous insulin administration, and (3) following a home meal.

Methods We randomly allocated T1D adults (diagnosed <30y, insulin since diagnosis, $eGFR \geq 60ml/min/1.73m^2$) to receive a standard MMTT (Test 1) followed by a MMTT with the subject's normal morning insulin (Test 2), at least 48 hours apart. sCP and UCPCR were measured at fasting and stimulation (90min for sCP, 120min for UCPCR). Postprandial UCPCR was collected 2h after the largest meal of the day between the MMTT study dates.

Results 28 adults (age of diagnosis median (IQR)17.5 (10.5-22.8y), diabetes duration 25.5(11.1-42.5y) were recruited:

(1) In Test 1, 120min UCPCR was highly correlated with 90min sCP (r=0.88 (95%CI: 0.75, 0.94), $p=7x10^{-10}$). The correlation persisted during Test 2 (r=0.95 (95%CI: 0.89, 0.98), $p=5.3x10^{-14}$), despite a lower glucose increment during the test (Test 1 glucose increment 9.5mmol v Test 2 4.4mmol/l, $p=8.3x10^{-6}$).

(2) There was a reduction in 90min sCP from Test 1 to Test 2 (range 0.0-0.43nmol/l, p=0.03). However, this made no difference to the relationship between 120min UCPCR in Test 2 (with insulin) and 90min sCP in Test 1 (no insulin) (r=0.90 (95% CI:0.79-0.95), $p=1.4x10^{-10}$).

(3) Postprandial largest meal UCPCR was well correlated with the MMTT 90minute serum C-peptide (r=0.78 (95% CI: 0.58, 0.89), $p=9.6x10^{-7}$).

Conclusions/Summary In young onset Type 1 diabetes, urinary C-peptide is a non-invasive alternative to serum following a mixed meal tolerance test. Coexistent insulin administration makes no difference to the mixed meal tolerance test. Urinary C-peptide is a useful reliable measure and may be better tolerated than the inpatient MMTT in younger patients.

B-46

Simultaneous analysis of testosterone, androstenedione, dehydroepiandrosterone sulfate and 17-hydroxyprogesterone by LC-MS/MS

N. K. Djedovic, S. Rainbow. *Northwick Park Hospital, Middlesex, United Kingdom,*

Introduction: Polycystic ovarian syndrome is the most common cause of androgen excess in women, but it is essential to exclude other causes of hyperandrogenism, such as late-onset congenital adrenal hyperplasia, androgen-secreting neoplasms and Cushing's syndrome. Serum testosterone is often the only androgen measured, but it is not always raised in these conditions. The difficulties of measuring testosterone in women by immunoassay are recognized and well-described. Immunoassays for this analyte have limited sensitivity and specificity. We report here a liquid chromatography tandem mass spectrometry method for the simultaneous measurement of testosterone (T), androstenedione (A4), dehydroepiandrosterone sulfate (DHEAS) and 17-hydroxyprogesterone (17-OHP).

Method: The current LC-MS/MS method for T was expanded to include DHEAS, A4 and 17-OHP. Protein precipitating agent (300 µL, 0.3 M zinc sulfate in 30% water:methanol (*v:v*)) was added to aliquots of 150 µL of standards, controls and patient serum. The precipitating reagent contained internal standards for each analyte (d_2-DHEAS, d_3-T, d_7-A4, and d_8-17-OHP). Samples were analysed on an API 3000 tandem mass spectrometer (Applied Biosystems), equipped with a TurboIonSpray source and an Agilent 1100 system HPLC system. Chromatographic separation was achieved using a BDS Hypersil C_8 column (50x2.1 mm, 3 µm), with a BDS Hypersil C_{18} guard column (10x2.1 mm, 5 µm). The MS instrument was operated in multiple reaction monitoring mode with negative electrospray for the measurement of DHEAS and switched to positive electrospray for T, A4 and 17-OHP. The analysis time per sample was 9 min.

Results: The assay was linear up to 24 µmol/L for DHEAS, 42 nmol/L for A4, 63 nmol/L for T and 62 nmol/L for 17-OHP. Validation of this method showed acceptable sensitivity (limits of quantitation of 0.2 µmol/L (DHEAS), 0.7 nmol/L (T), 0.4 nmol/L (A4) and 0.6 nmol/L (17-OHP)). The total imprecision of the method was less than 10% for each analyte. The androstenedione method was compared to an external LC-MS/MS method: y = 0.98 x + 0.20, r = 0.95. The 17-hydroxyprogesterone method was compared to a radioimmunoassay method (direct DPC-Coat-a-Count): y = 0.61 x - 0.68, r = 1.00. The dehydroepiandrosterone sulfate method was compared to an immunoassay method (Immulite 2000): y = 0.92 x + 0.37, r = 0.99.

Conclusion: We have developed a robust method for the simultaneous measurement of testosterone, androstenedione, dehydroepiandrosterone sulphate and 17-hydroxyprogesterone.

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Computational Methods are Significant Determinants of the Associations and Definitions of Insulin Resistance Using the Homeostasis Model Assessment (HOMA) in Women of Reproductive Age

F. H. Safar[1], O. A. Mojiminiyi[2], H. Al Rumaih[3], M. Diejomaoh[2]. *[1]Clinical Biochemistry, MAK Hospital, Kuwait, Kuwait, [2]Faculty Of Medicine, Kuwait, Kuwait, [3]IVF Unit, Maternity Hospital, Kuwait, Kuwait,*

Introduction: Insulin resistance (IR) is associated with obesity, metabolic syndrome (MS), hyperandrogenic states and plays an important role in the pathogenesis of polycystic ovary syndrome (PCOS). The precise definition of IR is complex as several methods are used for its estimation in different studies. The homeostasis model assessment (HOMA), which correlates well with gold standard clamp techniques, is the most frequently used surrogate marker of IR. The most widely used method of HOMA assessment is the original HOMA model formula (HOMA1); the computer model (HOMA2) which is available as an online calculator is being increasingly reported. There are no previous studies that have compared the two HOMA assessment methods in terms of their associations and definition of IR. The aim of this study was to determine the effect of HOMA computational method on the associations and identification of IR in metabolic syndrome (MS) phenotypes and PCOS.

Methods: Anthropometric measurements, follicular phase LH, FSH, estradiol, testosterone, androstenedione, DHEA-S, SHBG, fasting lipid profile, adiponectin, leptin, glucose and insulin were measured in 92 women with PCOS (Rotterdam criteria) and 108 apparently healthy control subjects. HOMA1 and HOMA2 were used to assess IR. Subjects were classified as MS positive and MS negative (International Diabetes Federation criteria). Bland-Altman analysis was used to evaluate agreement between the two methods.

Univariate and logistic regression analyses were used to find the associations of the two HOMA computation methods with different variables, MS and PCOS.
Results: There is significant difference in the mean estimated HOMA IR in the study population: HOMA1 mean = 2.79 and HOMA2 mean = 1.42. The difference in the computation methods (HOMA1 - HOMA2) was significantly ($p<0.05$) positively correlated with insulin (r =0.97), FBS (r =0.58), TG (r =0.37), WC (r =0.48), Free Androgen Index (r =0.24), leptin (r =0.37); and negatively correlated with HDL (r = - 0.35) and adiponectin (r = - 0.52). For each HOMA method, IR was defined as HOMA IR index in the upper 25% quartile. The cut-off points for definition of IR were 2.9 for HOMA1 and 1.7 for HOMA2. HOMA1 and HOMA2 showed comparable significant correlations with anthropometric, biochemical and metabolic variables but correlation coefficients were consistently higher with HOMA1 method. In addition, HOMA1 (but not HOMA2) showed significant correlations with systolic and diastolic blood pressures, LH and DHEA-S. Binary logistic regression analyses showed that odds ratio (OR) of the associations of HOMA1 and HOMA2 with MS were 5.7 and 4.2 respectively ($p<0.0001$) and the OR for PCOS were 3.7 ($p=0.007$) and 3.5 ($p=0.004$) respectively.
Conclusions: HOMA computational method significantly affects the associations and cut-off values used for definition of insulin resistance. The correlations of the difference in the computational methods suggest a metabolic basis for the difference. The difference also explains, in part, the wide variability of the cut-off values used in different studies. As precise identification of IR in patients with PCOS is of great practical importance, we suggest alignment and harmonization of computational methods within each population.

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Measurement of urine free cortisol and cortisone by LC-MS/MS with simultaneous detection of synthetic glucocorticoids

N. K. Djedovic, S. Rainbow. *Northwick Park Hospital, Middlesex, United Kingdom,*

Introduction: Supraphysiological administration of corticosteroids is a common cause of Cushing's syndrome, and prolonged use may also lead to suppression of endogenous corticosteroid production. One of the most widely used investigations for Cushing's syndrome is measurement of 24 hour urine free cortisol (UFC). Immunoassays are still commonly used for measurement of UFC, but their lack of specificity is well-known and they are increasingly being superseded by liquid chromatography tandem mass spectrometry. We report a LC-MS/MS method for the measurement of 24 hour urine free cortisol and cortisone with simultaneous detection of commonly prescribed steroid drugs (prednisolone, dexamethasone, betamethasone and beclomethasone dipropionate).
Method: Aliquots of 250 µL of standards, controls and patient urine were spiked with internal standard and extracted with dichloromethane. Samples were analysed on an API 3000 tandem mass spectrometer (Applied Biosystems), equipped with a TurboIonSpray source and an Agilent 1100 system HPLC system. Chromatographic separation was achieved using a BDS Hypersil C_8 column (50x2.1 mm, 3 µm). The MS instrument was operated with positive electrospray and multiple reaction monitoring. Two transitions were monitored for each analyte: 363.3 → 121.2 and 97.2 for cortisol, 361.3 → 162.9 and 105.2 for cortisone, 393.1 → 147.2 and 237.2 for dexamethasone and betamethasone, 361.2 → 147.2 and 279.2 for prednisolone and 521.4 → 115.0 and 338.0 for beclomethasone dipropionate. The ratio of intensities of product ion fragments was established for each compound. It was used to detect interference from isobaric compounds and to confirm the identity of the synthetic glucocorticoids. Dexamethasone and betamethasone are epimers, co-elute, and fragment in the same way under the chosen conditions. However, the ratio of the fragments differs.
Results: Cortisol and cortisone were quantitated using d_4-cortisol as internal standard, and the assay was linear up to 788 nmol/L and 777 nmol/L respectively. The method was validated with a limit of quantitation of 5.0 nmol/L for both analytes. The total imprecision of the method was 3.8% for cortisol and 7.9% for cortisone. The method was compared to a cortisol immunoassay (Immulite) method: y = 1.29 x + 26.6, r = 0.83. The analysis time per sample is 7 min. The synthetic glucocorticoids included in the acquisition method were not quantitated if detected, but they were detectable to less than 5 nmol/L. In an audit of 138 samples collected from 103 patients (38 male and 65 female), prednisolone was detected in 3 samples from 2 patients. Dexamethasone was detected in 3 samples from different patients, and betamethasone was detected in 1 sample. 2 of these 6 patients had completely suppressed cortisol and cortisone. Cortisol and cortisone were suppressed in 3 other patients, but we were unable to determine the cause. The effect of the synthetic corticosteroid will depend on time of administration and duration of therapy.
Conclusion: We have developed a sensitive and specific method for the analysis of urine free cortisol and cortisone, and additional information about possible glucocorticoid therapy will aid clinicians and improve patient management.

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Pattern of cytokines in Graves' disease after treatment with methymazol

L. IONESCU[1], A. E. STANCIU[2], R. TRIFANESCU[1]. *[1]"C.I.Parhon" National Institute of Endocrinology, Bucharest, Romania, [2]"Prof.Dr.Al. Trestioreanu" Institute of Oncology, Bucharest, Romania,*

Background: To assess methymazole's effects on inflammatory markers in patients with Graves' disease, after restoring normal thyroid function, just before surgery.
Patients and methods: 90 patients (11M/79F, aged M41.9±15.6, F42.1±11.4 years) with Graves' disease were enrolled in the study, 9 of them showing concomitant papillary carcinoma on pathology. Proinflammatory cytokines: interleukin 1β (IL-1β), tumor necrosis factor (TNF-α) and interleukin 10 (IL-10) were measured by ELISA; C reactive protein (CRP) and total antioxidant status (TAS) were determinated by chemiluminescent immunoassays, fibrinogen by coagulation method in citrated plasma, ESR by Westergreen method, acid uric and cholesterol using biochemical methods.
Results: There is a mild inflammatory status despite normalization of thyroid function: increased IL-10 1.79 ± 1.34 pg/dL, fibrinogen 464.3 ± 114.1 mg/dL. CRP 0.27 ± 0.46 mg/dL., ESR 11.5 ± 8.7 mm/h were in normal range. There is no difference between inflammatory markers in patients with Graves' disease and patients with incidentally discovered thyroid carcinoma. There is a significant correlation between fibrinogen and ESR ($r = 0.4$, $p < 0.0001$, $t = 3.96$), fibrinogen and CRP ($r = 0.5$, $p < 0.0001$, $t = 5.39$) and IL-10 and CRP ($r = 0.4$, $p < 0.0001$, $t = 0.38$). There is a gradual increase, in normal limits, of CRP, ESR and IL-10 with age in women. Among these groups there are differences regarding the followed parameters, after therapy.
Conclusion: Despite normalization of thyroid function, inflammatory markers remain increased in patients treated with methymazole. The status of oxidative stress is normal, like the endocrine response.

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Comparison of Results from Four Commercially Available FT4 Assays to Results Obtained After Equilibrium Dialysis

M. L. Gantzer, D. R. Paramore, R. T. Gorman. *Siemens Healthcare Diagnostics, Newark, DE,*

OBJECTIVE: Thyroxine (T_4) is one of the thyroid hormones and plays an important role in regulating metabolism. The majority of circulating T_4 (99.95%) is reversibly bound to various transport proteins, with the remainder free in the circulation (FT_4). It is well-recognized in the clinical laboratory that there is variation in results obtained by different FT_4 assays due to both a lack of standardization and variable biases due to endogenous or in vitro factors. We compared the results obtained from four commercially available assays to RIA after equilibrium dialysis (ED), often considered to be a "gold standard" method.
METHODS: Serum samples that had already been tested on an Ortho Clinical Diagnostics Vitros ECi Immunodiagnostic System with the Vitros® FT4 Assay were frozen at -70 C until the day of testing. Samples were thawed at room temperature for 30 minutes prior to testing on the following Siemens Healthcare Diagnostics systems: ADVIA Centaur® FT4 Assay on an ADVIA Centaur XP™ Automated Chemiluminescence System; FT4 Flex® Reagent on a Dimension® RxL Clinical Chemistry System with Heterogeneous Module; and FT4 Flex Reagent on a Dimension Vista® 500T System. An aliquot of each sample was also sent to Quest Diagnostics (Chantilly, VA) for ED followed by FT4 determination via RIA. Out of an original 51 samples, 36 were in the assay range for all 5 methods.
RESULTS: As shown by the slopes in the following table, all four methods had FT4 values less than those obtained by ED. The slopes from the ADVIA Centaur and Vista Systems were almost identical, while the Vitros and RxL Systems had slightly lower slopes. The ADVIA Centaur, RxL and Vista Systems had similar correlation coefficients; the Vitros correlation was much lower.

Assay	Technology	Assay Range (ng/dL)	Comparison to ED	
			Slope	Correlation (R^2)
ADVIA Centaur	Competitive immunoassay; Chemiluminescent detection	0.1-12	0.51	0.84
RxL	2 step EIA with colorimetric detection	0.2-6.5	0.44	0.90
Vista	Homogeneous, sequential chemiluminescent assay based on LOCI® technology	0.1-8.0	0.51	0.81
Vitros	Competitive Immunoassay; luminescent detection	0-6.99	0.48	0.69

CONCLUSIONS: The four commercial assays each show similar performance when compared to equilibrium dialysis.

B-51

Measurement of 1,25 dihydroxy vitamin D by LC-MS/MS

F. Strathmann, T. J. Laha, A. N. Hoofnagle. *University of Washington, Seattle, WA,*

Background: The measurement of 25-hydroxylated vitamin D is useful in determining vitamin D stores but provides no information about the active dihydroxylated metabolite of vitamin D. The level of the biologically active 1,25 dihydroxy vitamin D is an important component to monitoring patients with chronic kidney disease, oncogenic osteomalacia syndrome and acquired or inborn errors of phosphate homeostasis. The dihydroxylated form of vitamin D has been a challenging analyte to measure in the clinical laboratory due to the low levels present in serum and the poor characterization of cross-reactivity of available immunoassay detection methods with other forms of vitamin D. We sought to develop an in-house method for the analysis of 1,25 dihydroxy Vitamin D3 and D2 using a combination of immunoaffinity purification followed by high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Methods: We used off-line isopropanol protein precipitation of serum followed by an antibody-based extraction and 4-Phenyl-1,2,4-Triazoline-3,5-Dione derivitization for analysis by LC-MS/MS. We report here the current intra and inter-assay performance, analytical measurement range and clinical recovery.
Results: For $1,25(OH)_2D3$, the intra-assay precision (CV) at 50 pg/mL and 20 pg/mL was 16.2% and 13.8%, respectively. Inter-assay precision was 19.7% (50 pg/mL) and 25.7% (20 pg/mL). The analytical measurement range was 2 to 500 pg/mL. The clinical recovery was 96.3% at 50 pg/mL and 86.6% at 20 pg/mL. For $1,25(OH)_2D2$, the intra-assay precision at 50 pg/mL and 20 pg/mL was 18% and 28%, respectively. Inter-assay precision was 22.9% (50 pg/mL) and 28.2% (20 pg/mL). The analytical measurement range was 2 to 500 pg/mL. The clinical recovery was 83.7% at 50 pg/mL and 81.4% at 10 pg/mL.
Conclusions: Isopropanol protein precipitation followed by an antibody-based extraction is an effective method for sample preparation prior to LC-MS/MS analysis for $1,25(OH)_2D2$ and $1,25(OH)_2D3$.

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Determination of Urinary Metanephrine and Normetanephrine by LC-MS/MS Following Solid Phase Extraction

Z. D. Clark[1], M. L. Parnas[2], E. L. Frank[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT,*

BACKGROUND: The determination of urinary metanephrine (M) and normetanephrine (NM) concentrations is used in clinical diagnosis of pheochromocytoma, a rare but potentially fatal tumor arising primarily from the chromaffin cells of the adrenal medulla. In our laboratory, a GC-MS method is used to measure M and NM concentrations. Separation and detection are accomplished in 13 minutes. The assay is robust, although double derivatization is required to provide unique fragments for both analytes. Increased reagent cost and limited availability prompted our investigation of an alternate method. OBJECTIVE: The goal of this study was to develop a high-throughput LC-MS/MS method for the measurement of urinary M and NM to replace the current GC-MS based assay. METHOD: Urine specimens were subjected to acid hydrolysis at 90 °C for 30 min. M and NM were isolated using mixed mode cation exchange solid phase extraction and separated in 50 mM formic acid in water/methanol on a Restek Ultra II PFP column (2.1 x 100 mm, 3 µm) using an Agilent 1200 HPLC system. Gradient elution produced well-resolved peaks within 5 minutes. Detection was accomplished with an Applied Biosystems API 3200 triple quadrupole mass spectrometer with electrospray ionization in positive ion mode. Quantitation was achieved using deuterated internal standards and a five-point calibration curve. Data was acquired in the multiple-reaction monitoring mode: *m/z* 180 --> 165 for M, *m/z* 166 --> 134 for NM, *m/z* 183 --> 168, for d_3-M, and *m/z* 169 --> 137 for d_3-NM. RESULTS: Method validation results are displayed in the table below.

Parameter	Metanephrine	Normetanephrine
Limit of Quantitation	10 nmol/L	14 nmol/L
Analytical Measurement Range	50-7000 nmol/L	50-7000 nmol/L
Linearity	y= 0.995x + 24.6 Sy/x= 48.3	y= 0.998x + 12.1 Sy/x= 29.1
Precision Estimate - Low Control:		
Mean	1665 nmol/L	3920 nmol/L
Within Run CV	1.1 %	1.3 %
Between Run CV	1.0 %	0.6 %
Total CV	2.4 %	2.9 %
Precision Estimate - High Control:		
Mean	3558 nmol/L	6390 nmol/L
Within Run CV	0.8 %	0.8 %
Between Run CV	0.5 %	1.7 %
Total CV	1.4 %	2.5 %
Accuracy/Method Comparison	n= 25 y= 1.002x - 5.4 Sy/x= 215.5 R= 0.9956	n= 25 y= 0.969x + 668 Sy/x= 1510 R= 0.9887

CONCLUSION: We have successfully developed and validated an LC-MS/MS method for the measurement of urinary M and NM. Advantages of this method include higher analytical sensitivity and increased throughput compared to the GC-MS method in current use in addition to elimination of a cumbersome and costly sample derivatization step.

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Impact of using glycosylated hemoglobin (HbA1C) in screening for increased risk of diabetes

T. C. Aw, S. K. Pua, P. W. Wong, L. Yew, S. P. Tan. *Changi General Hospital, Singapore, Singapore,*

Background In 2003 the American Diabetes Association (ADA) revised downwards the cut-point for an impaired fasting glucose (IFG) from 6.1 to 5.6 mmol/L (Diabetes Care 2003; 26:3160). In 2009 the ADA Expert Committee recommended the use of HbA1c in the diagnosis of diabetes (Diab Care 2009; 32:1). ADA has since updated the Standards of Medical Care in Diabetes 2010 (Diabetes Care 2010; 33.Suppl 1;S11) to include the use of HbA1c for diagnosis of diabetes (HbA1C > 6.5%); HbA1C of 5.7-6.4% is considered at "increased risk for future diabetes". Pre-diabetes (IFG and impaired glucose tolerance - IGT) has also been included in this category of increased risk.
Methods We measured HbA1C using an automated immunoturbidimetric assay (Cobas 6000, Roche) in 139 subjects (aged 21-65, average 40.3 years) - 84 subjects with IFG (fasting glucose 5.6-6.9 mmol/L) and in 55 normal euglycemic subjects (fasting glucose 3.3-5.5 mmol/L). This assay is quite precise - interassay cv is 2.2% @ HbA1C values of 5.8% and 1.1% @ HbA1C concentrations of 9.6%.
Results Thirty-one of the 55 (56.4%) normal euglycemic subjects have HbA1C values between 5.7-6.2%. In the IFG group 57 of the 84 (67.9%) hyperglycemic subjects had HbA1C > 5.7%; 16 of these subjects had HbA1C values that are diagnostic of diabetes (>6.5%). In fact, the proportion of abnormal or "at risk" HbA1C values increase sharply beyond fasting glucose levels of 6.1 mmol/L (11 subjects versus 5 patients).
Conclusion. It is left to be seen what the longer-term outcomes of these subjects will be. In the mean time both laboratorians and clinicians can expect to see more people diagnosed as "at risk" or "overt" diabetes if we adopt the new ADA criteria.

B-54

Method Comparison of UniCel Dxi 600 Immunoassay System Versus Immulite 2000

Y. Qiu, T. Currington, J. R. Petersen, A. O. Okorodudu. *UTMB, Galveston, TX,*

Background: The UniCel DxI 600 Immuno System and Immulite 2000 are high throughput immunoassay systems with a broad menu for patient care diagnostic service. The DxI uses heterogeneous, enzyme-mediated assays with most tests completed in 15-30 minutes. Coated paramagnetic particles in the DxI react with target analyte in patient sample and assay-specific reagents to form immune complexes. A magnetic field immobilizes the bound immune complexes while the unbounded components are washed away. Chemiluminescent substrate then reacts with an enzyme label present in the bound complexes and releases light. Immulite 2000 has analytical throughput ranging from 45 - 60 minutes and uses specific antibody-coated polystyrene beads as solid phase. The two systems were evaluated for menu, analytical performance and throughput.
Methods: The menu from both systems was evaluated relative to our scope of service. Twelve tests (n = 20-50 samples per assay) were evaluated using samples selected to cover the entire analytical range. Results from the Dxi were compared to those obtained from

the Immulite. Each sample was tested in singleton at the same time on the two instrument systems. CLSI EP09-A2 protocol was used for method comparison. Linear regression was performed to determine the agreement of two instrument systems. The evaluation also included reference range validation by using 10 normal males and 10 normal females. The evaluation also included impact of analytical throughput for "STAT" assays (i.e., intra-operative PTH) was also considered.

Conclusion: The analytical performance indicated that results from both DxI and immulite correlated well with noted biases (as shown by slope and intercept) which are attributable to antibody specificity. The availability of the inhibin A assay in the DxI gave it the menu advantage. Also, the significantly faster analytical time for PTH in the DxI gives an edge.

Method Comparison

Test	Slope	Intercept	R	Reference Range
CEA	0.90	12.04	0.9620	0-10 ng/mL
Cortisol	0.72	2.99	0.8601	6.7-22.6 ug/dL
DHEAS	0.98	-1.58	0.9599	50-995 ng/mL (Prepubertal) 650-3400 ng/mL (Adult)
Estrodial	0.85	-1.02	0.9750	20-47 pg/mL (Male) 26-158 pg/mL (Female Follicular Phase) 69-364 pg/mL (Female Midcycle) 51-219 pg/mL (Female Luteal Phase) 0-47 (Female Postmeopausal)
FSH	0.93	3.3	0.9843	1-18 mIU (Male) 3-10 mIU/mL (Female Follicule Phase) 3-34 mIU (Female Midcycle) 1-12 mIU (Female Luteal Phase) 23-116 mIU (Female Postmeopausal)
hLH	1.18	-0.26	0.9898	1.24-8.62 mIU/mL (Male) 2.12-10.89 mIU/mL (Female Follicle Phase) 19.18-103.3 mIU/mL (Female Midcycle) 1.20-12.86 mIU/mL (Female Luteal Phase) 10.87-58.64 mIU/mL (Female Postmeopausal)
Insulin	1.17	-1.93	0.8838	1.9-23 uIU/mL
CA125	1.28	49.11	0.9841	0-35 U/mL
Progesterone	0.86	1.95	0.9152	0.27-0.90 ng/mL (Male) 0.33-1.2 ng/mL (Female Follicle Phase) 0.72-17.8 ng/mL (Female Luteal Phase) 0-1.0 ng/mL (Female Postmeopausal)
Prolactin	0.82	3.41	0.9830	2.64-13.13 ng/mL(Male) 3.34-26.72 ng/mL (Female)
PTH	0.73	28.62	0.9892	12-88 pg/mL

B-55

Development of the ADVIA Centaur® Enhanced Estradiol (eE2) Assay

B. O'Donnell, M. Smith, A. Shuaib, S. St-Onge. *Siemens Healthcare Diagnostics, Tarrytown, NY,*

Background: Measuring circulating levels of estradiol is important for assessing ovarian function and monitoring follicular development for assisted reproduction protocols. The aim of this study was to evaluate the performance of the ADVIA Centaur Enhanced Estradiol (eE2) assay.*

Method: The eE2 method was assessed for limit of blank (LoB) and limit of detection (LoD) according to CLSI EP17-A guidelines. Functional sensitivity was determined using multiple samples in the range of 5 to 55 pg/mL (18.4 pmol/L to 201.9 pmol/L). All samples were assayed in duplicate in each of 10 runs over the course of 5 days (n = 20 for each sample). Intra- and interday precision was estimated based on CLSI EP5-A2 guidelines with eight samples ranging from 41 to 2940 pg/mL. A study was also done to compare eE2 with ID-GC/MS with 30 samples across the range. Forty-five steroids/drugs were spiked into a base pool containing endogenous levels of estradiol.

Results: The ADVIA Centaur Enhanced Estradiol assay measured estradiol concentrations up to 3000 pg/mL (11010 pmol/L), with a limit of blank of 2.3 pg/mL (8.4 pmol/mL) and a limit of detection of 6.6 pg/mL (24.2 pmol/L). The functional sensitivity of the Centaur Enhanced Estradiol assay was 19 pg/mL (69.8 pmol/L). Within-run and total CVs for samples with estradiol levels between 200 and 2940 pg/mL were <5.6% and <8.8%, respectively. The ADVIA Centaur Enhanced Estradiol Assay is standardized using internal standards manufactured analytically which are traceable to gas chromatography-mass spectroscopy (GCMS). The following equation describes the relationship between samples tested by GC/MS and samples tested by the Enhanced Estradiol method throughout the range of the assay: ADVIA Centaur Enhanced Estradiol = 1.02 (GC/MS) + 15.2 pg/mL; r = 0.9974. The ADVIA Centaur Enhanced Estradiol assay showed high specificity for estradiol, as estrogens and their metabolites all cross-reacted less than 0.5% in the assay.

Conclusion: The ADVIA Centaur Enhanced Estradiol (eE2) assay is a reliable and rapid immunoassay with better precision and higher specificity and is capable of measuring free estradiol from 6.6 to 3000 pg/mL in human serum or plasma.

For investigational use only. The performance characteristics of this product have not been established.

B-57

Relationship between Osteoprotegrin Levels and Microvascular Complications in Patients with Type II Diabetes Mellitus

N. Eren, F. Turgay, O. Koca, S. Cigerli, Y. Altuntas, B. Aslan, H. Kocyigit. *Sisli Etfal Research and Training Hospital, Istanbul, Turkey,*

Osteoprotegrin (OPG) is synthesized in different types of vascular cells such as media layer of large arteries, coronary artery smooth muscles cells and endothelial cells. This diversion of production sites shows that it has a vascular function.

In this study we aimed to compare OPG levels of complicated and uncomplicated type II diabetes mellitus. 60 Type II diabetic patients and 21 healthy control subjects were involved in the study. To be able to investigate effects of both microalbuminuria and diabetic retinopathy (DRP) together, we also grouped same patients under the categories of patients having neither microalbuminuria nor DRP (Group 1, n: 28), patients having microalbuminuria but not DRP (Group 2, n: 17), and patients having both microalbuminuria and DRP (Group 3, n: 13).

In these groups, by comparing plasma OPG, Homocystein, lipid and HbA1c levels, we aimed to investigate relationship between plasma OPG levels and microvascular complications in patient with Type II diabetes mellitus.

In this study, we used "Bender MedSystems Human Osteoprotegrin Instant ELISA kit which has a reference range of 57.41 - 126.83 pg/ml for the measurement of OPG. We performed statistical analysis by using SPSS for Windows 15.0.

As a result of our statistical analysis, we found significantly higher OPG levels in Group 3 (184.16±83.95 pg/ml) than that of control group (84.74±20.96 pg/ml), Group 1 (108,62±54,45 pg/ml), and Group 2 (91,02±17,89 pg/ml) (p=0.0001). On the other hand, although OPG levels of Group2 was higher than that of the healthy control group, difference between these groups was not statistically significant (p=0,979). Because with the exception of 2 patients, all the patients having DRP had also microalbuminuria, we can say that increased OPG levels are more likely related to DRP positivity than microalbuminuria levels.

We also investigate if there are any correlation between plasma OPG levels and other parameters related to microvascular complications. We found positive correlation between plasma OPG levels and age (r=0.351, p=0.001), duration of diabetes (r=0.279, p<0.05), DRP (r=0.576, p<0,001) and Homocystein (r=576,p<0,001). There were weak positive correlation between OPG and microalbumin levels, but this correlation was not statistically significant (r=0,125, p=0,056)

As a result, we found most powerful relationships with plasma levels of OPG and DRP and duration of diabetes mellitus. These finding suggests that as the risk of developing complications increase, the plasma levels of OPD also increase. However, to be able to clarify if OPG has a potential use as an early marker for microvascular complications of diabetes, there is need for more detailed, long term studies with involvement of large number of patients.

B-58

Method Comparison of Progesterone Quantitation: Roche cobas 6000 versus Roche cobas e411

S. Trapp, J. B. Hoyne. *Mayo Clinic, Jacksonville, FL,*

Background: In order to offer progesterone testing at our clinic in Florida that yields results comparable to those at other sites within our health system, we conducted a method comparison of this assay run on a cobas 6000 at multiple sites and a cobas e411 at our site. Initial results were not acceptable despite the great similarities in instrumentation and identical reagent. Identifying the source of this unacceptable performance was complicated by the fact that method comparisons for this reagent were performed by Roche comparing performance on the Modular Analytics E170 versus the Elecsys 2010. Following instrument maintenance on the cobas e411 we obtained acceptable performance for the assay between sites. We are submitting this abstract to establish a literature citation for this information.

Method: Progesterone levels were determined on serum samples at 3 testing sites. 20 samples were run on a cobas 6000 in Rochester, MN. These samples were frozen, shipped, thawed and run on a cobas e411 in Jacksonville, Fl. A further 20 samples were

run on a cobas 6000 in Andover, MA, frozen, shipped, thawed and run on a cobas e411 in Jacksonville, Fl. All testing used Roche progesterone reagent (12145383) according to manufacturer's instructions.

Results: Method comparison between the cobas 6000 and the cobas e411 progesterone assays yielded a least squares regression line of y=1.0451x-0.4128 and an r^2 value of 0.9922 for comparison between systems at Rochester and Jacksonville. Method comparison between the cobas 6000 and the cobas e411 progesterone assays yielded a least squares regression line of y=1.0667x-0.3579 and and r^2 value of 0.9975 for comparison between systems at Andover (cobas 6000) and Jacksonville (cobas e411). The performance characteristics for the progesterone assay on the cobas e411 were acceptable: Precision, mean (CV) = 0.69 ng/mL (5.8%), 8.45 ng/mL (0.8%) ,10.23 ng/mL (3.7%), 18.29 ng/mL (1.7%), 23.62 ng/mL (4.6%). Linear range = 0.15 ng/mL - 60.0 ng/mL. Limit of Quantitation = 0.15 ng/mL.

Conclusion: Method validation for the Roche progesterone assay was originally performed on the Modular Analytics E170 versus the Elecsys 2010 by the vendor. The Modular Analytics E170 is a precursor of the cobas e601 module, which is the immunoassay component of the cobas 6000. The Elecsys 2010 is a precursor of the cobas e411. While nomenclature for the systems has changed over the past few years, the precursor systems are considered analytically identical to the cobas systems which replaced them. Our initial unacceptable results led us to wonder whether the differences in results were due to analytical differences or a maintenance issue with our cobas e411. After maintenance, progesterone determined on the cobas e411 correlated very well with results obtained on the cobas 6000. As these systems use very similar hardware and the same reagent, this result is in agreement with *a priori* assumptions. We hope that publication of this abstract will serve to clarify the relative performance of the cobas e411 and the cobas 6000.

B-59

Macroprolactinemia: Our Experience Of 3680 Cases

S. Ozdem, S. Dogan, H. Ucar, S. Kuloglu, G. Yucel, I. Mendilcioglu, H. Altunbas. *Akdeniz University Medical Faculty, Antalya, Turkey,*

Background: Hyperprolactinemia is the most frequent reason for galactorrhea, amenorrhea and infertility in women. Based on its molecular weight, prolactin is present in three forms in circulation as monomeric (23 kDa), dimeric (45-60 kDa), and multimeric (100 kDa). Macrolactin is a prolactin-IgG complex and constitutes a minor fraction of the prolactin in circulation. It is much more slowly cleared from the circulation compared to prolactin and is biologically inactive. Although it produces no symptoms in patiens, being an important source of interference in immunoassays it causes errors in assays and diagnostic tests. Polyethylene glycol (PEG) precipitation is a fast and cheap method for screening macroprolactinemia. Given that macroprolactin requires no diagnostic test or treatment, differentiation of macroprolactinemia is particularly important in cases with prolactinemia. In the present study we aimed to determine the prevalence of macroprolactinemia and thus the real ratio of hyperprolactinemia in cases with a lab demand for prolactin measurement.

Materials and Methods: The presence of macroprolactin was assessed using PEG precipitation technique in 3680 cases who applied to our lab with a demand for the measurement of prolactin level in serum between the dates of January 2006 and July 2009. Cases with a post-PEG precipitation prolactin level that is less than 60% of pre-PEG precipitation prolactin level were considered as positive for macroprolactin.

Results: The mean age of the patients were 34.11 ± 7.74 years (mean ± SD). Macrolactin was positive in 2,91% (107 out of 3680) of our cases. We determined real hyperprolactinemia in 905 cases (24.59**%).**

Conclusion: Determination of the presence of macroprolactin may be helpful in assigning the etiology in patients with hyperprolactinemia and may eliminate the need for intensive diagnostic tests/ hypophyseal imaging and unnecessary treatment. Therefore, we recommend macroprolactin screening in all patients with hyperprolactinemia.

B-60

Do We Need the Glucose Challenge Test (GCT)?

C. Schromm[1], D. Lantz[2], E. S. Pearlman[3]. *[1]Codman Square Community Health Center, Boston, MA, [2]East Boston Neighborhood Health Center, Boston, MA, [3]Boston Medical Center, Boston, MA,*

It is current practice in our institutions to screen patients for gestational diabetes mellitus (GDM) with a non-fasting GCT using a 50 gram glucose load and proceeding to a 3 hour OGTT using a 100 gram glucose load when the plasma glucose concentration (PGC) exceeds a pre-specified value (140 mg/dL). We considered two algorithms that might replace the GCT and simultaneously reduce the total number of required 3 hour OGTTs. The first algorithm applied the following rules:

1. If fasting PGC <85 mg/dL then stop.
2. If fasting FPG =/>100 mg/dL stop and diagnose GDM
3. If fasting PGC falls between 85 and 99 mg/dL do a 2 hour PG after intake of a 100 gram glucose load. Diagnose GDM if PGC is =/> 155 mg/dL.

The second algorithm specified an overnight fast followed by determination of PGC one hour after challenge with a 100 gram glucose load and then applied the following rules:

1. If 1-hour PGC <155 mg/dL then stop.
2. If 1-hour PGC is =/>180 mg/dL then stop and diagnose GDM.
3. If PGC falls between 155 and 179 mg/dL then refer the patient for a complete 3-hour OGTT.

We initially considered data from 167 consecutive patients referred for 3-hour OGTTs of whom 50 met ADA criteria for GDM. The median age of these patients was 30 YO [range: 16-46 YO] with that of the GDM patients (33 YO) being significantly greater than that of the non-GDM patients (29 YO) [p<.01; median test]. All PG determinations were performed on a Dade Xpand chemistry analyzer (Siemens; Newark, DE) according to manufacturer's instructions. The first algorithm would have detected 43/50 GDM patients and generated 9 FPs with an overall reduction of 70% in the number of PG determinations for this population. When applied to an independent sample of 28 patients from a second community health center, the first algorithm would have detected 7/9 patients who met ADA criteria for GDM and generated 2 FPs again with an overall reduction of 70% in the number of PG assays. The second algorithm would have detected 49/50 GDM patients with 8 FPs. An overall reduction of 57% in the number of PG determinations would have resulted with 48 OGTTs being required in place of the 167 that were actually done. When applied to the second sample, this algorithm detected all 9 GDM patients without any FPs and 8 (instead of 28) 3-hour OGTTs would have been required. This is a retrospective study subject to selection bias since a positive screen would have been the pre-requisite to referral for a 3-hour OGTT. Nonetheless it does suggest the possibility of developing an algorithm with acceptable sensitivity for the diagnosis of GDM with a significant reduction in the number of PGC determinations and patient visits. Also there is the possibility of improving algorithm performance by incorporating additional easily accessible data e.g. patient age, gestational age at the time of testing and BMI to develop a classification score using a multivariate statistical approach.

B-61

Subclinical Hypothyrodism in a Midsized Academic Medical Center: An Unrecognized Epidemic

M. Jeffries, K. E. Bllick. *Un of OK Health Sci Ctr, Oklahoma City, OK,*

Background: Subclinical hypothyroidism (SCH) is a poorly recognized and potentially devastating condition associated with such common primary care complaints as sleep disturbance, memory impairment, dyslipidemia, musculoskeletal problems, coronary artery disease, and psychiatric illness, all typically treated individually with a panoply of medications or surgeries. The diagnosis of SCH is based on TSH measurements in the subclinical "grey zone" traditionally defined as 5-10 mIU/L; however, there is a dearth of studies reporting SCH prevalence. Accordingly, TSH remains a frequently underutilized screening modality in the outpatient population, where it may be quite beneficial, and has been historically over utilized in the inpatient population, where it often does not reflect true thyroid status.

Objective: The objective of this study was to evaluate the frequency of TSH measurements in the low (0.0-1.0 mIU/L), euthyroid (1.0 mIU/L-5.0 mIU/L), grey zone (defined at our institution as 5.0 mIU/L-10.0 mIU/L), and high (>10.0 mIU/L) ranges of large groups of inpatients and outpatients at a mid-sized academic medical center.

Methods: All measurements were performed at the University of Oklahoma Health Sciences Center Clinical Laboratory on an Advia Centaur XP Immunoassay System instrument using third-generation TSH reagents. The system employs a direct-chemiluminescent sandwich assay, ferromagnetic bead labeling, and a two-cycle wash procedure. Data were collected from men and women aged ten to 99 years. The outpatient pool consisted of 3461 tests and inpatient consisted of 3031 tests.

Results: Outpatients had a markedly high prevalence of subclinical hypothyroidism as reflected by TSH values in the "grey zone": 201 (5.9%). If, as some have suggested, the threshold for SCH were lowered to 3 mIU/L, 648 (18.9%) of outpatients would fall into this category. Other data include low TSH: 885 (25.9%), euthyroid: 2269 (66.3%), and overtly high TSH: 69 (2.0%). Unexpectedly, the majority of inpatients' TSH values were in the lower range (0.0 to 1.0 mIU/L): 1801 (61.1%). Other inpatient findings include euthyroid: 1039 (35.1%), subclinical: 71 (2.4%), and overtly elevated: 41 (1.4%).

Conclusions: Our outpatient population data suggest that the prevalence of SCH is quite high, ranging from 5.6% to 18.9%, suggesting that patients presenting with the variety of clinical complaints listed above may, in fact, be expressing symptoms of a so-called subclinical hypothyroid state. These data suggest that a trial of thyroid replacement therapy, rather than more drastic medical or surgical treatment, should be considered in addressing these common presenting complaints. It is noteworthy that only 2% of our outpatients had TSH levels above the traditional 10.0 mIU/L cutoff. In contrast, only 2.4% of inpatients were found to have TSH levels in the subclinical range, with an even lower percentage (1.4%) having TSH values >10.0mIU/L. TSH is known to be of limited use to

clinicians in evaluating true thyroid status of inpatients in whom, as our data indicate, TSH levels in the low range are likely reflective of a euthyroid sick subpopulation.

B-62

Comparative study between diabetic patients with and without microalbuminuria installed.

C. E. Ferreira[1], M. L. C. H. Issa[2], I. K. Ueda[2], A. M. O. Machado[2], A. Andrilo[2]. *[1]Hospital Albert Einstein, São Paulo, Brazil, [2]Universidade Federal São Paulo - UNIFESP, São Paulo, Brazil,*

Introduction: The world population is aging and therefore the incidence of metabolic disorders as well. The assessment of microalbuminuria for initial assessment of loss of kidney function and evaluation of glycated hemoglobin in the assessment "chronic" in diabetics is increasingly rooted in clinical practice.
Objective: To compare diabetic patients with and without microalbuminuria installed.
Methods: 100 randomly selected diabetic patients type 2 with microalbuminuria installed (> 20 μ/m) and 100 patients without the presence of microalbuminuria (< 20 micrograms / minute). We evaluated the following parameters: age, sex, fasting glucose, total cholesterol and fractions. The biochemical measurements were performed at the Olympus 640 analyzer *Beckman Colter*. The determination of microalbuminuria was performed by immunoturbidimetric the same equipment. The measurement of glycated hemoglobin was performed by HPLC equipment TOSOH 2.2 *Scenika*.
Results: The mean dosage of microalbuminuria among groups were as follows (1-without microalbuminuria : 6.7 μ/m and 2- with microalbuminuria: 61.6 μ/m).The comparative analysis of two groups - table 1. Statistical analysis was performed by Student t test.
table 1

	Glucose	Cholesterol	LDL	HDL	HbA1c
1- without microalbuminuria	143	166	92,4	46,5	7,3
2- with microalbuminuria	171	181	102,2	47,6	8,5
Test t	**0,006**	**0,003**	**0,01**	0,32	**<0,0001**

Conclusions: We conclude that the group has installed microalbuminuria has a poor glycemic control. The levels of total cholesterol and LDL also shown higher in the microalbuminuria group installed. The only parameter that has not changed in the two groups was HDL.

B-63

Evaluation Of Adiponectin As A Potential Component Of The Criteria For The Metabolic Syndrome In Normoglycemic First-degree relatives (FDR) of patients with type 2 diabetes

O. A. Mojiminiyi[1], H. Al Mohammedy[2], N. Abdella[1]. *[1]Faculty of Medicine, Kuwait University, Kuwait, Kuwait, [2]Ministry of Health, Kuwait, Kuwait,*

First-degree relatives (FDR) of patients with type 2 diabetes (T2DM) have increased risk of developing diabetes because of aggregation of obesity-associated cardiometabolic risk factors. This study explores the potential use of adiponectin, an adipokine with anti-inflammatory and insulin-sensitizing properties, as a marker of the metabolic syndrome (MS) in normoglycemic FDR of T2DM patients. Fasting adiponectin, insulin, glucose, and full lipid profile were determined in 423 FDR and 53 healthy control subjects without family history of diabetes. Clinical and anthropometric data were recorded and subjects were classified on the basis of the degree of adiposity, insulin resistance (IR) (HOMA-IR) and the number of criteria of the MS (International Diabetes Federation). Adiponectin concentration was higher in females than males (mean 9.7 vs. 6.9 ug/ml) despite similar waist circumference (WC). In both FDR and controls, adiponectin was inversely correlated with WC and HOMA-IR and positively correlated with HDL-cholesterol (HDLC). Adiponectin showed stepwise decrease with increasing number of MS criteria. Binary logistic regression showed that the odds ratio of MS as predicted by adiponectin was 0.55 [95% confidence interval 0.41-0.73; $p < 0.0001$]. At cut-off points of 7.5 ug/ml, the diagnostic sensitivity and specificity of adiponectin for the MS were 90% and 70% respectively compared to 42% and 95% for triglycerides and 80% and 54% for HDLC at standard cut-off points. Receiver Operating Characteristic analysis showed that adiponectin (0.859) had significantly higher area under the curve compared with HDLC (0.745) and triglycerides (0.823) for detection of MS. We conclude that adiponectin should be an additional and useful criterion for identification of the MS.

B-64

Establishing Traceability to Isotope Dilution Gas Chromatography Mass Spectrometry (ID/GC/MS) on the Dimension Vista® System for Three Steroid Hormone Methods: Estradiol*, Progesterone*, and Testosterone*

D. P. Clark[1], L. M. Thienpont[2], D. Morey[1], E. Oduaran[1], B. Wessel[1], L. Geng[1], T. Gorzynski[1]. *[1]Siemens Health Care Diagnostics, Newark, DE, [2]Laboratory for Analytical Chemistry Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium,*

Traceability of methods to reference procedures is desirable (ISO 17511). The Joint Committee for Traceability in Laboratory Medicine (JCTLM) lists Isotope Dilution Gas Chromatography Mass Spectrometry (ID/GC/MS) reference procedures for estradiol, progesterone and testosterone. We have established traceability to ID/GC/MS for the steroids on the Dimension Vista® System. To establish traceability we prepared serum reference pools which spanned the assay ranges. All pools were prepared from endogenous serum units; none were spiked with steroids. Units were screened for appropriate values, pooled proportionately to prepare pools of different concentrations for each steroid. Using testosterone as an example; units with testosterone values of 25-26 ng/dL were used for pool 2, units from 1-355 ng/dL were used for pool 3, units from 286-314 ng/dL were used for pool 4, and units from 966-1269 ng/dL were used for pool 5. The same procedure was used for progesterone and estradiol pools. All pools for the steroids were sent to the University of Ghent, a JCTLM listed reference measurement laboratory, for quantitation by ID/GC/MS. Reference pool values for estradiol range from 59.3 to 1973 pg/mL, progesterone from 0.03 to 37.4 ng/mL, and testosterone from 22.5 to 1119.0 ng/dL. The pools with ID/GC/MS values were used to calibrate the methods on a Dimension Vista System and assign values to masterpools for each method. The instrument was calibrated with the masterpools to assign values to commercial calibrator products. To establish traceability to the reference procedure, 38-59 patient serum samples were tested at the University of Ghent by ID/GC/MS and in-house using the steroid methods on a Dimension. Based on these results, the steroids are deemed traceable to their reference procedures and will provide accurate and reliable results. *Product under development_ Not available for sale

Linear Regression Analysis

Method	N	Range by ID/GC/MS	r	Slope and Intercept
Estradiol	54	5.6-1404.0 pg/mL	0.99	Dimension Vista=(1.01*ID/GC/MS)+ 0.4 pg/mL; Sy/x 33.4 pg/mL
Progesterone	38	0.02-31.8 ng/mL	0.99	Dimension Vista= (1.00*ID/GC/MS) + 0.11 ng/mL; Sy/x 1.29 ng/mL
Testosterone	59	3.8-1458.8 ng/dL	0.99	Dimension Vista= (1.00*ID/GC/MS) + 2.5 ng/dL; Sy/x 39.6 ng/dL

B-65

Development and Performance of a LOCI® Testosterone Assay on the Dimension Vista® System

T. J. Gorzynski, P. D. Nagle, Z. Teng, M. Drinan, J. F. Pierson-Perry. *Siemens Healthcare Diagnostics Inc., Newark, DE,*

We describe a fully automated homogeneous competitive binding immunoassay for testosterone on the Dimension Vista® System based upon LOCI® technology. The TTST* method utilizes three reagents: two synthetic bead reagents and a biotinylated monoclonal antibody specific for testosterone. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitizer dye. The second bead reagent (Chemibeads) is coated with a testosterone analog and contains chemiluminescent dye. Sample is reacted with a displacer to release testosterone bound to endogenous sex hormone binding globulin, which then competes with Chemibeads for biotinylated antibody to form bead-analog-antibody immunocomplexes. Addition of Sensibeads leads to formation of bead pair complexes. Illumination of the complex by light at 680 nm generates singlet oxygen from Sensibeads, which diffuses into Chemibeads to trigger a chemiluminescent reaction. The resulting signal (measured at 612 nm) is directly related to analyte concentration.
The assay uses a 7 μL sample volume of serum or plasma and has an analytical range of 5-1200 ng/dL, with results traceable to an ID/GC/MS reference method. The time to first result is 23 minutes. Precision was evaluated per CLSI EP5 using serum pools and commercial quality control materials. Repeatability and within-lab precision were < 2.3 %CV and < 7.3 %CV, respectively, across the assay range. Good agreement was observed in patient sample method comparison studies versus three different systems: Dimension Vista = 1.00 * ID/GC/MS + 2.5 ng/dL (r = 0.99, n = 59), Dimension Vista = 1.00 * Roche ELECSYS® - 10.1 ng/dL (r = 0.95, n = 59), and Dimension Vista = 1.00 * ADVIA

Centaur® - 36.9 ng/dL (r = 0.96, n = 59). Minimal cross reactivity (< 10%) was observed with key compounds including: androstenedione, androsterone, 5a-dihydrotestosterone, corticosterone, 11-deoxycortisol, DHEA, DHEA-sulfate, 17b-estradiol, progesterone, cortisol, dexamethazone, danazol, 17a-methyltestosterone, 11b-hydroxytestosterone, and 11-ketotestosterone.

Conclusions. We conclude that use of LOCI technology provides acceptable sensitivity, precision, accuracy, turnaround time, and dynamic range suitable for measurement of testosterone in serum and plasma.

*Product under development - Not available for sale

B-66

Postal Urinary C-peptide creatinine ratio can discriminate Type 1 from Type 2 diabetes and identify Type 1 patients with persistent endogenous insulin production

T. J. Mcdonald[1], R. Besser[2], M. Shepherd[2], B. Shields[2], B. Knight[2], A. Hattersley[2]. *[1]Royal Devon and Exeter Hospital, Exeter, United Kingdom, [2]Peninsula Clinical Research Facility, Peninsula Medical School, University of Exeter, Exeter, United Kingdom,*

Introduction: Serum C-peptide is a measure of persistent endogenous insulin production useful in identifying diabetes subtypes. Urinary C-peptide creatinine ratio (UCPCR) is stable over 3 days at room temperature so offers a potential practical outpatient alternative.

Aims/Objectives: To determine if a patient posted postprandial UCPCR can:(1) discriminate Type 1 (T1D) and Type 2 (T2D) diabetes;(2) detect T1D patients with persistent endogenous insulin secretion.

Methods: Postprandial UCPCR was measured in adult Caucasian patients (diabetes duration ≥5y, eGFR ≥60mL/min/1.73m^2) with T1D (n=61; diagnosed<30y, insulin since diagnosis) or T2D (n=54; diagnosed ≥35y, not insulin treated in year following diagnosis, treated with diet (6), oral agents (30), or insulin (18)).

T1D patients with significant UCPCR and matched negative T1D controls, undertook a mixed meal tolerance test (MMTT).

Results: UCPCR was lower in T1D than T2D:(median(IQR) <0.02(<0.02-<0.02) v 2.47(1.36-4.13)nmol/mmol, p=3.7x10^{-21}). Receiver Operating Characteristic Curves identified a cut-off of UCPCR ≤0.2nmol/mmol to be 95% sensitive and 98% specific for discriminating T1D from T2D (area under curve 0.99 perfect test 1.0). All 3 T1D patients with UCPCR>0.2 remained positive on repeat testing. In a MMTT their stimulated serum C-peptide was higher than in the controls (mean 579 v 24pmol/l) with 2/3 exceeding the C peptide negative cut off (200pmol/L) despite being GAD positive and 25 and 31 years post diagnosis.

Conclusions: Posted postprandial UCPCR can discriminate Type 1 from Type 2 diabetes and detect patients with long duration Type 1 diabetes with significant insulin production. Practical and pragmatic classification using UCPCR may be more relevant to patient care than an aetiological approach in long duration diabetes.

B-67

Urinary C-Peptide/Creatinine Ratio (UCPCR) is a reliable measure of endogenous insulin secretion, even in patients with renal impairment

T. J. MCDONALD[1], P. Bowman[2], B. Shields[2], B. Shields[2], R. Besser[2], G. Githens-Mazer[2], G. Githens-Mazer[2], A. Hattersley[2]. *[1]Royal Devon and Exeter Hospital, Exeter, United Kingdom, [2]Peninsula Clinical Research Facility, Peninsula Medical School, University of Exeter, Exeter, United Kingdom,*

Serum C-peptide can be used to measure endogenous insulin secretion in people with diabetes. However, C-peptide is predominantly renally cleared from blood limiting its value when patients have renal impairment which is often found in type 2 diabetes. In contrast serum insulin is predominantly metabolised by the liver. UCPCR is a non-invasive alternative that measures urinary C-peptide relative to creatinine so should better reflect insulin production in renally impaired subjects.

We aimed to assess the utility of UCPCR in individuals with Type 2 diabetes (T2D) as an alternative to serum C-peptide for measuring insulin secretion.

21 T2D patients on two oral agents (median BMI 29.6 kg/m2 (IQR 25.3-34.8), HbA1c 7.1(6.7-8.0) % had a standard high carbohydrate meal on three occasions. We measured serum insulin and C-peptide every 30 minutes over 2hrs and 2hr post meal UCPCR. 6 patients had renal impairment (eGFR <60 mL/min/1.72m^2).

UCPCR was at least as reproducible as serum C-peptide (fasting CV mean (95%CI): 29(21-37%) v 31 (24-39)% and 2hr post meal 21 (16-26)% v 28 (21-35)%). Insulin secretion, measured as insulin area under the curve (AUC), was strongly associated with serum C-peptide AUC (r=0.63 p =0.02) and UCPCR (r=0.85 p=0.000001). In regression analysis the association between serum C-peptide AUC and insulin AUC differed between renally impaired and non-renally impaired patients (p= 0.02 for interaction). In contrast UCPCR association with insulin AUC was not affected by renal impairment (p=0.6)

UCPCR better reflects insulin secretion than repeated serum C-peptide measurement in patients with renal impairment. This convenient test therefore has a potential utility in measuring endogenous insulin production in patients with diabetes and renal impairment.

Tuesday PM, July 27

Poster Session: 2:00 pm – 4:30 pm
Electrolytes/Blood Gas/Metabolites

B-68

Comparison of Glucose and Electrolyte Concentrations among Plasma Samples Obtained from Various Types of the Combination between Lithium Heparin and Antiglycolytic Agents

W. Boonlert[1], R. Wiriyaprasit[2], N. Nuanmuang[1], S. Oo-puthinan[3], A. Chittamma[4]. *[1]Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand, [2]Regional Science Institute, Phitsanulok, Thailand, [3]Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand, [4]Clinical Chemistry Division, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand,*

Background: Lithium heparin (LH) plasma samples have been used recently in urgent cases for simultaneously measuring glucose and routine biochemical testing. Previous studies introduced several antiglycolytic agents and combined those with LH for using in clinical chemistry testing. There were many arguments that had been discussed on the efficiency of these agents. The objectives of this study were to compare glucose and electrolyte concentrations among the combinations between LH and various antiglycolytic agents.

Methods: Twenty millilitres of fasting blood samples were collected from venipuncture from 20 healthy volunteers and 20 diabetic patients. Each sample was aliquot into pain, sodium fluoride (NaF), LH, and Plasma glucose, sodium (Na^+), potassium (K^+), and chloride (Cl^-), concentrations were measured in serum, NaF, LH, LH plus various antiglycolytic agents, 10.0 mmol/L glyceraldehydes (GA), 9.8 mmol/L of tris-bromoacetate (BA), or 16.7 mmol/L of D-mannose plasma samples at 0^{th}, 2^{nd}, 4^{th}, 6^{th}, and 8^{th} hours after blood drawing. Pair t-test was used for data analysis.

Results: Blood samples treated with LH plus D-mannose and LH plus tris-bromoacetate could preserve glucose levels effectively for up to 8 hours and did not significantly differ from using plasma NaF (p>0.05). Most plasma samples obtained from LH plus antigycolytic agents could be used for electrolyte analysis, except for the LH plus BA plasma that showed slightly hemolysis at 4 hours.

Conclusions: Plasma LH plus 16.7 mmol/L of D-mannose was the most efficiency for glucose and electrolyte determinations. The effect of this combination on other biochemistry should be further investigations.

B-69

Urinary pH Levels between Healthy and Metabolic Syndrome Volunteers

W. Boonlert, S. Rapiya, A. Karaged, O. Narksing. *Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand,*

Metabolic syndrome (MetS) is characterized by a group of metabolic risk factors that present in an individual. MetS has been linked to the abnormal biochemical processes involved in the body. Thus the body must have homeostasis processes to filtrate by kidney and show this processes with pH in urine. The objective of this study is to compare urinary pH levels obtained from healthy and MetS volunteers. The MetS was classified by using the NCEP-ATP III guideline and defined as having at least three criteria in an individual. Twenty milliliters of first morning urine samples were collected from 100 volunteers (50 healthy and 50 MetS volunteers) and urinary pH levels were measured using a pH paper and a pH meter. The median of pH determined by a pH paper was not significant different from those measured by a pH meter (p>0.05). The median of pH in healthy and MetS volunteers were 6.5 and 6.00 respectively. The median of urinary pH obtained in healthy was statistically significant higher than those obtained from MetS volunteers (p<0.001). Sensitivity and specificity of urinary pH levels were 82% and 90% respectively when used a cut off point ≤ 6.00 measuring by pH papers and MetS risk increased 8.2 times when compared to lower pH levels. In conclusions, urinary pH was different between MetS and healthy volunteers. Urinary pH ≤ 6.00 measured by pH paper could be used to distingue MetS from healthy.

B-70

Prevalence of Falls and Fractures in Hyponatremic Patients Presenting to an Emergency Department

S. D. Thomas, G. H. White, P. S. Coates. *SA Pathology, Adelaide, Australia,*

Background: Hyponatraemia is the most common electrolyte disturbance and is caused by either salt and water loss or water retention. The condition has been associated with gait disturbances and falls. Identification of hyponatremia early in the elderly may be important in reducing morbidity and mortality. We undertook a clinical audit of emergency presentations by patients with hyponatremia.

Methods: All consecutive emergency presentations to a tertiary level hospital who had an initial plasma electrolyte evaluation with a Na≤129 mmol/L were selected (reference interval for plasma Na 135 - 145 mmol/L). Plasma osmolality, K, Cl, emergency and discharge diagnosis for each patient were collated.

Results: During Jan-Aug 2009, 375 specimens (from 300 patients) with Na≤129 mmol/L were received from the emergency department (ED). Only 59 specimens had an osmolality requested (16%). Forty one of 300 patients presented to ED with a history of recurrent falls (13.7%) of whom 18 (43.9%) sustained a fracture as a result of the fall. The mean plasma Na in the fracture patients was 126 (120-129 mmol/L) similar to the mean for all patients with falls (125.7, range 119-129 mmol/L; mean age 81, range 55-96y) but significantly less than age matched controls (P<0.001) admitted with conditions other than falls or trauma.

Conclusion: The incidence of hyponatremia in patients with fractures has been reported to be higher than other patients presenting to ED. However only a small proportion of these patients have their hyponatremia investigated further. Falls and fractures are more likely to occur in patients with mild to moderate hyponatremia, suggesting the need for careful follow up and correction of Na levels in the elderly in the community.

B-71

Abbott ARCHITECT ICT (ISE) Module Use-Life Response Characteristics

J. L. Seago, Q. Li, D. Pistone. *Abbott Laboratories, Irving, TX,*

Objective: Characterize the use-life performance of the Integrated Chip Technology (ICT), a solid-state ISE module before and after testing over 15,000 samples over 2 months.

Relevance: Electrolytes, especially Potassium, are critical STAT tests requiring quick, accurate results. Sodium, Potassium and Chloride results are available within 3 minutes from 15 µL of Serum, Plasma or Urine on the Abbott ARCHITECT Clinical Chemistry Systems. The ICT module is calibrated once per day and warranted for 15,000 samples (45,000 tests) or two months use.

Methodology: 24 hour Calibration Stability, Linearity and Precision were evaluated on modules that processed 12,000, 17,000 and 22,000 samples. NIST SRM 909b, Linearity Standards, Controls, revised ICT Calibrator and new concentrated ICT Sample Diluent (55 and 90 mL cartridges) automatically diluted by the instrument, were used to characterize the ICT Module for Linearity, Precision and Accuracy.

Validation: Improvements were evaluated for Precision (EP5-A2), Linearity (EP6-A) and Accuracy (Six Sigma metric). The 24-hour calibration interval imprecision data represents typical response from an ICT module after processing the indicated number of samples. Sigma values are from a module that tested 22,000 samples over two months with 3 reps of NIST SRM 909b.

Sigma metric: (Sigma=[(TEa(%)-Bias(%))/CV(%)] as per westgard.com using RiliBäk TEa targets.

Results:

	# Samples	Months	Na^+ (S)*	Na^+ (U)*	K^+ (S)	K^+ (U)	Cl^- (S)	Cl^- (U)
Precision – 5 day (%CV)	0	5	0.33	0.51	0.44	0.43	0.33	0.43
	12,000	7	0.49	0.62	0.71	0.75	0.68	0.69
	17,000	7	0.33	0.53	0.47	0.41	0.62	0.53
	22,000	7	0.47	0.87	0.50	0.83	0.64	0.73
Linearity	**Range** (mmol/L)		100 - 200	20 - 400	1.0 – 10.0	1 - 300	50 - 150	20 - 300
	0 - 17,000	5 - 7	≤5%	≤5% /3 mM	≤5% /0.25 mM	≤5% /3 mM	≤5%	≤5% /3 mM
	22,000	7	≤5%	≤5% /3 mM	≤5% /0.25 mM	≤5% /3 mM	≤5%	≤5% /3 mM
Sigma Analysis - (NIST SRM 909b)			Na^+ (L1)	Na^+ (L2)	K^+ (L1)	K^+ (L2)	Cl^- (L1)	Cl^- (L2)
Certificate Value (mmol/L)			120.76	141.0	3.424	6.278	89.11	119.43
NIST 909b Mean (n=3)			118.56	139.40	3.31	6.13	88.24	119.02
Bias (%)			-1.82%	-1.14%	-3.47%	-2.34%	-0.97%	-0.34%
% CV (Serum)			0.47%		0.50%		0.64%	
TEa (%) (RiliBäk)			5.0%		8.0%		8.0%	
Sigma			**6.8**	**8.2**	**9.1**	**11.3**	**11.0**	**12.0**

* S=Serum, U=Urine

Conclusion: The claimed ICT Module performance was confirmed after testing >15,000 samples over 2 months. Recent ICT improvements resulted in better accuracy and precision especially for Potassium with a 24-hour calibration interval. Sigma metrics demonstrate improved quality confirming the Abbott ICT Module is well suited for use in the Clinical Chemistry Laboratory over its intended use life. The new concentrated ICT Sample Diluent (700/1200 samples/cartridge) eliminates the need for multiple cartridges on-board the system, freeing up more reagent positions for laboratories to run additional assays in-house.

B-72

Evaluation of Total Bilirubin Assay on the GEM Premier 4000

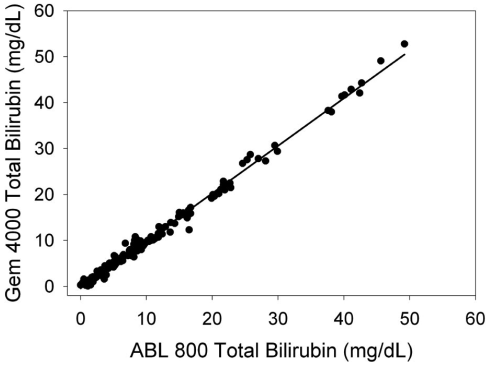
D. J. Dietzen[1], T. R. Wilhite[2], P. V. A. Pamidi[3]. *[1]Washington University School of Medicine, St. Louis, MO, [2]St. Louis Children's Hospital, St. Louis, MO, [3]Instrumentation Laboratory, Bedford, MA,*

Background: Unconjugated hyperbilirubinemia in newborns may lead to irreversible brain damage (kernicterus). Total bilirubin concentrations dictate the need for intervention (phototherapy, exchange transfusion) aimed at preventing neurological damage. The use of serum or plasma to measure bilirubin is complicated by limited availability of blood and high hematocrits characteristic of newborns. Alternatively, direct bilirubin may be measured in small volumes of whole blood by multiwavelength spectrophotometry. The aim of the current study was to compare the analytical performance of total bilirubin measurement on the Instrumentation Laboratory GEM Premier 4000 to that on the Radiometer ABL 800.

Methods: Excess heparinized blood from 100 neonates (age <60 days) and 100 adults was obtained from St. Louis Children's Hospital and Barnes-Jewish Hospital, respectively. Split samples were analyzed within 10 minutes of each other on the ABL 800 and GEM 4000 platforms. Imprecision was monitored through intelligent quality management (iQM) on the GEM and daily controls on ABL.

Results: Imprecision of controls over the three weeks of the study on the ABL was 3.3%, and 3.1% at 4.5 and 19.4 mg/dL, respectively (n = 117). Drift using iQM on the GEM did not exceed 0.05 mg/dL for the process checks at 10.4 mg/dL (n = 150) and 20.1 mg/dL (n = 50). Whole blood bilirubin concentrations ranged from undetectable to 45.6 mg/dL. The initial comparison revealed four significant outliers that were reconciled following a revised correction for turbidity on the GEM. Linear regression of the GEM vs. ABL (see Figure) yielded the following: GEM = 1.034 (ABL) -0.41, r = 0.997, S_{yx} = 0.84. Bias between the GEM and the ABL averaged -0.1 mg/dL (range -4.2 to 3.6 mg/dL).

Conclusions: GEM Premier 4000 shows substantially equivalent performance to the ABL 800 series in assessing the total bilirubin levels in both newborns and adults.



B-73

An LC-MS Method for the Quantification of 4 Bile Acid Species in Broncheal Washings from Lung Transplant Patients

A. N. Sireci, T. Thomas, B. Aramini, F. D'Ovidio, M. A. Pesce, S. Cremers. *Columbia University Medical Center, New York, NY,*

Context: Micro-aspiration may be a risk factor for the development of bronchiolitis obliterans syndrome (BOS) after lung transplantation. Total bile acids (TBA), measured by a spectrophotometric assay, in bronchial washings obtained during surveillance bronchoscopy are promising biomarkers in predicting outcomes in lung transplantation patients(1). However, little is known about which bile acid species are important in disease pathogenesis.

Objectives: To develop an LC-MS method for separating and quantifying 4 major bile acids species in bronchial washings from lung transplant patients.

Methods: Standard solutions of three primary bile acids (cholic acid (CA), deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA)) and one taurine conjugate (taurocholic acid (TCA)) were prepared in methanol. Bronchial washings found to be negative by an enzyme-based spectrophotometric assay for TBA (Trinity Biotech. Bray, Ireland) were spiked with standards, deproteinized with acidic methanol, redissolved in 55:45 (v/v) methanol: 0.01% acetic acid containing 0.05% ammonium acetate and subjected to LC-ESI(-)-MS (Waters Quattro Micro) in Selective Ion Recording mode over 14 minutes. The mobile phase was a gradient of water:methanol, both containing 0.5% formic acid and 0.05% ammonium acetate. Spiked bronchial washings were also subjected to the enzyme-based spectrophotometric assay.

Results: There was adequate baseline separation of all four species (RT: TCA 1.71min, CA 2.72 min, DCA 4.23min, CDCA 4.49min). Estimates of ionization suppression ranged from 13% (DCA/CDCA) to 50% (CA/TCA). Recoveries for each individual bile acid ranged from 65% for high concentrations of CA (50uM) to 95% for mid-range concentrations of TCA (5uM). The LC-MS method is linear from 0.05 to 100uM for all compounds (0.2-400uM TBA), exceeding the linear limits of the spectrophotometric method in bronchial washings (3.25-100uM TBA). The overall inter-injection and inter-assay precision for each bile acid species at high (50uM), medium (5uM) and low (0.5uM) concentrations were <3% and <10%, respectively. Inter-assay precision for TBA by LC-MS and trinity methods were comparable (50uM: 18% by LC-MS, 16% by Trinity; 5uM: 27% by LC-MS and 26% by Trinity). Correlation between the two methods on spiked samples was excellent (R2=0.96).

Conclusions: We have developed an LC-MS method for the quantification of 4 individual bile acid species in bronchial washings. Our method performs similarly to a commercially-available assay in the measurement of TBA in bronchial washings. It will be useful for future studies examining the role of specific bile acid species in the pathogenesis of BOS.

(1)D'Ovidio et al. J Thorac Cardiovasc Surg. 2005 May;129(5):1144-52

B-74

Standardized Enzymatic Creatinine on the Dimension Vista

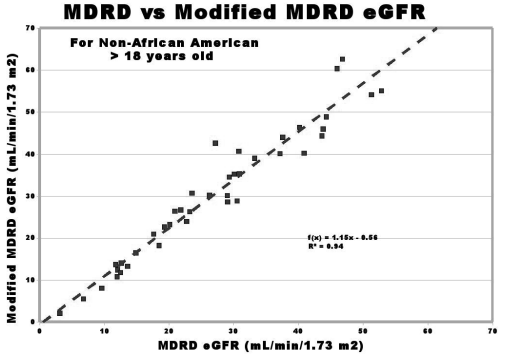
C. Feldkamp, M. Cosman, J. Zajechowski, J. Carey, V. I. Luzzi. *Henry Ford Hospital, Detroit, MI,*

Background: Serum creatinine concentration is used to estimate glomerular filtration rate (eGFR). In 2006, new recommendations by the laboratory working group of the National Kidney Disease Education Program (NKDEP) established that creatinine methods use to calculate eGFR should be standardized. Although the Jaffe method is commonly used in clinical laboratories to measure creatinine, it may overestimate this metabolite due to non-creatinine interferences. The enzymatic method also shows some interferences but it does not have the bias shown by the Jaffe method.

Objective: To evaluate the standardized enzymatic Dimension Vista creatinine assay (eCREA) and use it to calculate eGFR as recommended by the NKDEP. Method: De-identified serum specimens or commercially available material were used to investigate the accuracy by comparing the current creatinine assay (modified kinetic Jaffe) with the eCREA. Precision and linearity were also measured. Comparison of the eGFR using the Modification of Diet in Renal Disease (MDRD) or the modified MDRD was performed for eGFR below 60 mL/min/1.73m^2. EP evaluator or Microsoft Excel was used to analyze the data.

Results: The current creatinine assay compared very well with the eCREA (r=0.99; new=1.01*old-0.29). Within run precision at 1.02 mg/dL and 4.32 mg/dL had a CV of 1.06 and 0.39 % respectively. The precision profile demonstrated a CV of 18.9% at 0.11 mg/dL. The analytical measurable range was validated between 0.14-20 mg/dL with an average recovery of 98.4%. Linearity average recovery was 98.6%.

Correlation of the eGFR using the MDRD or the modified MDRD was good (new=1.15*old-0.15; R^2=0.94, figure). Conclusions: The new eCREA Dimension Vista and eGFR correlate well with the method and calculations currently used. Using the standardized method, our laboratory will be able to report the eGFR according with the NKDEP recommendations.



B-75

Closing the Gap: Correlation of D-lactate with Anion Gap and Metabolic Acidosis in Diabetic Ketoacidosis

Q. H. Meng[1], J. Lu[2], G. Zello[1], E. Randell[3], K. Adeli[4], J. Krahn[1]. *[1]University of Saskatchewan, Saskatoon, SK, Canada, [2]Wenzhou Medical College, Wenzhou, China, [3]Memorial University of Newfoundland, St Johns, NL, Canada, [4]University of Toronto, Toronto, ON, Canada,*

Objective: Diabetic ketoacidosis (DKA) is a severe acute complication of diabetes. Methylglyoxal is an intermediate glucose metabolite and markedly increased in diabetes**.** D-lactate is an intermediate metabolite of methylglyoxal. A high anion gap in DKA suggests that some unmeasured anions must contribute to the generation of the anion gap in addition to the ketone bodies. This study was to investigate the correlation of D-lactate with methylglyoxal, metabolic acidosis and anion gap in DKA.

Subjects and Methods: In this study, 48 diabetic patients without ketoacidosis (Diabetes), 47 patients with DKA and high anion gap and 43 healthy controls were included. Plasma methylglyoxal was assayed by liquid chromatography-mass spectrometry. Plasma D- and L-lactate were quantitated by high-performance liquid chromatography. Plasma glucose, β-hydroxybutyrate, bicarbonate, electrolytes and blood hemoglobin A1c (HbA1c) were determined on the chemistry analyzers in the clinical biochemistry laboratory.

Results: The plasma fasting glucose levels, β-hydroxybutyrate and blood HbA1c in DKA were significantly higher than in the Diabetes and in the controls ($P < 0.001$). The plasma MG levels in the DKA were highly elevated compared to the Diabetes and to the controls (325.41 ± 140.62 vs. 147.70 ± 59.26 and 325.41 ± 140.62 vs. 169.80 ± 93.67 nmol/l, respectively, $P < 0.001$). Plasma bicarbonate levels were significantly decreased in DKA compared to the Diabetes or to the control group (13.7 ± 5.9 vs. 26.4 ± 2.5 and 13.7 ± 5.9 vs. 27.8 ± 1.6 mmol/L, respectively, $P<0.001$). Furthermore, plasma anion gap was significantly increased in DKA compared to the Diabetes and to the control group (20.6 ± 6.4 vs. 6.5 ± 1.8 and 20.6 ± 6.4 vs. 7.5 ± 1.9, respectively, $P < 0.001$). Plasma L-lactate levels were elevated in DKA (2.14 ± 2.18 mmol/L). Most importantly, plasma D-lactate levels were markedly increased in DKA compared to the Diabetes and to the control groups (3.82 ± 2.50 vs. 0.25 ± 0.35 and 3.82 ± 2.50 vs. 0.47 ± 0.55 mmol/L, respectively, $P < 0.001$). In addition, plasma D-lactate levels in Diabetes were higher than in the controls. Regression analysis showed that both D- and L-lactate were associated with acidosis (bicarbonate). Further multiple linear regression analysis demonstrated that D-lactate, but not L-lactate was correlated with anion gap and an independent predictor of anion gap ($r = 0.686$, $P < 0.001$, $n = 80$). The contribution of D-lactate to diabetic acidosis and anion gap was almost equivalent to β-hydroxybutyrate. A positive correlation between plasma D-lactate and methylglyoxal levels was also observed ($r = 0.309$, $P < 0.02$, $n = 80$).

Conclusions: Our study demonstrates that plasma levels of glucose, methylglyoxal, β-hydroxybutyrate and blood HbA1c in DKA are significantly increased. Moreover, plasma D-lactate levels in DKA are highly elevated and make significant contributions to the metabolic acidosis and the high anion gap in DKA. There is a positive correlation between plasma methylglyoxal and D-lactate levels. Laboratory monitoring of D-lactate levels will provide a significant value for DKA and should be implemented in laboratory service.

Keywords: Diabetes, diabetic ketoacidosis, anion gap, methylglyoxal, D-lactate

B-76

Imprecision Study of Dimension Vista and Comparability with Vitros 5,1 FS

D. Coenen, V. Verhaeghen, K. Van Brussel, V. Van Hoof. *Antwerp University Hospital, Edegem, Belgium,*

Objective: Dimension Vista® 1500 (Siemens, Newark, DE) instruments, linked to an Advia LabCell® (Siemens) system, will replace Vitros® 5,1 FS (Ortho-Clinical Diagnostics, Rochester, NY) instruments as part of a total laboratory automation project in our hospital. Vista integrates four technologies (photometry, electrolyte detection, nephelometry, chemiluminescence). Here, we describe validation results on Vista and the comparability with Vitros for 27 common biochemical analytes in serum.

Methodology: Three Vista instruments were validated and compared with three Vitros instruments. Repeatability (within-run precision) was carried out on Vista by analyzing ten replicate samples of control material (Multiqual, Bio-Rad, Hercules, CA) at three concentrations. Reproducibility (within-laboratory precision) was studied on both Vista and Vitros by analyzing control material (Multiqual, Bio-Rad) at three concentrations twice daily for ten days on all instruments. Means and CVs for each individual instrument and for each type of instrument were calculated. For Vista, these estimates of precision were compared with analytical goals defined by the minimum performance for imprecision based on a biological variation database (Ricos). Reproducibility estimates were compared for both types of instruments. For evaluating the degree of correlation and agreement between Vista and Vitros, a comparison of patient samples experiment was done. Each analyte was measured on both instrument types in at least 30 serum samples covering the measuring interval.

Results: Within each instrument type, individual instrument means and CVs were comparable, and individual instrument results and pooled results within an instrument type were evaluated. Repeatability and reproducibility of 18/27 analytes on Vista met minimal performance criteria; reproducibility for albumin, amylase, iron, LDL-cholesterol, magnesium, total bilirubin and total protein was better on Vista; alkaline phosphatase, AST, calcium, chloride, creatinine, glucose, HDL-cholesterol, lipase, phosphate, total CO2, triglycerides and uric acid were more reproducible on Vitros; ALT, BUN, cholesterol, CK, GGT, LDH, potassium and sodium had equal reproducibility. Of the 9 analytes not meeting the proposed analytical goals for reproducibility on Vista, alkaline phosphatase and creatinine did meet their goal on Vitros. Reproducibility on Vista was problematic for creatinine (Jaffé, low level CV 10,5%) and chloride (3 levels, CV 2.3% - 3.5%). Pearson correlation coefficients between Vista and Vitros ranged from 0.961 to 0.998; r was > 0.990 for 13 analytes. For evaluating the degree of agreement, Bland-Altman plots were drawn and Passing-Bablok regression was used to determine proportional and constant bias via, respectively, slopes and intercepts of the regression lines. BUN, cholesterol, glucose, iron and sodium showed close agreement between both instrument types. Significant deviation from the target value of 1.00 for slopes was observed for 19 analytes ($P < 0.05$). The slope for LDH (0.39) showed a large deviation from 1.00. Significant deviation from the target value of 0.00 for intercepts was observed for 18 analytes ($P < 0.05$).

Conclusion: although reproducibility was better on Vitros, over-all imprecision of Vista instruments is acceptable for routine use, but extra attention will be needed with regard to daily QC and follow-up. Some methods need improvement. Vista and Vitros results were well correlated, but the numerical values can differ considerably.

B-77

Novel one-shot reagent for colorimetric measurement of sodium

I. Mueller[1], R. A. Kaufman[2], P. Schu[1], J. Schrantz[1], E. Metzmann[1], T. Hektor[1]. *[1]DiaSys Diagnostic Systems GmbH, Holzheim, Germany, [2]Specialty Assays, Inc., Hillsborough, NJ,*

Sodium is the third-most frequent inorganic ion in the human body after calcium and potassium. Sodium ions are necessary for regulation of blood and body fluids, transmission of nerve impulses**,** heart activity, and certain metabolic functions. Therefore, plasma sodium concentrations are an important emergency parameter in the clinical laboratory. The normal sodium concentration in the serum is about 135 to 155 mmol/L. Two electrolyte disturbances are known: severe hypernatremia (elevated sodium level in the blood by dehydration) at > 158 mmol/L, severe hyponatremia (medical illnesses in which fluids rich in sodium are lost by vomiting or diarrhea, e.g.) at < 120 mmol/L. There are two established ways for the measurement of sodium: the atomic absorption spectroscopy (advantage: reference method, high accuracy; disadvantage: low throughput) and the ion selective electrode (advantage: high throughput; disadvantage: high maintenance effort).

We developed a new liquid-stable one-shot-reagent for the determination of sodium in serum and plasma to be used in small and medium sized laboratories on photometers or small routine photometric analyzers which lack an ISE. The new approach uses the complexation of sodium with the chelator 3,6-bis[(o-phosphonophenyl)azo]-4,5-dihydroxy-naphtalen-2,7-disulfonic acid (Phosphonazo III). Phosphonazo III is a phosphorous analog of Arsenazo III wherein the two arsenite groups have been substituted by phosphonate. The complexation causes a change in the Phosphonazo III spectrum which can be measured by an absorption increase at 660 nm. Phosphonazo III forms complexes with several mono- and divalent metal ions. The specificity for sodium has been achieved by choosing alkaline buffer conditions. The application on a routine Hitachi 917 analyzer requires a sample volume of 2 µL which is followed by the addition of 180 µL of reagent. After incubation for 4 minutes at 37 °C the complexation reaction is completed and a one point measurement is taken. The absorbance increase of the measurement is linear proportional to the sodium concentration in the sample. The CV (%) for the precision in series (CLSI guideline EP5-A2) for a patient in the physiological range is 1.19 % within run, 0.60 % between day, 0.89 % between run and 1.61 % total precision. Linearity is given from 40 to 180 mmol/L sodium. No interference is observed at bilirubin concentrations up to 20 mg/dL, triglycerides up to 2000 mg/dL, ascorbate up to 30 mg/dL, haemoglobin up to 1000 mg/dL, potassium from 3.9 to 7.7 mmol/L, and magnesium from 0.76 to 7.7 mmol/L. A method comparison of Sodium Phosphonazo III assay against flame atomic absorption spectroscopy with n=50 patient samples showed a correlation coefficient of r=0.90 and a slope of y=1.00 between the two methods. Our results demonstrate, that the colorimetric Sodium Phosphonazo III assay offers a suitable photometric method for small and medium-sized laboratories which can be used manually as well as on routine clinical analyzers with a similar performance as ISE and AAS.

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Analytical Performance of Ion Selective Electrode (ISE)

On Beckman Coulter AU5800® Clinical Chemistry Analysers*
S. O'Mahoney[1], M. Kono[2], A. Fujimura[1], J. Siegele[1], M. O'Neill[1], M. D. McCusker[1]. *[1]Beckman Coulter Inc., Co. Clare, Ireland, [2]Beckman Coulter Inc., Shizuoka, Japan,*

Background: The Beckman Coulter AU5800 clinical chemistry analyser is the latest system from Beckman Coulter. It is a fully automated, random access analyser, designed for ultra high throughput laboratories. This system is designed to suit varying workloads and is available in different configurations, from a one-photometric module AU5810, up to a four-photometric module AU5840. Electrolyte measurement is performed using a single or double cell Ion Selective Electrode (ISE).
Methods: The single cell option processes 300 serum samples/hour, the double cell option processes 600 serum samples/hour. Indirect measurement of sodium, potassium and chloride is performed using electrode technology which is in common with other members of the AU family. This study evaluated the analytical performance of sodium, potassium and chloride in serum and urine on 2 double cell AU5800 ISE units. Each double cell contains 2 individual ISE flow cells, which are used alternately during sample testing.
Results: The analytical performance for one double flow cell is presented in the table below, where within-run precision, accuracy of each flow cell and recovery difference between each flow cell are illustrated. Total precision (CLSI EP-5A) met the acceptance criteria and method comparison/bias estimation (CLSI EP-09) against the AU5400 ISE system confirmed substantially equivalent data performance. Linearity (CLSI EP-6) was tested using the following ranges: sodium serum from 50-200mEq/L, sodium urine from 10-400mEq/L, potassium serum from 1.0-10.0mEq/L, potassium urine from 2.0-200mEq/L, chloride serum from 50-200mEq/L, chloride urine from 15-400mEq/L. Based on the ranges specified, linearity testing confirmed reportable ranges for each electrolyte.
Conclusions: This study confirms equivalent precision and accuracy performance of the AU5800 ISE compared to other AU systems. Analysis of the AU5800 ISE performance test data demonstrates the ISE reliability and consistency.

Serum N=20	**Target** (mEq/L)	**Within Run Precision** (% CV's)		**Accuracy vs Target** (mEq/L)		**Recovery Difference Between Cells** (mEq/L)
		Cell 1	**Cell 2**	**Cell 1**	**Cell 2**	
Na	**118**	0.32	0.42	0.43	0.30	0.14
	152	0.21	0.16	1.10	0.00	1.10
K	**3.81**	0.39	0.40	-0.02	-0.03	0.01
	6.36	0.33	0.32	0.07	-0.05	0.12
Cl	**89.9**	0.32	0.29	0.10	-0.26	0.36
	118	0.24	0.17	0.94	-0.43	1.37

* Analyser under evaluation and currently not available for clinical use.

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Use of serial ICU patient data to evaluate the goodness of iSTAT and RapidPoint electrolyte testing

G. S. Cembrowski[1], R. B. Schifman[2], D. V. Tran[1]. *[1]University of Alberta Hospital, Edmonton, AB, Canada, [2]Southern Arizona VA Health Care System, Tuscon, AZ,*

Background Method stability and analytical imprecision are two of the most important criteria for instrument selection. We have devised a data-mining statistic (within-patient imprecision [WPI] regressed to zero-time between specimens) that summarizes the average short term analytic imprecision (s_a) and minimizes biologic patient variation (s_b). Unlike the short term analytic imprecision that is derived from quality control data, this statistic can summarize the analytic imprecision over many reagent lots and calibrations. Acute care hospitals and intensive care units provide adequate data to generate this imprecision statistic. This statistic can be used to compare the analytic performance of different analyzers operating in similar patient care environments.
Methods We analyzed 18 and 22 months of serial patient Rapidpoint 400(Siemens, Deerfield IL) and iSTAT (Abbott, Abbott Park, IL) results, respectively, operated by nurses and respiratory therapists in intensive care units at the Tucson VA Hospital. For sodium and potassium (done by both iSTAT and Rapidpoint) and bicarbonate and glucose (done only by Rapidpoint), we tabulated the measurements of paired intra-patient samples drawn within 12 hours of each other. After outlier removal, we calculated the standard deviations of duplicates (SDD) of the intra-patient pairs grouped in two-hour intervals: 0-2 hours, 2-4 hours, 4-6 hours, 6-8 hours, 8-10 hours and 10-12 hours. The SDDs were then regressed against the midpoint time intervals of 1 to 11 hours; extrapolation to zero time (y-intercept) represents the average variation $(s_a^2 + s_b^2)^{1/2}$.
Results The Table summarizes our findings.
Conclusions The Rapidpoint offered better performance for sodium and potassium. The performance of these two iSTAT analytes is roughly equal to that offered by central laboratory analyzers like the Roche Hitachi 917 and the Beckman LX-20.

Test	Rapidpoint Pairs (12 h)	SDD at t = 0 (WPI), Rapidpoint	iSTAT Pairs (12 h)	SDD at t = 0 (WPI) iSTAT	Diff (Rapidpoint-iSTAT)
Glucose, mmol/L	3162	0.8162			
Potassium, mmol/L	3783	0.2235	1117	0.3009	-29.5%
Sodium, mmol/L	3975	1.0928	1284	1.3899	-23.9%
HCO3-, mmol/L	3593	1.415			

B-80

Dialysate Method Comparison Using the Dialysate Sample Mode on the RAPIDPoint® 350 Blood Gas Analyzer

T. Hotaling. *Siemens Healthcare Diagnostics Inc., Norwood, MA,*

Background: Dialysis treatment in renal patients replaces the filtration function of the kidneys. Dialysate is the aqueous chemical solution used in conjunction with a filter to remove waste products and excess fluid from the bloodstream while maintaining proper electrolyte balance. Dialysate can be either bicarbonate or acetate based and is normally prepared by mixing appropriate concentrations of the substance(s) with distilled water. Measuring the dialysate fluid components aids in verifying and controlling the electrolyte concentrations in the dialysis liquid, most significantly those of Na^+, K^+, and Ca^{++}. Presented is a method comparison of the new dialysate measurement option under development for selected markets on the RAPIDPoint 350 blood gas analyzer.
Method: Bicarbonate-based dialysate samples from five sources and acetate-based dialysate samples from a single source were prepared, encompassing the entire reporting range for each parameter. The specimens were measured in triplicate in the dialysate sample mode on multiple RAPIDPoint 350 systems. The same specimens were immediately measured on a reference or comparative device. Six RAPIDPoint 350 units were used to analyze Na^+, K^+, pH, and pCO_2; three devices were used to analyze Ca^{++}. Flame photometry was used as the reference method for Na^+ and K^+, while Ca^{++}, pH, and pCO_2 were compared against the RAPIDLab® 348 (RL348) system.
Results: Simple linear regression of the grouped data was employed to determine the method comparison statistics on each of the measured parameters for both the bicarbonate- and acetate-based material (see table).
Conclusions: Dialysis fluid measurement on the RAPIDPoint 350 system showed good correlation to reference and comparative methods, particularly for Na^+, K^+ and Ca^{++}. It affords the clinician the ability to verify dialysate composition and/or monitor the patient's whole blood electrolytic balance on a single portable device.

Table 1. RAPIDPoint 350 dialysate mode method comparison summary.

Analyte	**Fluid**	**Method**	**n**	**r^2**	**Slope**	**Intercept**
Na^+	Bicarb	Flame	1620	0.999	0.999	+0.101
K^+	Bicarb	Flame	1620	0.999	0.999	+0.004
Ca^{++}	Bicarb	RL348	810	0.991	0.994	+0.013
pH	Bicarb	RL348	1260	0.984	0.983	+0.121
pCO_2	Bicarb	RL348	1260	0.997	0.997	+0.200
Na^+	Acetate	Flame	324	0.999	1.012	-0.942
K^+	Acetate	Flame	324	0.996	0.990	+0.025
Ca^{++}	Acetate	RL348	162	0.986	0.995	-0.003
pCO_2	Acetate	RL348	252	0.997	1.088	-7.896

B-81

Comparability of Test Results and Sigma Metrics for the Abbott ARCHITECT Family of Clinical Chemistry and Immunoassay Systems

A. DeFrance, C. Lo, D. Armbruster, Y. Lemma, D. Petty, T. Sparks, J. Browning. *Abbott Laboratories, Irving, TX,*

***Objective*:** Nine clinical chemistry analytes and one immunoassay analyte were tested for patient result comparability across three ARCHITECT clinical chemistry and two immunoassay systems. Twenty day precision and patient method comparison against a

reference system was performed and Six Sigma metrics were calculated.

***Methodology*:** The ARCHITECT Clinical Chemistry (c4000, c8000 & c16000) and Immunoassay (i1000SR & i2000SR) Systems use identical reagents and methods, are fully automated, and can be operated as stand-alone instruments or integrated systems. The same set of fresh patients were tested for nine clinical chemistry analytes on the three chemistry analyzers and one immunoassay analyte on the two immunoassay analyzers to demonstrate equivalent results across systems. Twenty day precision was measured for each system using the same controls. Method comparison against a reference system was performed by testing the same patient specimens. Results were used to calculate Sigma metrics.

***Results*:** Results were compared by calculating the grand mean for each analyte across three platforms for clinical chemistry assays and two platforms for immunoassay. The individual platform means were then compared to the grand mean. In addition, minimum and maximum differences for each individual sample for each analyte on each system were determined. Sigma metrics for assays with comparable methodologies between ARCHITECT and the reference system consistently demonstrated high analytical quality for the clinical chemistry assays.

Comparison of Patient Results Across a Family of Systems

Assay	Grand Mean Result	c4000		c8000		c16000		Range of Samples (N)	Min to Max diff
		% diff*	Sigma Value**	% diff	Sigma Value	% diff	Sigma Value		
Urea Nitrogen (mg/dL)	35.4	1.76	8.3	-0.53	15.5	-1.23	13.9	5.3-112.4 (41)	0.0-3.8
Magnesium (meq/L)	1.68	0.86	NC	2.11	NC	-2.97	NC	0.2-2.5 (40)	0.0-0.11
Calcium (mg/dL)	8.7	0.56	9.8	0.06	10.4	-0.62	10.8	7.0-10.7 (39)	0.0-0.23
CO2 (meq/L)	18.6	-1.58	NC	-1.79	NC	3.36	NC	7.7-28.7 (43)	0.01-1.15
Creatinine (mg/dL)	1.87	-0.55	6.4	1.96	6.1	-1.41	9.6	0.57-11.75 (41)	0.0-0.25
Glucose (mg/dL)	120.0	-0.01	19.8	0.12	15.8	-0.11	13.2	19-344 (40)	0.0-1.5
Sodium (mmol/L)	142.3	0.32	8.9	-0.64	6.5	0.32	9.3	129.2-166.0 (41)	0.1-4.2
Potassium (mmol/L)	4.47	0.99	6.6	-1.22	9.4	0.23	10.4	3.1-6.2 (40)	0.01-0.17
Chloride (mmol/L)	109.0	0.63	9.8	-0.25	5.3	-0.38	13.9	97.7-137.9 (41)	0.02-3.56
		i1000sr		i2000sr					
		% diff*	Sigma Value	% diff*	Sigma Value				
TSH (ng/mL)	4.27	-0.92	NC	0.92	NC			0.02-42.1 (38)	0.0-0.61

*% diff = difference between Grand Mean and individual systems means

**Sigma Values calculated using bias from reference instrument and twenty day precision.

NC -Not Calculated - Sigma values were not calculated for assays using a different methodology from ARHCITECT.

***Conclusion*:** The ARCHITECT Clinical Chemistry and Immunoassay Systems Family demonstrated result comparability and excellent Six Sigma performance when compared outside of the ARCHITECT family of systems to a commercially available reference system. Patient specimens may be tested for the same analyte on any ARCHITECT system interchangeably and equivalent results will be obtained. The ability to integrate ARCHITECT clinical chemistry and immunoassay modules provides the capability to perform testing for most routine analytes on one system.

B-82

Validation of platelet collection and pleural fluid pH using the Radiometer ABL800 series blood gas analyzers.

C. R. McCudden[1], C. M. Parker[2], S. Koenig[2], Y. A. Park[1]. *[1]University of North Carolina, Chapel Hill, NC, [2]University of North Carolina Hospitals, Chapel Hill, NC,*

Background: Laboratories frequently receive requests to analyze non-standard specimens for clinical purposes. Many of these specimens are rare or variable making it challenging to validate their measurement. This study focused on pH determination of pleural fluid and platelet collections. The pH of platelet collections is occasionally used as a quality control measure for blood banking, where platelets with a pH <6.2 are considered unusable due to presumed contamination. Pleural fluid pH is used to identify parapneumonic effusions that require draining, where a pH <7.2 indicates drainage is required.

Objectives: The goals of this study were 1) to evaluate the ability of the Radiometer ABL800 series to measure pleural fluid pH using the dedicated pleural fluid pH measuring mode; 2) to validate pH measurement of platelet collections using the ABL835 FLEX.

Material and Methods: *Pleural fluid*: Thoracocentesis specimens were collected from 25 different patients and stored at -20°C. For analysis, samples were thawed at 4°C and then spiked with acetic acid to yield a total of 45 specimens spanning a pH range of 6.95-7.60. The pH of each specimen was measured in duplicate using Radiometer ABL835 and ABL837 analyzers and compared against an FDA 510(k)-approved reference instrument (Roche Omni S). The Radiometer dedicated pleural fluid pH measuring mode is traceable to the IFCC reference method for measuring pH. Whole blood specimens and liquid controls were also compared between the instruments. *Platelets*: The pH of 20 different platelet collections was measured using the Radiometer ABL835 and compared with a pH meter (accumet XL15, Fisher Scientific) as the reference method. Measurements were done in duplicate within 2 minutes of each other using syringe-drawn aliquots from platelet collection bags. Results of pleural fluid and platelet experiments were analyzed using Deming regression and Bland-Altman difference plots.

Results: Regression analysis indicated that the Radiometer ABL800 series had proportional and constant bias (y=1.3 - 2.2) in comparison to the Roche Omni S. At pH 7.4, values were identical, whereas at pH <7.2 the ABL800 series were ~0.1 units lower. Analysis of liquid controls revealed a similar difference (y=1.1 - 0.6) between the ABL800 series and Omni. There was good agreement between the Omni and ABL800s for whole blood pH measurements (y=1.0 - 0.5). For platelet pH determination, the pH meter and ABL835 blood gas analyzer demonstrated excellent agreement (y=1.0 - 0.2).

Discussion: The results of this study demonstrate constant and proportional differences between the Roche Omni reference pleural fluid pH method and the Radiometer 800s. Clinically, these differences are acceptable for pleural fluid analysis as there was no significant difference between instruments as to whether patients would be treated or not (pleural effusion drainage) using the standard pH 7.2 cutoff. The observed differences in pleural fluid pH measurement likely reflect differential handling of liquid specimens between the Roche Omni and the ABL800 series; these differences were evident when comparing liquid controls, but not whole blood specimens. This study confirms the validity of the Radiometer ABL800 series blood gas analyzers to measure platelet and pleural fluid pH.

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Next Generation Magnesium Assay Application for the Abbott ARCHITECT® *c*Systems™

J. Salazar, C. Kasal, B. Combs, L. Harris. *Abbott Diagnostics, Irving, TX,*

Background: Magnesium is an essential nutrient involved in many biochemical functions. Hypomagnesemia results in the impairment of neuromuscular function and may develop in severe prolonged diarrhea, malabsorption syndromes, hyperaldosteronism, and diuretic therapy. Hypermagnesemia is seen in renal failure and diabetic coma. The Abbott Next Generation Magnesium assay uses an Enzymatic methodology, and is standardized to the National Institutes of Standards and Technology (NIST) standard reference material (SRM) 956. Next Generation Magnesium is a liquid-ready-to-use, 2-part reagent, which will be available in two kit sizes.

Methodology: Magnesium is measured by the use of the enzymatic reaction with Isocitrate Dehydrogenase. Magnesium present in the sample is a cofactor for the reaction. The reaction is monitored via the formation of NADPH. The rate of increase in absorbance at 340nm, due to the formation of NADPH, is directly proportional to the magnesium concentration in the sample.

Objective: Assay was evaluated on the Abbott ARCHITECT *c*Systems for the quantitation of magnesium in human serum, plasma, and urine. The calibration and on-board stability will be a minimum of 30 days. The serum application will achieve the performance requirements of Total Error </=15.0%, Bias </=7.5%, and Total Precision (%CV) </=3.75%, per the equation %Total Error (TE) = %Bias + 2xTotal Precision. Representative performance data for key attribute serum testing are summarized below.

Study	Protocol	Results* (mg/dL)		
Calibration/ On-Board Stability	% Recovery to Day 0	Minimum of 30 days		
Bias	% Difference to SRM 956c CofA Target Values, Levels 1, 2, & 3	**Target: 3.031 mg/dL** Reagent Lot1: -0.4% Reagent Lot2: -0.4% Reagent Lot3: -0.7%	**Target: 2.084 mg/dL** Reagent Lot1: -0.7% Reagent Lot2: -0.2% Reagent Lot3: -0.2%	**Target: 1.143 mg/dL** Reagent Lot1: 1.5% Reagent Lot2: 3.2% Reagent Lot3: 1.5%
Precision	CLSI EP10-A2 (5-day, N=50)	**Mean: 1.58 mg/dL** Within Run 0.88%CV; Total 1.56%CV **Mean: 3.82 mg/dL** Within Run 0.49%CV; Total 1.33%CV		
Linearity	CLSI EP6-A	0.60 - 20.00 mg/dL		
Sensitivity	CLSI EP17-A	Limit of Detection (LOD) 0.15 mg/dL		Limit of Quantitation (LOQ) 0.60 mg/dL
Interference: Bilirubin Hemolysis Lipemia	Dose Response, Serum Mg Levels 1, 2, & 3	**Target: 1.69 mg/dL** 60 mg/dL Conj Bili: 94% 60 mg/dL Unconj Bili: 102%	**Target: 3.35 mg/dL** 60 mg/dL Conj Bili: 94% 60 mg/dL Unconj Bili: 101%	**Target: 5.63 mg/dL** 60 mg/dL Conj Bili: 94% 60 mg/dL Unconj Bili: 101%
		Target: 1.52 mg/dL 500 mg/dL Hb: 105%	**Target: 2.98 mg/dL** 500 mg/dL Hb: 102%	**Target: 5.07 mg/dL** 500 mg/dL Hb: 101%
		Target: 1.59 mg/dL 1000 mg/dL Intralipid: 100%	**Target: 3.04 mg/dL** 1000 mg/dL Intralipid: 100%	**Target: 5.26 mg/dL** 1000 mg/dL Intralipid: 100%

*In development

Conclusion: The Next Generation Magnesium assay on the Abbott ARCHITECT *c*Systems provides an Enzymatic method, standardized to NIST SRM 956. The reagent is a liquid-ready-to-use 2-part format with an extended calibration/on-board stability of at least 30 days. Representative data indicates excellent assay performance with </=3.2 %Bias, </=1.56 Total %CV, and 0.60 - 20.00 mg/dL linearity. Study also supports robustness against common interferents such as bilirubin up to 60 mg/dL, hemoglobin up to 500 mg/dL, and intralipid up to 1000 mg/dL.

B-84

Variations in the serum Creatinine determinations in Greek laboratories. - Impact on GFR estimation (eGFR).

K. Makris[1], I. Drakopoulos[1], O. Panagiotakis[2], D. Rizos[3], A. Haliassos[4].
[1]Clinical Biochemistry Department, KAT Hospital, Kifissia, Greece, [2]ESEAP, Athens, Greece, [3]Hormone Laboratory "Aretaieion" University Hospital, Athens, Greece, [4]Diamedica SA, Athens, Greece,

Glomerular filtration rate (GFR) is the best index of kidney function used to diagnose stage and treat chronic kidney disease. International organizations recommend the use of GFR estimating equations based on serum creatinine measurements and other demographic and clinical variables to assess kidney function. Due to substantial interlaboratory variation creatinine based equations require the calibration of the creatinine assays to be traceable to the laboratory that created the equation, otherwise a systematic bias occurs in the eGFR.
In order to access these creatinine calibration variations we used data from the Greek External Quality Assessment Scheme (ESEAP). The number of participating laboratories was 266. The consensus mean was calculated for each sample (ranging from 0.81 to 2.19mg/dL) and the absolute percentage of deviation was calculated for all laboratories.
Our goal was to determine the level of accuracy and reproducibility of measurements among Greek laboratories and if this can be tolerated without compromising the clinical utility of the eGFR equation. For this purpose we focus on creatinine range between 1.00 and 1.55mg/dL which corresponds to eGFR values near the therapeutic decision threshold of 60ml/min/1.73m^2. The table summarizes our results:

Sample number	1	12	3	4	10	5	8	11	13	6	9	2	14	7
m+2sd	1.09	1.20	1.15	1.31	1.72	1.35	1.47	1.53	1.68	1.83	2.14	1.99	2.32	2.49
mean	0.81	0.86	0.93	***1.01***	***1.06***	***1.07***	***1.19***	***1.29***	***1.34***	***1.55***	1.62	1.77	2.04	2.19
m-2sd	0.53	0.52	0.71	***0.71***	***0.40***	***0.79***	***0.91***	***1.05***	***1.00***	***1.27***	1.10	1.55	1.76	1.89
sd	12.82	19.34	6.64	***11.4***	***29.26***	***8.8***	***11.5***	***10.9***	***12.08***	***9.6***	14.14	8.5	9.1	7.9
mean absolute (%) difference	11.36	13.75	8.57	***10.04***	***12.75***	***9.25***	***11.1***	***10.5***	***9.61***	***8.41***	7.8	7.4	7.1	6.9
Number of labs with bias>15% from consensus mean	56	84	41	***51***	***53***	***53***	***42***	***44***	***49***	***32***	21	26	28	30
(%)	21.05	31.58	15.42	***19.18***	***19.93***	***19.93***	***15.79***	***16.54***	***18.42***	***12.03***	7.89	9.77	10.53	11.28

The samples were prepared from pooled human sera from donors by MCA-unit (Queen-Beatrix hospital, Netherlands). Reported values were subjected to a 3-SD test for the elimination of outliers and less than 5 labs were removed. The mean bias of all participating labs ranged from 7.9 to 32.6 in all samples, but only from 12.3 to 19.9 in the region of measurements within the critical range. The number of laboratories that deviated more than 15% in this area ranged from 32 to 52. Our preliminary results cannot support the reporting of eGFR based on these creatinine measurements and we must support the current effort of IFCC and IVD manufacturers to better harmonize creatinine measurements.

B-85

Development of an Enzymatic Creatinine Assay on the Dimension Vista® Intelligent Lab System from Siemens Healthcare Diagnostics

S. T. Salyer, J. Strauss, C. R. Jesse. *Siemens Healthcare Diagnostics, Newark, DE,*

Introduction and Objective: We describe the development and analytical performance of a fully automated assay for the measurement of creatinine in serum, plasma, and urine on the Dimension Vista® System using our enzymatic method. Our objective was to develop a standardized creatinine assay which has improved precision and accuracy.
Relevance: This assay addresses healthcare providers need for standardized creatinine measurement which is necessary to diagnose and treat renal disease.
Method: This method is based on the determination of sarcosine after conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase. The liberated hydrogen peroxide is measured photometrically at 540nm via a modified Trinder reaction. The new assay is traceable to isotope dilution mass spectrometry (ID-MS). The enzymatic creatinine calibrator is traceable to working standards prepared with National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 914 and the assigned values are confirmed using NIST SRM 967 material, whose values are assigned by ID-MS. The method uses a 2.7ul sample size. **Results:** Time to 1st result is 7.7 minutes and calibration is stable for 90 days. The assay range for serum and plasma is 0.14 to 20.0 mg/dL; for urine the assay range is 2.8 to 400 mg/dL. Samples above the assay range can be quantitated using the onboard auto-dilution feature. Limit of detection is 0.14 mg/dL. Repeatability and within-lab reproducibility on serum pools were measured to be 2.9 and 4.4 %CV at 0.61 mg/dL, and 1.4 and 2.4%CV at 1.57 mg/dL creatinine respectively per the CLSI EP5-A2 protocol over a 20 day testing interval. Recovery of NIST 967 material (levels 1 and 2) is within 5% of the NIST certified values. Split sample correlation (n=130) between the Dimension method (DV) and Roche CREA plus® kit run on Hitachi 717 Analyzer gave a Deming regression fit as follows: slope = 1.03, intercept = -0.18, and correlation (r) = 1.00. The correlation study included serum and plasma samples which were visibly lipemic and icteric. The Deming regression for a split sample study with urine is: slope = 1.06, intercept = -6.11, and correlation = 0.99. No significant interference (<10%) at a creatinine concentration of 1.02 mg/dL is found at 30 mg/dL bilirubin, 1000 mg/dL triglyceride, 500 mg/dL hemoglobin, or 25 mg/dL creatine.
Conclusion: We conclude that the new standardized enzymatic creatinine assay has excellent precision, accuracy, and a dynamic range suitable for measurement of creatinine in serum, plasma, and urine.

B-86

Assessment of a Whole Blood Neonatal Bilirubin Method on the RAPIDPoint® 405 Instrument versus a Plasma Method on the VITROS® 950 Chemistry System

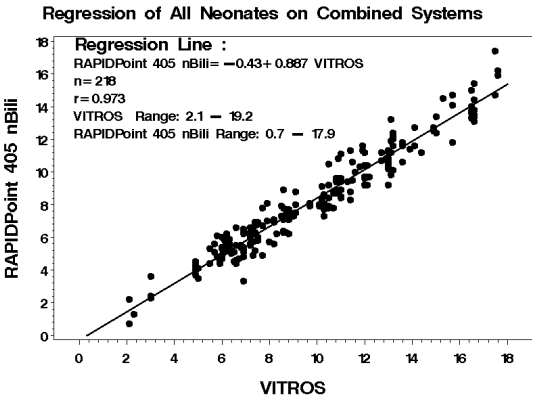
T. Hotaling, M. Lynch. *Siemens Healthcare Diagnostics Inc., Norwood, MA,*

BACKGROUND: In the neonate, bilirubin is measured as an aid for assessing jaundice and the risk of kernicterus. A fast turnaround time on minimal sample volume is often required. We present an initial hospital evaluation of a new whole blood neonatal bilirubin (nBili) method under development for the point-of-care, cartridge-based Siemens RAPIDPoint 405 (RP405) blood gas analyzer compared to plasma nBili analysis on the Ortho-Clinical Diagnostics VITROS® 950 chemistry system.

METHOD: Heparinized whole blood specimens from neonates (0-14 days old) were obtained over an 8-day period. Samples from each specimen were spun and measured as plasma on the VITROS system; remnant volume was measured as whole blood on up to two RP405 instruments (n=218). Imprecision was monitored daily on the RP405s using aqueous controls. No adjustments to the RP405 bias to reference correction algorithms were applied at this time.

RESULTS: The RP405 total imprecision (%CV) of controls was 3.1%, 2.9%, and 2.9% at 19.8, 9.6, and 5.1 mg/dL (n=26), respectively. Least squares regression comparing the RP405 (without final bias to reference correction) versus the VITROS system yielded a slope of 0.89 and intercept of -0.43, with r=0.973 across the nBili range of 2.1-19.2 mg/dL (see figure). No significant change in RP405 nBili bias was observed as a function of specimen total hemoglobin (tHb; range of 11.6-22.0 g/dL). RP405 nBili bias to reference was also not significantly affected by specimen pH (range of 7.31-7.76). The RP405 system reports nBili results in approximately 1 minute from sample introduction.

CONCLUSIONS: The RAPIDPoint 405 whole blood neonatal bilirubin method gives analytical values comparable to those of the VITROS plasma chemistry method without the need for preanalytical centrifugation. It may present a quick time-to-patient-result alternative for monitoring neonatal bilirubin.



B-87

Evaluation of the i-Smart 30 electrolyte analyzer: A smart electrolyte analyzer for point-of-care and laboratory testing

J. Shin[1], T. Y. Kang[1], H. Kim[1], M. Kim[1], J. Ha[1], H. Nam[2], G. S. Cha[2]. *[1]i-SENS, Inc., Seoul, Korea, Republic of, [2]Kwangwoon University, Seoul, Korea, Republic of,*

The i-Smart 30 analyzer, developed by i-SENS, is a fully automated electrolyte analyzer that can accurately measure the concentrations of sodium, potassium, and chloride ions and hematocrit in blood for point-of-care and laboratory testing.

The analyzer uses 60 µl of whole blood, serum, or plasma for analysis and reports the results in fifty seconds from sample introduction on the color screen of the analyzer. The analyzer offers complete portability with light weight (5.5 Kg) and battery power option (the built-in rechargeable battery allows uninterrupted use without the main power for up to two hours). Further, the analyzer operates on an embedded PC with Microsoft Windows® XP Embedded Operating System and provides many convenient features such as touch screen operation, voice and pop-up instructions, easy data entry using barcode scanner or virtual keyboard on the screen, and USB ports for data download.

Besides many advantageous features mentioned above, the i-Smart analyzer is the only electrolyte analyzer available in the market that uses a closed cartridge including electrochemical sensors, calibration solutions, and all fluidic components from a sample introducing probe to a waste bag. This cartridge reduces the maintenances of the analyzer and relating labor, time, and expense.

The performance of the electrolyte analyzer was evaluated with four i-Smart 30 analyzers at i-SENS, Inc. facility in Seoul, Korea. For sodium, potassium, and chloride ions, heparinized whole blood samples were collected from healthy individuals and altered by spiking or dilution technique. For hematocrit, heparinized whole blood samples were centrifuged to separate plasma from red blood cells and the plasma and the red blood cells were re-mixed at appropriate ratios to span the hematocrit range. Whole blood samples were analyzed on the i-Smart 30 analyzers and an ABL 825 blood gas analyzer (Radiometer) for comparison. For hematocrit, the results were also compared to the centrifuged microhematocrit method. A total of 84 whole blood samples were tested in duplicate for electrolyte parameters and a total of 72 samples in duplicate for hematocrit over two weeks of the cartridge uselife. Good agreement was found between the i-Smart and the ABL. The linear regression results yielded a slope of 0.908 with an intercept of 14.2 (r^2=0.994) over a range of 120 ~ 180 mM for sodium, a slope of 0.971 with an intercept of -0.07 (r^2=0.999) over a range of 2 ~ 13 mM for potassium, a slope of 0.968 with an intercept of -0.5 (r^2=0.998) over a range of 90 ~ 170 mM for chloride, and a slope of 0.999 with an intercept of -3.3 (r^2=0.987) over a range of 10 ~ 60 % for hematocrit, As compared to the microhematocrit method, a slope of 0.955 with an intercept of -0.6 (r^2=0.987) was obtained.

In conclusion, the i-Smart 30 electrolyte analyzer shows good correlation in whole blood with the ABL 825 blood analyzer for sodium, potassium, chloride, and hematocrit.

B-88

Correlation Studies Of The Creatinine Enzymatic Method In The Roche Modular® And Roche Cobas® 6000 Analyser Systems

M. F. Aumentado, S. Pang, S. Cosio, A. Omar, M. Wong. *Alexandra Hospital, Singapore, Singapore,*

Alexandra Hospital is a 400-bed acute care general hospital. The Department of Laboratory Medicine uses two main chemistry analytical platforms, namely the Roche Modular and the Roche COBAS 6000 analysers (Roche Diagnostics, Basel, Switzerland). The COBAS 6000 processes serum, plasma and urine samples while the Roche Modular is designated to run serum and plasma samples only. Our laboratory measures serum/plasma and urine creatinine levels using a modified Jaffé method which has a higher sensitivity and better precision than the original Jaffé method. Creatinine is produced endogenously from creatine and creatine phosphate as a result of muscle metabolic processes and excreted by glomerular filtration during normal renal function. In 2009, Roche introduced an IDMS-traceable enzymatic method, based on the established determination of sarcosine after conversion of creatinine with the aid of creatinase, creatinase and sarcosine oxidase. The liberated hydrogen peroxide is measured via a modified Trinder reaction. Optimization of the buffer system and the colorimetric indicator enables the creatinine concentration to be quantified both precisely and specifically. We evaluated the technical performance of this new assay on both analysers. A total of 151 serum/plasma samples were analysed on both instruments while 101 urine samples were analysed on the COBAS 6000 analyser. Imprecision studies using 2 concentrations of material for serum/plasma on the Roche Modular yielded intra-assay CVs of 2.06-2.58% and inter-assay CVs of 0.93-1.67%. The COBAS 6000 yielded intra-assay CVs of 1.35-1.56%, and inter-assay CVs of 1.37-1.59%. The intra-assay and inter-assay CVs for urine samples on the COBAS 6000 were 1.20-1.29% and 1.29-1.75% respectively. Correlation studies between the enzymatic and Jaffé methods on the COBAS 6000 and Roche Modular derived a relationship of y=1.0236x-2.4107 (R2=0.9965) and y=1.0314x-6.0687 (R2=0.998) respectively. Correlation studies on urine samples showed a relationship of y=1.0587x-0.1918 (R^2=0.998). The correlation between the two instruments on the other hand yielded a relationship of y=1.0247x-0.4595 (R^2=0.9993). We collected serum samples from 199 healthy individuals, comprising 159 females and 40 males, to derive reference ranges in our local population. Female and male reference ranges were 41-79 µmol/L and 64-111 µmol/L respectively. Our study shows that precision studies for the creatinine enzymatic method are acceptable. In addition, the new method correlates well with the creatinine Jaffé method.

B-89

An Automated Turbulent Flow Liquid Chromatography - Isotope Dilution Mass Spectrometry (LC-IDMS) Method for Quantitation of Serum Creatinine

R. Harlan[1], W. Clarke[1], J. M. Di Bussolo[2], M. Kozak[2], J. Straseski[1], D. L. Meany[1]. *[1]Johns Hopkins Medical Institutions, Baltimore, MD, [2]Thermo Fisher Scientific, Franklin, MA,*

Background and Objective: Proper estimation of Glomerular Filtration Rate (GFR) requires accurate measurement of serum creatinine. When creatinine concentrations determined by routine clinical assays are in question, reference measurement procedures

aid in investigation. Currently, all creatinine reference measurement procedures require different degrees of clean-up of serum samples before analysis. The clean-up procedures of some methods are so labor-intensive that it may prevent their implementation in a routine clinical laboratory setting. Here, we developed an automated, fast, accurate and reliable turbulent flow liquid chromatography (TFLC) - isotope dilution mass spectrometry (IDMS) method for quantitation of serum creatinine. The novelty of this method lies in the use of TFLC using a cation exchange column for on-line serum sample clean-up to automate serum analysis.

Methods: Human serum samples were mixed with an internal standard solution of deuterated creatinine (creatinine-D_3) and were injected onto a Cyclone MCX TurboFlow™ column (0.5 x 50 mm, Thermo Scientific, Franklin, MA). The extracted creatinine and creatinine-D_3 were eluted onto a Hypercarb column (5 µm particle size, 3 x 50 mm, Thermo Scientific, Bellefonte, PA) and separated using a 5-95% gradient of methanol with 0.1% ammonium hydroxide over 2.5 minutes. Creatinine and creatinine-D_3 were ionized using electrospray ionization and measured by selective ion monitoring of m/z 114 and 117, respectively, using a TSQ Quantum Access mass spectrometer (Thermo Scientific, San Jose, CA). Precision, linearity, limit of detection (LOD) and limit of quantitation (LOQ) of this TFLC-IDMS method were evaluated. Method comparison between the Roche Creatinine Plus enzymatic assay and this method was achieved using a Deming fit and Bland-Altman analyses on the creatinine results of 134 patients' serum samples.

***Results*:** Precisions of this method were less than 8% (1.0-2.5mg/dL). LOD was 0.05 mg/dL and LOQ was 0.3 mg/dL. Average recovery was 111.0% in the range of 0.41 to 6.5 mg/dL. Creatinine concentration of the National Institute of Standards and Technology (NIST) Standard Reference Material 967 levels 1 and 2TF were 0.77 ± 0.01 mg/dL (mean ± SD,standard deviation, n=3) and 4.01 ± 0.04 mg/dL (n=3), respectively. Based on the Student's t test, these values were not significantly different from their certified concentrations of 0.75 ± 0.01 mg/dL and 3.92 ± 0.04 mg/dL. Both the Deming fit analysis and Bland-Altman plot indicated that the TFLC-IDMS method and the Roche Creatinine Plus enzymatic assay compared well over the range of 0.33-6.5 mg/dL creatinine (n=134). The Deming fit analysis produced a slope of 1.07 (95% CI 1.05 to 1.09) and an intercept of -0.04 (95% CI -0.07 to -0.01) with Pearson correlation coefficient (r) of 1.00. The Bland-Altman plot demonstrated that 95.5% of the samples (128/134) were within the 95% limits of agreement (-13.0% to 19.4%), which were calculated based on the 2 Standard standard deviation limits of the differences between the methods.

Conclusion: We have developed an automated and accurate method for quantitation of serum creatinine. It has demonstrated potential as a quick and simple alternative to current creatinine reference methods.

Tuesday PM, July 27

Poster Session: 2:00 pm – 4:30 pm
Molecular Pathology/Probes

B-90

Molecular analysis of SLC2A9 gene associated with serum uric acid levels in the Chinese male Han population by high-resolution melting

M. Guan, Y. Chen, H. Zou. *Huashan Hospital, Fudan University, Shanghai, China,*

Background Uric acid is the end product of either dietary or purine metabolism, and hyperuricemia might be caused by an overproduction of or by disturbances in the elimination of uric acid.SLC2A9 is a novel identified urate transporter influencing uric acid metabolism. It has been suggested that the single nucleotide polymorphisms (SNPs) in SLC2A9 may affect the serum UA levels. The present study was designed to investigate rs6855911 polymorphism in intron 7 of SLC2A9 in a total of 372 Chinese male subjects.
Methods We examined 166 gout patients, as well as 206 healthy male volunteers in this study. DNA was purified from peripheral blood and the rs6855911 polymorphism was evaluated using high resolution melting (HRM) analysis and direct sequencing. Serum uric acid, BUN, creatinine, total cholesterol, triglyceride, HDL-C and LDL-C were measured using a clinical analyzer. Demographic and clinical data obtained from the patients and controls among the genotype groups were analyzed.
Results A/A and A/G genotypes were unambiguously distinguished with HRM technology. The occurrence of the homozygous type (G/G) was completely absent among the study population. The prevalence of the A/A and A/G genotype was 96.0% and 4.0%, respectively. Genotyping based on HRM was fully concordant with sequencing. Serum uric acid levels in the A/G genotype subjects were significantly lower than those with the A/A genotypes.
Conclusions The polymorphism rs6855911 in SLC2A9 may be a genetic marker to assess risk of hyperuricemia among Chinese male Han population.
Table Clinical data for subjects grouped by rs6855911 SNP genotypes

Indexes	rs685591 genotypes		*P*
	A/A	A/G	
Subjects(%)	357(96.0)	15(4.0)	
Age (y)	55.3±13.5	53.5±14.4	0.61
BMI (kg/m^2)	25.4±5.7	24.5±4.0	0.54
Systolic blood pressure (mm Hg)	125.4±13.8	120.8±14.6	0.21
Diastolic blood pressure (mm Hg)	74.8±8.9	73.2±7.8	0.50
BUN(mmol/L)	5.7±2.4	5.6±3.1	0.81
Creatinine(μmol/L)	100.3±21.3	94.5±19.6	0.30
Total cholesterol (mmol/L)	4.8±2.0	4.6±1.8	0.66
Triglyceride (mmol/L)	1.9±0.9	1.9±1.1	0.86
HDL-C(mmol/L)	1.3±0.5	1.3±0.3	0.82
LDL-C(mmol/L)	3.5±1.0	3.2±1.2	0.31
Uric Acid (μmol/L)	380.7±130.8	284.5±97.6	< 0.01

Data are expressed as mean±S.D.

B-91

Detection of *Mycobacterium tuberculosis* resistant genotypes by PCR-SSCP at the Public Health System in southern Brazil

L. K. Grutzmacher, C. M. M. Cordova. *FURB - Universidade Regional de Blumenau, Blumenau, SC, Brazil,*

Background: One of the most ancient infectious diseases on earth, tuberculosis (TB) remains as one of the biggest challenges to be beaten as a public health issue around the globe. Emergency of *Mycobacterium tuberculosis* (MT) strains that are resistant to the traditional therapeutical schemes constitutes an issue of considerable concern.
Methods: In the present work we have aimed to evaluate the prevalence of MT strains with a genotype profile harboring mutations that could result in resistance to the main drugs used in TB treatment, namely rifampicin, isoniazid, pyrazinamide, and ethambutol. We have directly amplified DNA purified from sputum samples of patients seeking for the public health service of our city with medical requests for MT diagnosis, also to evaluate the effectiveness of the approach of routine active MT resistance detection with a fast and reliable method such as the PCR-SSCP. We have amplified hotspot mutation regions of the genes *rpoB, ahpC, embB, katG, inhA*, and *pncA*, which are responsible for the majority of mutations associated to phenotypic resistance to the forementioned antibiotics.
Results: Among sputum samples from 117 patients suspected of having pulmonary tuberculosis, 2.56% (03) presented a positive baciloscopy result and also a positive PCR result with a pair of primers to a 123 bp fragment of the insertion element *IS*6110, and 7.69% (09) presented *Mycobacterium* sp. growth in culture (*P*= 0.1384). From the original DNA purified from the sputum of these nine samples that were positive by culture, we could also amplify DNA fragments of the forementioned drug resistance genes in seven (7) cases by PCR. All have presented a SSCP pattern similar to a native, non resistant genotype, with the ATCC strain 25177 as a control, except by one sample, which presented an SSCP profile demonstrating mutations at the *embB* gene, which could lead to an ethambutol resistant phenotype.
Conclusion: Our results are consistent with the empirical clinical observations of the physicians treating TB patients in our region, of a low prevalence of cases which are refractory to conventional treatment schemes. These findings may support the idea that an active resistant MT detection program routinely applied to all patients seeking TB diagnosis may not be cost effective. However, the availability of a reference laboratory capable of detection of MT resistant strains by molecular methods is of outstanding importance, as these methods are well known to have a much better turn-around time than culture methods for MT resistance detection. Support: FAPESC grant n. 14594/2005-7.

B-92

Time To Complete Molecular Remission Corresponds To Outcome In Infant Acute Lymphoblastic Leukemia With Mll Gene Rearrangements

G. Tsaur[1], A. Popov[2], T. Nasedkina[3], Y. Yakovleva[1], T. Riger[4], A. Ivanova[4], E. Shorikov[1], L. Saveliev[2], L. Fechina[1]. *[1]Regional Children's Hospital # 1, Yekaterinburg, Russian Federation, [2]Ural State Medical Academy, Yekaterinburg, Russian Federation, [3]Engelgardt Institute of Molecular Biology, Moscow, Russian Federation, [4]Research Institute of Cell Technologies, Yekaterinburg, Russian Federation,*

Background. Predictive value of early complete molecular remission (cMR) in infant acute lymphoblastic leukemia is not clear so far.
Objective. To define a time point (TP) of fusion gene transcript (FGt) monitoring by reverse-transcriptase PCR (RT-PCR), that clearly predicts outcome in infants with *MLL*-rearranged ALL, enrolled onto MLL-Baby protocol where conventional chemotherapy is augmented by administration of all-trans retinoic acid (ATRA) (L. Fechina et al, 2007).
Methods. Monitoring of FGt was performed in 18 infants with defined *MLL* translocation partner genes who had more than 4 follow-up samples. Median of follow-up in the observed group was 30 months. Presence of *MLL* rearrangements was detected by FISH and confirmed by long-distance inverse PCR (C. Meyer et al, 2005). cMR was defined as absence of FGt at nested RT-PCR with sensitivity 5E-05. Detection of FGt in bone marrow (BM) was performed by qualitative nested RT-PCR as previously described (A. Borkhardt et al.,1994, J. van Dongen et al., 1999, N. Palisgaard et al., 1998). Among 18 infants there were 11 *MLL-AF4*-positive patients (pts), 3 *MLL-MLLT10*-positive pts, 2 *MLL-EPS15*-positive pts, one *MLL-MLLT1*-positive patient and one *MLL-MLLT3*-positive patient. BM samples were obtained at diagnosis, at day 15 of remission induction (time point (TP) 1), at the end of remission induction (TP2) and after each course of ATRA administration (TP3-TP9). Informed consent was obtained in all cases. **Results**. FGt elimination speed did not correlate with any known prognostic factors such as initial WBC count, age at diagnosis, number of blast cells at day 8 of dexamethasone prophase, CNS status, type of *MLL* partner gene. Retrospectively, pts were divided into two groups. First group included 14 pts who achieved cMR by TP4, where two relapses occurred (in *MLL-AF4*-positive and *MLL-EPS15*-positive cases). The second group consisted of four *MLL-AF4*-positive pts, who had not achieved cMR by TP4. In this group there were 3 relapses. Number of relapses was significantly higher in the second group (odds ratio 18,00; 95% CI 1.19-271.47; p=0.044). Cumulative incidence of relapse in the first group was 0.15, in the second group 0.75 (p=0.022). 75-months event-free survival in the first group was 0.84, in the second group 0.25 (p=0.023).
Conclusions. Slow achievement of cMR corresponds to poor outcome in infant ALL with *MLL* rearrangements. Persistence of FGt at TP4 allows defining patients with high risk of relapse.

B-93

Evaluate the Treatment Efficacy of Cryptococcus Neoformans Meningitis by Real-time PCR Quantitative Analysis of VAD1 mRNA

L. Jiang, L. Dai, Q. Ou. *The department of medical laboratory science of the first affiliated hospital of Fujian medical university, Fuzhou, Fujian Province, China,*

Objective To establish a new approach for the quantitative detection of VAD1 mRNA in cryptococcus neoformans (CN) by real-time fluorescent quantitative PCR (RT-FQ-PCR), and appraise its value in the evaluation of the treatment efficacy of patients who suffered from cryptococcus neoformans meningitis (CNM).
Methods The primers and TaqMan probe were designed according to the published sequence of VAD1 mRNA (GenBank) for the establishment of RT-FQ-PCR. Cerebrospinal fluid from 25 CNM patients and 30 controls were detected to evaluated the sensitivity and specificity of this method. VAD1 mRNA concentration of csf from both acute phase and recovery phase of 25 CNM patients were also detected for the evaluation of the significance during treatment.
Results RT-FQ-PCR for the detection of VAD1 mRNA resulted in the correlation coefficient in -0.9979 for standard curve and the detection limit in 10^1 copies/ul. The CV values for high, medium and low concentrations were 0.65%, 0.89% and 1.23% respectively, and the sensitivity was 96% when its specificity was 100%. VAD1 mRNA concentration in acute phase were higher than that of in recovery phase patient (3.042±0.906 Vs 2.187±0.665, $P<0.01$). The levels of VAD1 mRNA were positively correlate with cryptococcus counts ($r=0.870$, $P<0.01$) and CSF intracranial pressure ($r=0.770$, $P<0.01$),negatively correlate with Glucose($r=-0.429$, $P<0.05$).
Conclusions The established RT-FQ-PCR method for the detection of VAD1 mRNA exhibits a good sensitivity, specificity and reproducibility, which might fit for the detection of VAD1 mRNA. The expression level of VAD1 mRNA is related to the treatment efficacy of CNM.

B-94

Effect of High Fat and High Fructose Diet on the Expression of Class I and II Histone Deacetylases in the Diet Induced Liver Steatosis

I. Kirpich, S. Barve, C. McClain. *University of Louisville, Louisville, KY,*

Introduction. Nonalcoholic fatty liver disease is the most common form of liver disease and is associated with obesity, diabetes, and insulin resistance. A complex interplay between environmental, nutritional and genetic factors is involved in the pathogenesis of these diseases. Recently, it has been shown that epigenetic mechanisms, such as chromatin remodeling, plays an important role in energy homeostasis and insulin synthesis. Histone deacetylases (HDACs) are known to globally coordinate gene expression and may influence genes involved into *de novo* lipogenesis and fatty acid □-oxidation. Accordingly, the **goal** of the present study was to investigate the levels of HDAC gene expression, both class I and II, in response to high fat and high fructose diet, and to establish their potential role in diet induced obesity and liver steatosis.
Materials and Methods. C57/BL6 mice were fed high fat lard (HFLD, 60% lard), high fructose (HFrD), or control diet (CD) for eight weeks. Liver steatosis was confirmed by histopathology and hepatic triglycerides (TAG) biochemical measurement; liver injury was evaluated by plasma alanine aminotransferase (ALT) activity. HDAC gene expression pattern was identified by qPT-PCR approach and calculated using the $2^{-\Delta\Delta Ct}$ method.
Results. At the end of the experiment, mice in both experimental groups (HFLD and HFrD) developed fatty liver. Hepatic TAG were significantly higher in HFLD and HFrD fed mice compare to CD (148.0 ± 6.8 and 93.74±24.81, respectively *vs* 42.0 ± 2.9 mg/g of liver, $p > 0.05$). Plasma TAG were found increased only in response HFLD feeding (80.38 ± 4.37 vs 56.39 ± 4.29 mg/dL, $p < 0.05$). Liver injury, confirmed by increased plasma ALT activity was noticed only in mice fed HFLD (58.3 ± 7.4 *vs* 26.2 ± 1.8 U/L, $p > 0.001$). Analysis of hepatic HDAC gene expression showed that expression of HDACs 1, 2, 3, 8 (class I), two members of HDACs class II - HDACs 4 and 9 and HDAC 11 were significantly increased in HFLD fed mice compare to CD. The same trend was noticed in HFrD group, but did not reach statistical significance.
Conclusion. Hepatic steatosis in response to HFLD and HFrD feeding is associated with increased liver HDAC gene expression, including, class I (HDACs 1, 2, 3, 8), class II (HDACs 4, 9), and HDAC 11. Potential pathogenic mechanisms of HDACs overexpression and possible therapeutic role of HDAC inhibitors in the diet-induced obesity and fatty liver need to be investigated.

B-95

CLU and PICALM with Schizophrenia: a Study of the Association in Chinese Population

Z. yi, s. xingbo, l. siyue, w. jun, y. binwu. *West China hospital of Sichuan university, Chengdu, China,*

Background: Disorganized thinking is a widely sympotom in Schizophrenia patients and alzheimer's disease(AD) patients. Increasing evidence indicates that there is a significant relationship between CLU gene and PICALM gene with AD, which may be due to variability of lipid transportation and endocytosis in neuron. So there is a hypothesis presented that CLU or PICALM may be also the target for Schizophrenia. It is not surprise that variability of these genes cause the malnutrition to neuron and imparied thinking.
Objective: This study aimed to evaluate the association of rs11136000 in CLU gene and rs3851179 in PICALM gene with Schizophrenia through a case-control study in Chinese population.
Materials and methods: 190 Schizophrenia patients and 120 healthy pepole were inclued in our study.The high-resolution melting (HRM) method was optimized on a lightcycler 480 machine, and genotypeing was performed by Gene Scan software.
Results: The HRM method was able to genotyping samples in the two single nucleotide polymorphisms(SNP). Rs11136000 was not found to be statistically sighificantly associated with schizophrenia(T allele frequency=19.21% in cases vs. 22.08% in controls; P>0.05). At the genotype level, the difference of the TT genotype frequency between cases and controls was also not significant(TT vs. TC+CC, 4.73%VS 5.00%;P>0.05). And no association was found between cases and controls in rs3851179 (G allele frequency =62.89% in cases vs. 58.33% in controls; P>0.05).
Conclusions: The HRM is efficient method for detection of polymophisms. Based on the results our study did not support rs11136000 in CLU gene and rs3851179 in PICALM gene may play a major role in schizophrenia.

B-96

Clinical Significance of the BCR-ABL Fusion Gene subtype in Chronic myelogenous leukaemia and Acute Lymphoblastic Leukemia

Y. Yuanxin, L. Xiaojun, s. xingbo, y. binwu. *West China hospital of Sichuan university, Chengdu, China,*

Background and Objective: At diagnosis, 90-95% of cases of CML have the characteristic t(9;22)(q34;q11) cytogentic abnormality that results in the Bcr-Abl fusion gene. It is also recommend that the BCR-ABL fusion gene is a rare but important prognostic indicator in acute lymphoblastic leukemia and the site of the breakpoint, which results in different subtypes that produce abnormal fusion protein P190,P210and P230, may influence the phenotype of the disease. This study was the first time to examine the association between BCR-ABL Fusion Gene subtype and those two disease.
Material and Methods: 354 CML patients before treatment and 72 ALL patients before treatment that detected as BCR-ABL fusion gene positive were involved in this study. Relative concentration of BCR-ABL fusion gene were detected through FQ-PCR . RT-PCR and gel electrophoresis were optimized for subtypeing P190 and P210
Results: For 354 patients with CML, 151patients (42.66%) showed subtypes associated with P210, 25 patients (7.06%) showed subtypes associated with P190. 173 patients (48.87%) showed subtypes associated with both P210 and P190. 5 patient s(1.41%) showed subtypes associated with neither P210 nor P190. For 72 patient with ALL, the rate were 11.1%, 51.39%, 36.11% and 1.39%. There were significant difference between CML and ALL($p<0.05$).
Conclusions: This study identifies the BCR-ABL gene as an important factor in CML and ALL. The result suggests that subtype P210 associated more with CML while subtype P190 associated more with ALL. The relationship with other subtypes is still under discussion.

B-97

Concordance study of direct-to-consumer (DTC) genetic testing

K. Imai[1], L. J. Kricka[1], P. Fortina[2]. *[1]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, [2]Department of Cancer Biology, Kimmel Cancer Center, Thomas Jefferson University, Jefferson Medical College, Philadelphia, PA,*

Background: A number of different companies offer direct-to-consumer (DTC) genetic testing to evaluate ancestry and health (e.g., carrier status, risk). This type of massive scale testing of thousands of SNPs is not without error and such errors could translate into, for example, misclassification of risk that could in turn provide a false sense of security or unnecessary anxiety in an individual. Previous studies have compared the concordance of raw data from 23andMe and Navigenics and shown a 99.7% agreement (1).

Objective: To undertake a more wide-ranging study, we have compared results on the same individual from three DTC services and a genomics service based on DNA microarray or Taqman analysis.
Method: DNA samples from the same individual were tested at three DTC services: 23andMe (Illumina HumanHap550+ BeadChip, ~578k SNPs), deCODEme (Illumina Human1M-Duo BeadChip, ~1.1 million SNPs), Navigenics (Applied Biosystems Taqman Genotyping Assays, ~120 SNPs) and a genomics service: Expression Analysis (Affymetrix SNP Array 6.0, ~909k SNPs).
Results: Comparisons between DNA microarray assays showed the following degree of agreement: 23andMe vs deCODEme - 99.95% (551,115 SNPs compared); 23andMe vs Expression Analysis - 99.70% (162,312 SNPs); deCODEme vs Expression Analysis - 99.63% (288,676 SNPs). Comparisons between DNA microarray and Taqman analysis showed the following degree of agreement: 23andMe vs Navigenics - 100% (74 SNPs); deCODEme vs Navigenics - 100% (74 SNPs); Expression Analysis vs Navigenics - 100% (38 SNPs). The DTC services assign disease risk based on the SNP data. Although there was agreement for the relative risk assessment: e.g. Crohn's disease, there were also instances of considerable disagreement (see table).
Conclusion: The concordance of the DTC genetic testing is considered to be generally high. However, there were some marked differences in the assessed relative risk for certain diseases between the different services.

Disease	Relative risk reported for the same individual		
	23andMe	deCODEme	Navigenics
Atrial fibrillation	1.73	0.81 (*1)	1.81
Celiac disease	0.54	0.24	0.50
Crohn's disease	1.62	1.51	1.59
Macular degeneration	1.61	1.10	1.13
Prostate cancer	0.93	0.65	0.59
Psoriasis	0.62	0.37	0.83
Rheumatoid arthritis	1.28	1.86	0.94
Type 2 diabetes	0.89	0.80	0.72
*1: Two out of five SNPs used in the risk calculation were unavailable.			

Reference: 1. Ng PC et al., Nature 2009; 461: 724-6.

B-98

Application of Genotype MTBDRplus in rapid detection of the Mycobacterium tuberculosis complex as well as its resistance to isoniazid and rifampin in a high volum laboratory in Southern China

L. Zhang, Y. Ye, L. Duo, T. Wang, X. Song, B. Ying, L. Wang. *West China Hospital of Sichuan University, Chengdu, China,*

Background: There were 9.4 million incident cases of tuberculosis (TB), 11.1 million prevalent cases of TB and 1.82 millions deaths from TB in 2008 globally according to the WHO 2009 reports. TB ranks second only to human immunodeficiency virus as a cause of death from an infectious agent. The situation is further complicated by increasing emergence of drug resistant TB. The alarmingly worsening epidemics of drug-resistant TB call urgent need for a simple method for the rapid detection of drug-resistant TB in clinical settings, especially in China mainland where no identification and drug sensitivity program for Mycobacterium tubeculosis is routinely carried out in most of clinical microbiology laboratories in large general hospitals.
Methods: In an attempt to establish a rapid procedure for laboratory diagnosis of TB and investigate the local TB epidemiology, a molecular line probe assay of the Genotype MTBDRplus was used to identify Mycobacterium tuberculosis complex (MTBC) and detect mutations conferring resistance to two most active first-line drugs against TB: Rifampin and Isoniazid. 96 acid-fast bacillus (AFB) smear-positive sputum specimens and 18 PCR-positive non-sputum specimens have been determined for the MTBC and resistance to Rifampin and Isoniazid.
Results: The MTBC detection rates in two sources of specimens are 93.8% and 77.8% respectively. The overall drug resistance (Rifampin or Isoniazid) occurred in 34.6%. Resistance to rifampin (RMP) is 28.8% and 25% is to Isoniazid (INH), in which high level drug resistance account for 88.5% and low level drug resistance for 7.7%. Multidrug resistance (MDR), defined as resistant to both RMP and INH, was found in 19.2% of clinical samples, which is double that of official statistics. In the RMP-resistant TB, 33.3% (10/30)are sensitive to INH and 66.7%(20/30)are resistant to INH. Among the INH-resistant TB, 23.1% (6/26)are sensitive to RMP and 76.9% (20/26) are resistant. It indicates that multidrug resistance is more common than monoresistance to either RMP or INH . 63.3%(19/30)RMP-resistant mutations are identified in the region of RpoB 530~533, among which 57.9%(11/19)are the S531L mutation. 84.6%(22/26)of resistance to INH is mediated by Kat S315T1 mutations which confers the high-level resistance to INH. Finally, the overall turnaround time from specimen receipt to reporting of result of susceptibility was dramatically shortened to roughly 4 - 5 hours compared to weeks to months with traditional culture-based assays.
Conclusions: As a simple, quick and efficient method for rapid laboratory diagnosis of TB, the multiplex PCR-based Line Probe Hybridization of Genotype MTBDRplus successfully reveals the high burden of drug resistant TB in local region of a city in Southern China. Its application and popularization will help better solve the long-standing problem of laboratory diagnostic delay and insufficiency in China. The establishment of new approaches into which the Genotype MTBDRplus is incorporated will greatly improve the level of diagnosis and treatment of tuberculosis.

B-99

Development of a nylon membrane-based oligonucleotide array for genotyping 7 SNP loci associated with type 2 diabetes mellitus

G. Deng, X. Zhou. *Wuhan University Zhongnan Hospital, Wuhan, China,*

Objective: Type 2 diabetes mellitus (T2DM) is a common, chronic, complex disorder of rapidly growing global importance. Despite strenuous efforts over the past two decades to identify the genetic variants that contribute to individual differences in predisposition to T2DM, the story until recently was characterized by slow progress and limited success. Recent progress in whole genome SNP array techniques has made whole genome association studies feasible for molecular biology laboratories, thus promoting our understanding of various common multifactorial diseases like type 2 diabetes mellitus (T2DM), improving the chances of identifying common low risk variants and rare high risk variants. Several novel SNP loci were identified to be associated with type 2 diabetes mellitus(T2DM), but the application of these SNPs for large sample association studies was still impeded by the great cost of whole genome SNP arrays or the cumbersome procedure of conventional methods. Our aim was to develop a rapid, precise, medium-throughput and low-cost oligonucleotide array-based method for genotyping SNPs associated with T2DM to facilitate the use of these SNPs in large sample association studies.
Methods: We selected 7 SNP loci (INS rs689; JAZF1 rs864745; DCD rs1153188; IGF2BP2 rs1470579; TCF7L2 rs7501939; TSPAN8,LGR5 rs7961581; rs9465871) which are reported to be associated with T2DM to demonstrate the feasibility of the protocol. We designed PCR primers to amplify the region around each SNP locus. For convenience and throughput, each pair of primers was adjusted to obtain a common annealing temperature of 58 degrees Celcius for all 7 pairs of primers. Biotin-conjugated-nucleotide was added to the PCR reaction mixture to complete the labeling and amplification process concurrently. For parallel genotyping, genotyping probes for each allele were designed. The probes were immobilized by UV-crosslinking on Hybond N+ nylon membrane, and hybridized overnight at 42 degrees Celcius with pre-amplified and pre-labeled target DNA. After non-stringent and stringent washing of the nylon membrane, genotyping results were obtained by developing AP-avidin was added to the nylon membrane for incubation at 37 degrees Celcius for 30min. After extensive washing, NBT/BCIP was added for development. Genotyping results were obtained by comparing the intensity of different allele spots, and confirmed by DNA sequencing.
Results: The oligonucleotide array-based method clearly discriminate different alleles of the 7 T2DM associated SNP loci, and genotyping results correspond with DNA sequencing results.
Conclusion: The oligonucleotide array-based method is a simple, reliable, medium-throughput and relatively low-cost SNP genotyping method, which is a promising method for use in large sample association studies.

B-100

A Ligation Detection Reaction-Universal Array (LDR-UA) for parallel genotyping of 18 T2DM associated SNPs

Y. Xie, X. Zhou. *Wuhan University Zhongnan Hospital, Wuhan, China,*

Objective: The last 20 years have seen the identification of millions of single nucleotide polymorphisms (SNPs), which represent a large proportion of common genetic variations among the humans. It is an important objective to correlate these molecular genetic variations with complex multifactorial diseases like type 2 diabetes mellitus (T2DM). Whole genome SNP arrays provide such a route for comprehensively access information about the genetic constitution of individual patients. However, it is still a challenge to confirm SNP array genotyping results. Conventional genotyping methods like PCR are labor-intensive and time consuming, while newer techniques like Sequenom are too expensive. Recent development of the DNA microarray technology has added a high throughput option. However, to gain good probe specificity, very careful probe design and repeated experimental set up optimization would be required. Here we adopted an LDR-UA approach, that combines a cycled ligase detection reaction with hybridization on a Universal DNA array for parallel genotyping of 18 T2DM associated SNPs.
Methods: As described by Barany and co-workers, this procedure is based on the

discriminative properties of the DNA ligation reaction. Three oligos were designed for each SNP locus: two discriminating oligos, each consisting of a biotin conjugate at the 5' end, and 18-25bp allele discriminating sequence, and one common oligo which contains the 18-25bp sequence immediately downstream the SNP locus and an allele specific tag sequence. The universal tag array was prepared by UV-immobilizing the reverse complement sequence of the tags on Hybond N+ nylon membrane (Amersham). Ligation Reaction was carried out in a final volume of 20 μl containing 20 mM Tris-HCl (pH 7.5), 20 mM KCl, 10 mM MgCl2, 0.1% NP40, 0.01 mM ATP, 1 mM DTT, 2 pmol of each discriminating oligo, 2 pmol of each common oligo and 20ng genomic DNA. The reaction mixture was preheated for 2 min at 94°C and centrifuged in a microcentrifuge for 1 min; then 4U Pfu DNA ligase (Stratagene, La Jolla, California) was added. The LDR was cycled for 40 rounds of 94°C for 30 sec and 64°C for 4 min in a GeneAmp 9700 cycler (Applied Biosystems). The ligation product was preheated at 94°C for 2 min and chilled on ice, and was then added to the array. Hybridization was carried out at 42°C overnight in a UVP hybridization oven (UVP, USA). After non-stringent and stringent washing of the nylon membrane, AP-avidin was added to the nylon membrane and incubated at 37 degrees Celcius for 30min. After extensive washing, NBT/BCIP was added for development. Genotyping results were obtained by comparing the intensity of different allele spots, and confirmed by DNA sequencing.

Results: The LDR-UA array-based method clearly discriminate different alleles of the 18 T2DM associated SNP loci, and genotyping results correspond with DNA sequencing results.

Conclusion: The LDR-UA array-based method for parallel genotyping 18 T2DM associated SNPs is a simple, reliable, multiplex SNP genotyping method, which may be useful for confirming whole genome SNP array genotyping results.

B-101

Association study of single nucleotide polymorphisms in pre-miRNA and rheumatoid arthritis in a Han Chinese population

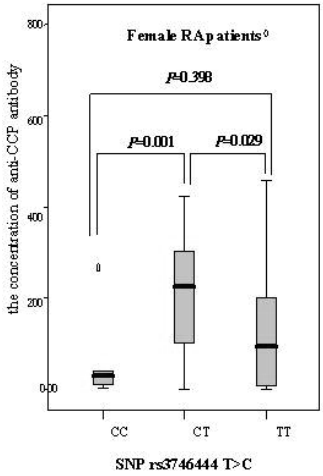
b. yang[1], J. Zhang[1], Y. Shi[2], D. Li[1], Z. Huang[1], B. Cai[1], X. Song[1], L. Li[1], B. Ying[1], L. Wang[1]. *[1]Department of Laboratory Medicine, west china hospital of sichuan unversity, chengdu, China, [2]Department of Nephrology,west china hospital of sichuan unversity, chengdu, China,*

Background: The genetic variants in many genes have been confirmed associated with the development of RA in many studies. MicroRNA can causes the degradation or translational repression of its target mRNA. Small variation in miRNAs may have an effect on thousands of target mRNAs and result in diverse functional consequences.

Objective: The aim of this study was to perform an association study between two single nucleotide polymorphisms (SNPs) rs2910164 G>C and rs3746444 T>C in pre-miRNA (hsa-mir-146a and hsa-mir-499), a negative feedback during the activation of innate immune responses and rheumatoid arthritis (RA) in a Han Chinese population.

Methods: 208 Han Chinese patients with RA and 240 healthy controls were included in this study. The SNPs in pre-miRNA was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). anti-cyclic citrullinated peptide (anti-CCP) antibody was measured by enzyme linked immunosorbent assay (ELISA) and RF was measured by rate nephelometry. The genotype frequencies between cases and controls were compared by $\chi 2$ analysis.

Results: No significant association between the SNPs(rs2910164 and rs3746444) and RA was observed in this study (P=0.631 and P=0.775, respectively).The SNPs didn't show any association with the RF-positive(P=0.631 and P=0.775, respectively).But there was significant difference on the level of anti-CCP antibody between different genotypes in rs3746444 (P=0.007). For the female RA patients, there was significant difference on the level of anti-CCP antibody between different genotypes in rs3746444 (P=0.003). The heterozygote CT have higher level of anti-CCP antibody compared with homozygote CC and TT(P=0.001 and P=0.029) (Figure)**.**



Conclusion: We first investigated the association between the SNPs(rs2910164 G>C and rs3746444 T>C) in the pre-miRNA (hsa-mir-146a and hsa-mir-499) and RA in a han Chinese population. We did not find a significant association between the SNPs and the susceptibility to RA.. The SNP rs3746444 may be a candidate biomarkers for predict joint damage in RA.

B-102

Association between polymorphisms of gap junction protein-alpha 8 gene and age-related cataract

M. Ke, L. Liu, F. Zheng, S. Guo, Q. Chen. *Zhongnan Hospital of Wuhan University, Wuhan, China,*

Background: Previous studies suggest mutation of the gap junction protein-alpha 8 (*GJA8*) gene is associated with hereditary congenital cataracts. Since *GJA8* plays an important role in lens growth and transparency, we speculate that polymorphism of this gene is implicated in age-related cataract. In this study, we investigated the association between two single nucleotide polymorphisms (SNPs) of *GJA8* and age-related cataract.

Methods: The SNPs (rs1495960 and rs9437983) were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and DNA sequencing in 96 age-related cataract patients and 208 gender and age matched healthy controls.

Results: There were no significant differences in genotype and allele distributions of rs1495960 polymorphisms between cases and controls (p>0.05). The allele distribution of rs9437983 was different between cases and controls; no difference was detected in the genotype distribution of rs9437983. A significantly lower G-G haplotype frequency (4.9% versus 15.5%, p=0.0001) and a significantly higher G-A haplotype frequency (45.6% versus 36.4%, p=0.030) were detected in cases than controls. A contrasting linkage disequilibrium (LD) pattern was observed between cases and controls. The polymorphic information content (PIC) value of rs1495960 and rs9437983 was 0.3746 and 0.3738, respectively. The PIC value of their haplotype is 0.692.

Conclusions: The *GJA8* gene may be a novel susceptibility gene for age-related cataract. These two SNPs had medium PIC values, and merging these two SNPs constituted a qualified genetic marker.

B-103

Connexin50S276F Mutant in Dominant Congenital Cataract Inhibits Gap Junction Channel and Hemichannel Function

Y. Liu, M. Yan, F. Zheng, X. Zhou, Q. Chen. *Zhongnan Hospital of Wuhan University, Wuhan, China,*

Purpose: In order to understand the mechanism underlying the autosomal dominant congenital cataract caused by mutant Cx50S276F, we designed this study to detect the mutant Cx50S276F expression, subcellular localization, detergent resistance, and channel and hemichannel functions.

Methods: Wild-type Cx50 and mutant Cx50S276F were expressed in HeLa and human lens epithelial (HLE) cells. The functional characteristics of mutant Cx50S276F were examined using immunoblot assay, confocal fluorescence microscopy and dye transfer analysis in comparison with wild-type Cx50.

Results: Mutant Cx50S276F and wild-type Cx50 were expressed in equal levels and could efficiently localize to the plasma membrane without transportation and assembly problems

in HeLa and HLE cells. Scrape loading dye transfer was evident in cells expressing wild-type Cx50, but were not evident in cells expressing mutant Cx50 and cells co-expressing wild-type and mutant Cx50. The dye uptake was found to be significantly lower in cells expressing mutant Cx50 than in cells expressing wild-type Cx50 and cells co-expressing wild-type and mutant Cx50. No statistically significant differences of dye uptake were detected between cells expressing wild-type Cx50 alone and cells co-expressing wild- type and mutant Cx50. The transfected HeLa and HLE cell lines showed similar performance in all the experiments.

Conclusions: The results indicated that mutant Cx50S276F inhibits the function of gap junction channel in a dominant negative manner, but inhibits the hemichannel function in a recessive negative manner.

B-104

Detection method for methylation in promoters of MLXIPL, PMVK, TRIB3 Genes

N. Yang, F. Zheng, S. Guo, R. Qian. *Zhongnan Hospital of Wuhan University, Wuhan, China,*

Objective: Recently, the most popular method for detecting the methylation in special DNA regions is methylation-specific PCR(MS-PCR). But it can only show the methylation states of part of the CpG islands in the target DNA fragments. In order to study the relationship between DNA methylation and disease, it is necessary to know methylation of every CpG in the gene promoter. The aim of the study is to establish time-efficient and sensitive method for detection of the methylation of PMVK, TRIB3 genes promoter.

Methods: The blood samples were collected from people in Medical Examination Center whose kinds of relative index are normal. DNA was extracted by NaI and Proteinase K methods, and treated using sodium bisulfite and hydroquinone. Based on the promoter sequence of PMVK, TRIB3 genes in UCSC Genome Browser Home, two sets of primers were used to amplify each target DNA fragments by nest PCR combined with touch-down PCR. The PCR products were further cloned and sequenced.

Results: The sequencing results showed that unmethylated cytosine residues (C) were transformed to uracil (U), while methylated cytosine (mC) remained unchanged after treatment of sodium bisulfite and hydroquinone.

Conclusions: In the present study, methods for the detection of methylation states of PMVK, TRIB3 genes promoters were successfully established, which transformed different methylation states of DNA regions into different base sequence. It can distinctly detect methylation of every CpG island. The study provides a technique for exploring the relationship between disease and the methylation of these genes.

B-105

Linkage analysis and mutation screening of candidate gene in a family with autosomal dominant retinitis pigmentosa

M. Yan, X. Cai, G. Song, F. Zheng, J. Zhang. *Zhongnan Hospital of Wuhan University, Wuhan, China,*

Objective: To localize the pathogenic gene and screen the gene mutation associated with autosomal dominant retinitis pigmentosa in a Chinese family.

Methods: Families were ascertained and patients underwent comprehensive ophthalmic examinations. Blood samples were collected and genomic DNA was extracted. Genetic linkage analysis was performed on the all known genetic loci for ADRP by using 36 polymorphic microsatellite markers, and lod scores were calculated. Subsequently, the mutation screening of candidate gene (rhodopsin) was conducted by polymerase chain reaction (PCR) analyses and sequencing.

Results: Two-point linkage analysis showed the LOD score reached 3.6671 and 3.1148 at marker D3S1292 and D3S1569 at recombination fraction θ=0.00. Meanwhile, other loci were also excluded. This region harbors the common candidate gene (rhodopsin). The results of direct DNA sequencing revealed a C→G transversion mutation at codon 53 in exon 1 of rhodopsin gene, which resulted in a proline to arginine change (Pro53Arg) in 12 patients, but not in the unaffected members of the family. The proline at codon 53 is conserved in mammalian RHO proteins, suggesting that this mutation is deleterious.

Conclusions: The heterozygous missence mutation Pro53Arg in rhodopsin gene cosegregated with the RP disease. Therefore, we could regard it as the pathogenic gene of this Chinese ADRP family. In 1992, Inglehearn found the CCC to CGC (Pro to Arg) mutation in codon 53 of rhodopsin gene. We also identified this mutation, in Chinese population for the first time.

B-106

Cell-free DNA in Patients with Disorder of Sex Development

X. Song, X. Lu, B. Ying, Y. Ye, L. Lin, L. Wang, L. Zhang. *West China Hospital of Sichuan University, Chengdu, China,*

Background: Cell-free DNA, also called circulating DNA, has widely been studied and correlated with diagnosis, prognosis or monitoring of various disorders in recent years, since the enormous attraction of realizing noninvasive testing is conspicuous, although the underlying mechanisms of its production and clearance in vivo is yet to understand. Althought the field of exploration has extended extensively first from fetal medcine and oncology then to other pathological states including trauma, sepsis, myocardial infarction, stroke, transplantation, diabetes mellitus, and hematologic disorders, there are no investigation of cell-free DNA into patients with disorder of sex development (DSD). This study uses quantitative realtime PCR to measure cell-free DNA in patients with DSD.

Methods: 25 patients with DSD at diagnosis (16 with 46XY,DSD; 4 with 46XX,testicular DSD;3 with Sex chromosome DSD and 2 with 46,XY complete gonadal dysgenesis) were enrolled in this study, as well as a control group of 28 healthy males matched by age. EDTA-anticoagulated venous blood specimens were collected from the patients with DSD and controls. All patients were sampled before start of any treament. Plasma samples were obtained by two-step centrifugation for completely eliminating the contamination of cellular DNA. Cell-free DNA was assessed by quantitative PCR amplification of the human β-globin gene (HBB) and the sex determining region of the Y chromosome (SRY) after plasma being subjected to the NucliSENS easyMAG (BioM′erieux, Marcy l'Etoile, France), an automated nucleic acid isolation system based upon the silica extraction technology.

Results: Circulating β-globin gene was determined in all DSD patients (427±2487.3 ng/ml) and controls (68.2±77.2 ng/ml). Levels of circulating β-globin gene in patients with DSD were significantly higher than in health controls (P< 0.001). Circulating SRY gene was quantified in 22 patients (189.5±1729.9 ng/ml) and all controls (107.5±100.5 ng/ml). Patients with DSD also present significantly higher concentrations of circulating SRY gene than health controls (P = 0.009). Additionally, the ratio of circulating SRY gene/β-globin gene was calculated and found significantly different between DSD patients (0.62±1.3) and health controls (1.8±1.8) (P = 0.008).

Conclusion: Our data suggested that cell-free DNA may play a potential role in assessment of DSD patients. However, further research is warranted to better define the value of present results and unveil the underlying mechanism.

B-107

Frequency of Covert *CYP2D6* Recombinants in Clinical Samples

D. L. Walker, D. J. O'Kane, M. Harmandayan, D. Mrazek, J. L. Black. *Mayo Clinic, Rochester, MN,*

Objective: Cytochrome P450 *2D6* (*CYP2D6*) is a polymorphic gene that has been observed to be deleted, duplicated and involved in recombination events with *CYP2D7* pseudogene. *CYP2D6* enzyme is involved in the metabolism of many drugs to either active or inactive forms and is routinely tested to predict metabolism of tamoxifen and other drugs. We recently reported the discovery of several new alleles and genomic variants involving *CYP2D6*.

The objective of this study was to extend previous research by determining the frequency of newly described genomic variants in a large clinical sample thus determining the risk of these variants going undetected. The results of this research will be informative to new and existing genotyping platforms as well as *CYP2D6* full gene sequencing or full genome sequencing analyses.

Methods: 776 clinical samples originally genotyped for **4* and **10* alleles or any homozygous genotype were analyzed.

Analyses for three genomic variants containing *CYP2D6-2D7* hybrids were done on samples containing **4* and **10* alleles to determine if **4N* + **4-like, *68* + **4-like,* or **36* + **10B* arrangements were present.

Analyses for the presence of *CYP2D7-2D6* hybrids, *e.g. *16*, were done in all homozygous samples.

Phenotype for each sample was predicted based upon results.

Analysis was by long-range PCR amplification, PCR fragment analysis, and DNA sequencing. Additional genotyping of amplicons was done by allele-specific primer extension assay.

Results: 1. Of 246 samples containing homozygous genotypes, 3 (1.2%) harbored a *CYP2D7-2D6* arrangement, e.g. *16. Phenotype prediction changed from extensive metabolizer to intermediate metabolizer status in all three cases. 2. Of 490 samples with a **4* allele, 79 (16.1%) had **68* + **4-like* and 6 (1.4%) had **4N* + **4-like* arrangements. Phenotype prediction did not change in any of these since **4N, *68*, and **4N*-like are thought to be null alleles. 3. Of 17 samples with a **10* allele, 3 (17.6%) harbored a **36* + **10B* arrangement. Phenotype prediction did not change appreciably in any of these cases

since **36* and **10* are both thought to have reduced function.
Conclusion: 1. The primers designed for this research can be used to reliably detect recombinant alleles. 1. The allele specific primer extension-based genotyping platform used does not identify rare *CYP2D7-2D6* alleles. When these recombinants are not detected, phenotype prediction may be inaccurate depending on the allele present on the other chromosome. 3. **4N* and **68* are frequently found in samples originally genotyped with **4* alleles and **36* is frequently found in samples originally genotyped with **10* alleles. These alleles do not change phenotype prediction. 4. Interpretation of results from future genotyping platforms including full gene sequencing of *CYP2D6* or full genome sequencing will need to use this information to adequately interpret raw data obtained.
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B-108

Association of pre-microRNAs genetic variants with susceptibility in Systemic lupus erythematosus

z. junlong, Y. Bin, Y. Binwu, L. Dongdong, S. Yunying, S. Xingbo, C. Bei, H. Zhuochun, W. Yongkang, W. Lanlan. *West China Hospital, Sichuan University, Chengdu, China,*

MicroRNAs(miRNAs) are noncoding RNAs that inhibit the expression of protein-coding genes by either translational repression or messenger RNA degradation. Growing evidences have suggested that miRNAs regulate a wide range of biological processes, including development, cell differentiation, proliferation and apoptosis. Sequence variations have been found to affect the processing and/or target selection of human miRNAs, even a single nucleotide polymorphism (SNP) sequence may significantly alter miRNA expression and function. The aim of this study was to investigate genetic polymorphisms(SNPs) of the miRNAs, and their relationships with various autoantibodies present in SLE patients. A total of 213 SLE patients and 209 healthy individuals of Chinese population were enrolled in the study. Case-control studies were performed for selected two miRNA polymorphisms (hsa-mir-146a rs2910164 G>C, and hsa-mir-499 rs3746444 T>C). The SNPs variants were analyzed by PCR-RFLP. Serum anti-ribonucleoprotein (anti-RNP), anti- Sm nuclear antigen (anti-Sm) antibodies were determined by an Anti-ENA kit and serum anti-double-stranded DNA(ds-DNA) was assessed by indirect immunofluorescence. We found the genotype frequencies of rs2910164 (GG, CC and GC) were 16%, 37% and 47% in SLE, 11%, 39% and 50% in health controls (p=0.397), rs3746444 (CC, TT and TC) were 3%, 74% and 23% in SLE, 3%, 76% and 22% in health controls (p=0.892). The G and C allele frequencies of rs2910164 were 39% and 61% in SLE, 36% and 64% in health controls (p=0.990), the C and T allele frequencies of rs3746444 were 15% and 85% in SLE, 14% and 86% in health controls (p=0.702). In addition, there was no difference in the distribution of rs2910164 and rs3746444 genotypes in each of the three antibodies. Although microRNAs may play important roles in SLE, hsa-mir-146a rs2910164 and hsa-mir-499 rs3746444 polymorphisms were no significant associated with SLE susceptibility. However, the positive frequencies of anti-RNP, anti-Sm, and ds-DNA were no difference with rs2910164 and rs3746444 genotypes.

B-109

Circulating miR-122 as a biomarker of liver disease

C. M. Lockwood[1], O. F. Laterza[2], K. M. Korenblat[1], M. G. Scott[1].
[1]Washington University, St. Louis, MO, [2]Merck Research Laboratories, Rahway, NJ,

Background: Serum alanine aminotransferase concentrations are often considered the standard laboratory indicator of acute liver damage. However, ALT concentrations do not always correlate with histomorphologic studies and extra-hepatic injury may also increase ALT. MicroRNA (miR) molecules have recently emerged as promising biomarkers for early detection of disease. miR are noncoding RNAs of approximately 22 nucleotides that function as regulators of mRNA translation and degradation. Tissue expression profiles have shown that miR-122 is specifically and abundantly expressed in the liver. The discovery of miR-122 in plasma suggested potential as a biomarker. Rat models of toxin-induced acute hepatitis demonstrated time- and dose-dependent elevations in plasma miR-122 plasma concentrations. Furthermore, miR-122 increased > 6000 fold compared to 70 - 80 fold increases in ALT, suggesting that miR-122 may be a more sensitive biomarker of liver injury. This small, proof of principle, case controlled study examined plasma miR-122 in humans with liver disease.
Objectives: To determine: 1) the amount of miR-122 in the plasma of 15 healthy subjects; 2) whether the amount of miR-122 is elevated in 30 patients with documented histories of liver disease; 3) whether the amounts of miR-122 in human blood correlate with concentrations/enzyme activities of traditional serum liver damage markers in liver disease patients.
Methods: IRB approval was obtained for this study and all participants provided informed consent. 15 healthy volunteers and 30 patients with documented histories of liver disease were recruited. Liver function tests (alkaline phosphatase, total and direct bilirubin, ALT, aspartate aminotransferase) were performed in the Barnes-Jewish Clinical Chemistry laboratory on a Roche P-Modular analyzer. Total RNA was isolated from EDTA plasma samples with TRIzol (Invitrogen) followed by nucleic acid precipitation. TaqMan Reverse Transcription Reagents (Applied Biosystems) were used for cDNA synthesis with 100 ng of total RNA reverse-transcribed using hairpin primers designed to target miR-122. TaqMan PCR was performed in quadruplicate with 20 ng cDNA on the ABI Prism Sequence Detection System 7900 HT (Applied Biosystems).
Results: Patients with histories of liver disease had diagnoses of: hepatitis C, 16/30, autoimmune hepatitis, 4/30, aminotransferase elevation of unknown origin, 4/30, steatohepatitis, 2/30, and other, 4/30. ALT was elevated above the reference interval in 2/15 (13%) healthy subjects and 17/30 (57%) liver disease patients. In healthy subjects, the lower and upper quartiles for miR-122 plasma concentrations were 32.8 and 93.0 copies/20 pg RNA, respectively (range 16.0 - 312.0). The miR-122 concentrations in liver disease patients for the lower and upper quartiles were 79.4 and 443.8 copies/20 pg RNA, respectively (range 20.9 - 1160.0). The difference between median miR-122 concentration in healthy subjects and liver disease patients (51.7 and 202.3 copies/20 pg RNA, respectively) was statistically significant (Mann-Whitney test, P=0.0016).
Conclusions: Our data suggest a role for miR-122 as a plasma biomarker in liver disease, and support the further investigation of miRNA-based diagnostic assays in a variety of pathological conditions to establish the specificity and sensitivity of circulating plasma miR-122.

B-110

Validation of a New Freeze-Dried PCR Universal Master Mix

M. Gramegna[1], E. Castiglioni[2], A. Moiana[1], M. Zanussi[3], M. Ferrari[2].
[1]Sentinel CH, Milano, Italy, [2]Vita-Salute San Raffaele University, Milano, Italy, [3]Diagnostica e Ricerca San Raffaele SpA, Milano, Italy,

Objectives. Sentinel Ch. has recently developed a new proprietary freeze-drying system for the manufacturing of ready-to-use PCR Universal master mixes. Usually the PCR mixes need to be stored at -20°C or + 2/8 °C. This means request for refrigerators and freezers in the labs, and controlled temperature in case of shipment. The new system is able to maintain the activity of the mix with no loss of performance at room temperature. The aim of the present study is to validate the performances of the freeze-dried mixes for the main molecular applications in research or diagnostic laboratories. **Design.** Each test tube contains components (reaction buffer, dNTPs, $MgCl_2$, Hot Start Polymerase, preservatives and stabilizers) in the freeze-dried form. The mixes are manufactured in a ready-to-use format with all the necessary components already included, also the enzyme. The storage of the mix is at room temperature.
Methods. Here we describe the protocols used for the five evaluated applications. For each application we compared the Sentinel CH. system with the system in use in the laboratory (HSR). For the present work we selected 16 different DNA samples, with a concentration of 20ng/µl each. Amplified products were analyzed by electrophoresis on a 2% agarose gel, before sequencing analysis (Applied Biosystems 3730 DNA Analyzer). Each PCR product was purified using MultiScreen HTS Vacuum Manifold system (Millipore). In parallel, we also evaluated the performance of Universal Master Mix reagents by the use of Rotor Gene 6000 (Corbett Life Science). This instrument allowed 3 different applications in the same analytical session: a real time PCR in presence of a fluorescent double strand specific dye (EvaGreen, Biotium), a melting curve and a high resolution melting analysis. QF PCR analysis includes amplification, detection and analysis of chromosome-specific DNA microsatellites using an automated genetic analyzer (Applied Biosystems 3730 DNA Analyzer).
Results and conclusions The system showed a good performance and accuracy for the five applications evaluated. In particular the freeze-dried form guaranteed high quality standard of PCR products, eliminating the presence of conventional additive that could disturb and delay the purification step, and generating sufficient amounts of amplified products for subsequent sequencing analysis. For sequencing analysis, real time PCR, melting analysis and QF PCR we obtained a perfect agreement (16/16 samples), while HRM analysis allowed the correct identification of 14/16 DNA samples. This problem could be due to a not fully optimized protocol and could be avoided by the use of other alternative commercial florescent dyes (Syto9, Invitrogen; LCGreen Plus, Idaho Technology, etc.) or by the variation of any cycling conditions. In conclusion, the ready-to-use format, and the room storage, allows an easy and useful application for different molecular systems and requirements, reducing the time needed for preparation of the amplification mix, and reducing risk of contamination.

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Simple and rapid molecular test for prediction of breast cancer prognosis with a capillary-based multiplex RT-PCR technology

S. Takahashi[1], R. Nomiyama[2], T. Fukui[2], S. Yokoyama[2], N. Gondo[2], C. Ishioka[1]. *[1]Department of Clinical Oncology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan, [2]FALCO Biosystems, Kyoto, Japan,*

[Background]: Breast cancer is one of the common malignant tumors affecting women with an increasing incidence rate in many countries. The prognosis of the breast cancer has been improved with multidisciplinary adjuvant treatments including chemotherapy, hormone therapy, and radiotherapy. To assist patient management, new molecular based prognostic tests for early breast cancer have been developed. However, utilization of these existing assays are still limited because their techniques are complicated, time-consuming, and high standard cost. In this study, we focused the gene expression profile that could predict TP53 mutations known as a poor prognostic factor in breast cancers to develop a simple, rapid and reliable prognostic test.

[Subjects and Methods]: 38 Japanese patients with invasive ductal carcinoma of the breast were collected. Only tumorous cells of the frozen tumor tissues were microdissected. Genomic DNA and total RNA were extracted from the tumorous cells. TP53 mutations were examined by Sanger-based DNA sequencing. The gene expression patterns of the 25 genes (16 up-regulated genes in mutant TP53, 9 down-regulated genes in mutant TP53) identified in our previous microarray study were determined by the GenomeLab™ GeXP Genetic Analysis System, the capillary-based multiplex RT-PCR system (Beckman Coulter). The expressions of each 25 genes were confirmed by single-plexed real-time RT-PCR to understand the quantitative performance of the capillary-based multiplex RT-PCR system. [Results]: The expression result of our multiplexed system had a good correlation with single-plexed real-time RT-PCR. In addition, the expression pattern of the 25 gene set could predict the TP53 mutation status. Furthermore, our assay had been shown to have the ability to recurrence-free survival.

[Conclusion]: We have developed a simple, rapid and reliable diagnostic assay to be able to predict the prognosis of the breast cancer with ease of use for clinical laboratories.

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***Matrix Metalloproteinases MMP*-9 and *MMP*-2 gene polymorphisms influence allograft survival in renal transplant recipients**

R. D. Mittal, R. Singh, A. Srivastava, R. Kapoor. *Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India,*

Background: Matrix metalloproteinases (MMPs) especially basement membrane degrading MMP-9 and MMP-2 play an important role in immune mediated tissue destruction of the allograft by allowing influx of leukocytes and mononuclear cells into the graft.Objective: The present study was therefore undertaken to investigate the association of functional polymorphisms in MMP-9 (836A>G, 1721G>C, 2003G>A) and MMP-2 (-735C>T) genes with risk of allograft rejection in renal transplant recipients of North India.

Methods: 306 renal transplant recipients categorised into 228 non-rejecters and 78 rejecters were genotyped for MMP-9 and MMP-2 polymorphisms by PCR-RFLP methodology.

Results: Mutant allele carriers for MMP-9 2003G>A (GA+AA- OR=0.45, 95% CI, 0.24-0.85, P=0.014) and MMP-2 -735C>T (CT+TT- OR=0.40, 95% CI, 0.18-0.91, P=0.029) demonstrated significantly reduced risk for allograft rejection. The mean time to first rejection episode was significantly higher in GA/AA and CT/TT genotype recipients for MMP-9 2003G>A (Log P=0.026) and MMP-2 -735C>T (Log P=0.003) respectively. Haplotypes with mutant alleles for MMP-9 1721C>G-2003G>A and mutant allele genotype combinations for both MMP-9 2003G>A and MMP-2 -735C>T conferred significantly reduced risk for allograft rejection.

Conclusion: Mutant alleles for MMP-9 2003G>A and MMP-2 -735C>T was observed to be associated with reduced risk for allograft rejection and improved allograft survival in North Indian transplant recipients and could serve as an ideal marker to predict pre-transplant allograft outcome.

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Stabilization of Cell-Free RNA in Plasma for Non-Invasive Diagnosis and Prognosis

R. Fernando, S. Norton, W. Ryan. *Streck Inc., La Vista, NE,*

Background: The discovery of the presence of cell-free nucleic acids in blood (1) and subsequent demonstrations that cell-free nucleic acids were elevated in certain disease conditions (2) suggested the usefulness of cell-free nucleic acids in non-invasive diagnosis and prognosis. The clinical utility of cell-free mRNA in blood as diagnostic and prognostic markers was first demonstrated in cancer patients (3). The presence of fetal cell-free mRNA in maternal blood was initially reported in 2000 by Poon et al. (4). Following this discovery many other clinically important fetal/placental cell-free mRNA molecules were found to be present in maternal blood. These discoveries have presented us with opportunities to develop non-invasive diagnostic and prognostic tools based on cell-free RNA. Due to its labile nature, there are inherent disadvantages of using cell-free mRNA in blood as biomarkers. Since plasma is rich in nucleases, cell-free mRNA tends to degrade during sample handling and processing. Another disadvantage is the release of non-target background RNA from blood cells during sample processing and storage. This kind of background increase hampers the detection of rare mRNA targets.

Objective: To develop a standardized blood collection device that preserves cell-free RNA in plasma and prevents the release of non-target background RNA from blood cells at ambient temperature.

Methods: Blood samples were drawn from healthy donors into K_3EDTA and into a collection tube developed by Streck Inc., (Cell-Free RNA™ BCT) and kept at ambient temperature until further processed. At different time points, plasma was separated, cell-free RNA was extracted and mRNAs for p53, c-fos, glyceraldehyde-3-phosphate dehydrogenase, (G3PD) and18S rRNA were quantified by reverse transcriptase real-time PCR.

Results: Blood drawn into K_3EDTA tubes showed a steady increase in mRNA copy numbers for c-fos, G3PD and 18S rRNA whereas mRNA for p53 showed a steady decrease over three days of *ex vivo* incubation at ambient temperature. However, blood drawn into Cell-Free RNA BCT tubes showed no significant such change in mRNA copy number. The mRNA copy number was constant throughout the three days of *ex vivo* incubation period for all mRNAs tested at ambient temperature.

Conclusion: Streck's Cell-Free RNA BCT blood collection device provides preservation and stabilization of cell-free mRNAs in plasma for at least for three days at ambient temperature. In doing so, the genetic expression patterns of specific genes are preserved. This allows for the development of non-invasive diagnosis and prognosis methodologies based on cell-free RNA in blood.

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Association of TLR2, TLR4, TNF-α and IL-6 gene polymorphisms and expression with atherosclerosis in type 2 diabetes patients

M. H. Hirata[1], F. A. Silva[1], S. Himelfarb[1], M. F. Sampaio[2], L. G. Lima-Neto[1], M. Saleh[2], M. C. Bertolami,[2], I. Del Monaco[2], S. S. Arazi[2], A. A. Rezende[3], D. Armaganijan[2], S. Q. Doi[4], A. G. M. Sousa[2], R. D. C. Hirata[1]. *[1]University of Sao Paulo, Sao Paulo, Brazil, [2]Instituto Dante Pazzanese de Cardiologia, Sao Paulo, Brazil, [3]Federal University of Rio Grande do Norte, Natal, Brazil, [4]Uniformed Services University of Health Sciences, Bethesda, MD,*

BACKGROUND: Individuals with type 2 diabetes mellitus (T2DM) have a proinflammatory status that increases the risk for atherosclerosis. Toll like receptors (TLRs), mainly the TLR4, involved in immune innate response, and pro-inflammatory cytokines such as IL-6 and TNF-α may be involved in the activation of early stages and progress of the atherosclerotic lesions in T2DM. We have investigated the relationship between TLR2, TLR4, TNF-α and IL-6 gene expression and polymorphisms with T2DM and atherosclerosis.

METHODS. Sixty-one T2DM and 39 normoglycemic (NG) individuals were selected at the Coronary Session of the Instituto Dante Pazzanese de Cardiologia (Sao Paulo, Brazil). Carotid artery ultrassonography was used to evaluate the atherosclerotic status. Polymorphisms of the TLR4 (Thr399Ile), TLR2 (Arg753Gln), and IL6 (-174G>C) genes were detected by PCR-RFLP. TLR4 (Asp299Gly) and TNFA (-308G>A) gene polymorphisms were detected by HRM real time PCR. Blood leukocytes mRNA expression was measured by real time PCR using GAPD as a reference gene.

RESULTS: In T2DM group, individuals carrying *IL6* -174C allele had higher total cholesterol, VLDL-c and triglycerides than the genotype GG carriers (respectively, p=0.007, p=0.006 and p= 0.030). TLR4 mRNA expression was higher in T2DM than in NG individuals (p=0.029). In T2DM group, TNFA -308GG genotype was associated with increased TNF-α mRNA expression levels (p=0.031) and higher fibrinogen levels than those carrying -308A allele (GA+AA genotypes) (p=0.020). In individuals with atherosclerosis *TLR4* 299Gly allele was associated with high post-test glucose levels

(p=0.012) and those carrying *IL6* -174C allele had higher total cholesterol and LDL-c than the GG genotype carriers (both p<0.001). The polymorphism IL6 -174G>C was related with risk for atherosclerosis (OR= 3.0, p<0.05) and the male sex, atherosclerosis and menopause were related with risk for T2DM (OR= 3.4, 16.97 and 3.02, respectivelly) . **CONCLUSION:** *TLR4* Asp299Gly polymorphism is likely to be associated with insulin resistance, in individuals with atherosclerosis, and increased *TLR4* expression is associated with T2DM status. IL-6 -174G>C polymorphism showed to be related with altered lipid profile in T2DM and individuals with atheroscerosis. TNFA -308 G>A polymorphism seems to be related to increased plasma fibrinogen and TNF-□ mRNA expression levels in T2DM.

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Molecular Testing for IDH1 and 2 Mutations in Bone Marrow, Blood, and FFPE.

V. Leung-Pineda, A. Mohammadi, J. A. Schmidt, T. J. Ley, J. E. Payton.
Washington University School of Medicine, Saint Louis, MO,

Relevance: The IDH1 and IDH2 genes have recently been demonstrated to contain one of several somatic substitution mutations at codon 132 (IDH1) and codons 140 and 172 (IDH2) in 10-20% of acute myeloid leukemia (AML) cases and 70% (IDH1) and 5% (IDH2) of grades II and III oligodendrogliomas and astrocytomas. In AML, these mutations have been associated with poorer prognosis (decreased 5-year disease-free survival, fewer patients achieved complete remission). In brain tumors, however, the mutations are associated with increased survival. In addition, although undetectable during remission, previously detected IDH1 mutations were again detected upon AML recurrence, suggesting that the mutation may be a useful clinical marker for detecting disease relapse. In both glioma and AML, knowledge of the IDH1/2 status will provide a more accurate prognosis and help guide therapeutic strategies.
Objective: Our goal was to develop a clinical test for identification of IDH1 and IDH2 mutations in bone marrow, blood, or FFPE brain tumor specimens.
Methods: In order to decrease the number of specimens requiring sequencing, which will decrease cost and turn-around-time, we developed a two-step assay to detect mutations in IDH1 and IDH2. DNA was isolated from 20 formalin-fixed paraffin-embedded (FFPE) grades II and III oligodendrogliomas and astrocytomas and 28 fresh frozen AML blood or bone marrow specimens. We performed melting curve analysis with unlabeled probes surrounding the regions of interest, using a LightCycler 2.0 (Roche) to differentiate normal from mutant IDH1 and IDH2. Specimens with melting curves consistent with the mutant alleles were subjected to direct Sanger sequencing using an ABI 3130 to identify the specific mutations present. Furthermore, all specimens were sequenced to confirm accuracy of the melting curve method.
Results: Our results demonstrate that our method can rapidly and sensitively detect mutations in IDH1 and IDH2 regardless of sample origin (bone marrow, blood, or FFPE) without sequencing wild-type samples. This approach avoids unnecessary sequencing when specimens do not contain mutations of interest, leading to improved efficiency and decreased turnaround time.
Conclusions: Implementation of this test will accurately detect IDH1/2 mutations and help guide diagnosis and treatment of glioma and AML patients.

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Rapid detection of two variants near the *IL28B* locus associated with HCV clearance and treatment response.

R. Melis[1], C. Fauron[1], G. A. McMillin[2], E. Lyon[2], P. Slev[2]. [1]*ARUP Laboratories Institute of Clinical and Experimental Pathology, Salt Lake City, UT,* [2]*ARUP Laboratories Institute of Clinical and Experimental Pathology; Department of Pathology, University of Utah, Salt Lake City, UT,*

Introduction: Hepatitis C (HCV) is a major public health problem affecting 3% of the world population; 80% of individuals infected with HCV become chronic carriers and are at risk for developing cirrhosis and hepatocellular carcinoma. Standard therapy for chronic HCV consists of pegylated- interferon-alpha and ribavirin that results in a sustained virological response (SVR) in only 50% of patients infected with HCV genotype 1. Not only is treatment response variable but the treatment itself is difficult to tolerate. It is therefore critical to identify factors that can enhance prediction to HCV treatment outcome. Recent genome-wide association studies have identified several genomic variants associated with both spontaneous HCV clearance and treatment response. Two variants, rs8099917 T>G and the rs12979860 T>C located up stream from the start codon of the *IL28B* interferon-lambda gene have been shown to have the strongest association, when studied independently. However, no study has investigated the effect of the combined (compound) genotypes.
Objective: The objectives of this study were to develop a genotyping method for simultaneous detection of the rs8099917 T>G and the rs12979860 T>C polymorphisms using real time PCR and dual Fluorescence Resonance Energy Transfer (FRET) probes on the Light Cycler instrument (Roche), and to evaluate the combined frequency of these alleles in Caucasians.
Methods: A multiplexed asymmetric PCR reaction amplifies two fragments of interest 175-bp for the rs12979860 and 273-bp for the rs8099917 in a single tube. The FRET probes, specific to each polymorphism, are designed to hybridize to the amplicons and the temperature at which the probes melt from the amplicon is characteristic for each allele. Because the FRET probes are labeled with different dyes, the melting curves are analyzed simultaneously in different channels on the Light Cycler.
Results and Discussion: 84 DNA samples from the Coriell human variation panel-Caucasians, were analyzed using the Light Cycler assay, and genotypes results were confirmed by mini-sequence (single base extension). The total time required for assaying one run that includes 29 samples plus controls is less than one hour. The frequencies of rs12979860 C and rs8099917 T alleles, both associated with increased likelihood of SVR were of 61% and 77%, respectively. Conversely, the risk alleles rs12979860 T and rs8099917 G were found in 39% and 23% of the samples, respectively. The observed genotype frequencies were consistent with Hardy-Weinberg equilibrium (p = 0.59). Compound genotypes rs12979860 CC and rs8099917 TT; and rs12979860 TT and rs8099917 GG were detected in 39% and 7% of the samples, respectively. Although the two polymorphisms were reported in Caucasians in strong linkage disequilibrium (D^2 = 0.99) the following compound heterozygous genotypes were also detected at the following frequencies: rs12979860 CT- rs8099917 GT (24%), CT-TT (19%), TT-GT (7%), TT-GG (7%) and TT-TT (4%). This is the first time that the frequencies of the compound genotypes have been described.
Conclusion: This assay supports rapid identification of the rs12979860T>C and rs8099917T>G polymorphisms. The detection of these two variants simultaneously should be very useful for further understanding the role of host genetics and response to HCV treatment.

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Relationships among CYP3A4 and CYP3A5 haplotypes, mRNA Expression, CYP3A activity and Atorvastatin response in Hypercholesterolemic Individuals

M. A. V. Willrich[1], F. D. V. Genvigir[1], A. C. Rodrigues[1], S. S. Arazi[1], R. Oliveira[1], M. H. Hirata[1], E. L. Dorea[2], R. D. C. Hirata[1]. [1]*Faculty of Pharmaceutical Sciences - University of Sao Paulo, Sao Paulo, Brazil,* [2]*University Hospital - University of Sao Paulo, Sao Paulo, Brazil,*

Introduction: Polymorphisms in CYP3A4 and CYP3A5 genes have been associated with variations in gene expression and enzyme activity that can modify the metabolism of several drugs, and therefore modify their efficacy and safety. Statins are inhibitors of cholesterol synthesis that are very effective in reducing low density lipoprotein (LDL) cholesterol. Variability of the response to statins have been mainly related to polymorphisms in genes involved in cholesterol homeostasis.
Objective: To investigate the relationship between CYP3A4 and CYP3A5 polymorphisms and mRNA expression, CYP3A enzyme activity and cholesterol-lowering response to atorvastatin.
Material and Methods: One-hundred-and-thirty-nine unrelated individuals with hypercholesterolemia (LDL cholesterol > 160 mg/dL) were selected at the University of Sao Paulo Hospital, Sao Paulo, Brazil and treated with atorvastatin (10mg/day/4weeks). Blood samples were collected for biochemical measurements and DNA and RNA extraction. Morning urine samples were collected to measure cortisol to 6βOH-cortisol ratio (6βOH/Cortisol), a clinical marker to detect CYP3A activity, since the conversion of cortisol to its hydroxy derivative is carried out by CYP3A4/CYP3A5. Serum lipids and apolipoproteins were measured by standard methods using an ADVIA 1650® analyzer. Genomic DNA was extracted by a salting out method and CYP3A4*1B (-290A>G), CYP3A5*3C (6986A>G) and CYP3A5*1D (31611C>T) polymorphisms were analyzed by direct sequencing using the Megabace 1000® automated sequencing system. mRNA levels in peripheral blood mononuclear cells (PBMC) were measured by TaqMan® Real Time PCR. Urine samples were prepared to detect simultaneously 6βOH-cortisol and cortisol by HPLC using a SHIMADZU® LC-2010A HT equipment.
Results: Linkage disequilibrium showed that CYP3A4*1B is linked with CYP3A5*3C (D' 0.7078) and CYP3A5*3C with CYP3A5*1D (D'0.8955). Three groups of subjects were analyzed based on their haplotypes: Group (1) Subjects carrying CYP3A4 functional allele only (CYP3A4 -290AA or -290AG and CYP3A5 6986GG and 31611TT); Group (2) CYP3A5 functional allele subjects (CYP3A4 -290GG and CYP3A5 6986AA/AG - 31611CC/CT); Group (3) Subjects carrying both CYP3A4 and CYP3A5 functional alleles (CYP3A4 -290AA or -290AG and CYP3A5 6986AA/AG - 31611CC/CT). Total cholesterol and LDL cholesterol reduction (%) after atorvastatin treatment was similar among three haplotypes groups (p>0.05). CYP3A4 and CYP3A5 mRNA expression, assessed in PBMC, did not change with statin treatment (p>0.05). CYP3A4 was approximately 4.5 times more expressed than CYP3A5 in all HC individuals (p<0.001).

CYP3A4 mRNA expression was correlated with CYP3A5 expression before (r=0.622 at baseline, p<0.001) and after(r=0.693, p< 0.001) atorvastatin treatment. When haplotype groups were analyzed, no association was established between mRNA expression and lipid reponse (p>0.05). Basal CYP3A activity measured as 6βOH/Cortisol ratio in urine samples presented a high inter-individual variability (84.3%). Differences in these values were not detected after atorvastatin treatment (p>0.05). There was no association of 6βOH/ Cortisol ratio values with haplotype groups (p>0.05) or with lipid levels or atorvastatin response(p>0.05).
Conclusions: These results reveal that despite the presence of genetic variants, CYP3A4 and CYP3A5 mRNA expression and CYP3A activity were not related to lowering-cholesterol response to atorvastatin treatment in vivo. This might suggest that the explanation of variability to statin response involves other genes that take part in the complex metabolism of statins.

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Association between urinary morbidity / erectile dysfunction induced by radiotherapy and SNPs of radiosensitivity-relevant genes in prostate cancer patients

Y. Zhu, D. Marshall, E. Garrett-Mayer, D. Zhou, J. Jenrette. *Medical University of South Carolina, Charleston, SC,*

Background: Radiotherapy is an effective treatment modality for localized prostate cancer, but it may cause normal tissue injuries such as urinary toxicity and erectile dysfunction. Increasing evidence indicates that there is significant inter-subject variability in the adverse radiotherapeutic effects (AREs), which may be due to single nucleotide polymorphisms (SNPs) of genes involved in DNA repair, cell growth regulation, cellular antioxidant defense, and telomere maintenance. The objective of this study is to preliminarily investigate the association between radiation-induced urinary morbidity/erectile dysfunction and 6 SNPs of radiosensitivity-relevant genes in prostate cancer patients.
Methods: We recruited 31 patients treated with external beam radiotherapy. Genomic DNA was extracted from blood cells using QIAamp® DNA Blood Mini Kits (QIAGEN). Six SNPs of radiosensitivity-relevant genes including TGF□1 -509C>T, ATM 5557G>A, XRCC1 399Arg>Gln (28152G>A), XRCC3 241Thr>Met (18067C>T), SOD 16Val>Ala (47T>C), and hTERT -245C>T were genotyped using TaqMan SNP genotyping assays (Applied Biosystems). The incidences of urinary morbidity graded according to Merrick dysuria severity scores (MDSS) and American Urology Association (AUA) prostate symptom scores and erectile dysfunction graded based on Mount Sinai Erectile Function (MSEF) scale were extracted from patient charts. Fisher's exact test was used to compare the incidences of urinary morbidity / erectile dysfunction between patients with wild-type and variant alleles.
Results: The proportions of patients with variant SNPs (heterozygous and homozygous variant) of TGFβ1 -509C>T, ATM 5557G>A, XRCC1 399Arg>Gln (28152G>A), XRCC3 241Thr>Met (18067C>T), SOD 16Val>Ala (47T>C), and hTERT -245C>T were 0.50, 0.09, 0.41, 0.53, 0.69, and 0.94 in these 31 patients. The incidences of AREs were 0.16, 0.10, and 0.26 respectively according to MSEF, MDSS, and AUA prostate symptom scores. The incidences of AREs in the groups with wild-type (CC) and variant (CT and TT) TGF β -509C>T SNP were significantly different (0.00 vs. 0.27, $P = 0.0434$) according to MDSS scores. Furthermore, we found that the incidences of AREs in patients with wild-type (CC) and variant (CT and TT) hTERT -245C>T were 1.00 and 0.10 respectively based on MSEF scores, and the difference is statistically different ($P = 0.0215$). In addition, the incidence of AREs determined by AUA scores in patients with wild-type XRCC3 (18067 C > T) (CC) was 0.43, while in patients with variant XRCC3 (CT) it was 0.12, although the P value did not reach statistically significant level of 0.05 ($P = 0.0971$).
Conclusions: The preliminary results show that the T allele of TGF β1 -509C>T SNP is associated with urinary morbidity and the T allele of hTERT -245C>T SNP is associated with erectile dysfunction resulting from radiotherapy in prostate cancer patients. No significant association was found between urinary morbidity / erectile dysfunction and other SNPs. Since the sample size was small in this preliminary study, further study is needed to determine the relationship between urinary morbidity / erectile dysfunction and these SNPs in prostate cancer patients treated with radiotherapy.
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Development of PCR-microplate hybridization assay for detection of *Mycobacterium tuberculosis*

J. Kim[1], I. Lee[1], Y. Cho[1], E. Cho[2], S. Cho[3], H. Lee[1], T. Kim[1]. *[1]College of Health Science, Yonsei University, Wonju, Korea, Republic of, [2]International Tuberculosis Research Center,, Masan, Korea, Republic of, [3]College of Medicine, Yonsei University, Seoul, Korea, Republic of,*

While tuberculosis caused by *Mycobacterium tuberculosis* (MTB) still remains to be the most dreadful infectious disease affecting almost every country in the world, infections caused by non-tuberculosis mycobacteria (NTM) also have become increasingly widespread in recent years. In order to treat patients effectively, the chemotherapeutic regimens used for diseases due to NTM should be different from that for MTB. In the present study, we have developed a simple and rapid detection assay for differentiation of MTB from NTMs using PCR-microplate hybridization assay. In order to develop microplate hybridization assay which can detect MTB as well as differentiate MTB from NTM simultaneously, a selective region of the *rpo*B gene was used to design PCR primers, and MTB-specific (MTB probe) and Mycobacterium genus-specific probe (Myc probe) molecules. The specificity of the microplate hybridization using newly designed PCR primers and probe molecules were confirmed using 15 mycobacterial reference strains including MTB and 12 non-mycobacterial reference strains. The cut-off value used was background signal value + 3 standard deviations. The background signal value corresponded to the mean absorbance measured in a well that contained specific probes and PCR negative conrol products. Accordingly, the cut-off value was set at 0.2. The results from this study suggested that two specific probes used in this microplate hybridization assay could specifically detect MTB and differentiate it from NTM as well. To determine the sensitivity of MTB-specific probe, serial 10-fold dilutions of reference strain of MTB genomic DNA were used for PCR amplification. The PCR products were then visualized both using agarose gel electrophoresis and microplate hybridization assay. The results showed that while agarose gel electrophoresis detected PCR products with 1 pg of genomic DNA, the microplate hybridization assay detected PCR products with 100 fg of genomic DNA. These results suggest that the microplate hybridization assay can detect the specimens which could have been false negative by agarose gel electrophoresis assay. Subsequently, the performance of microplate hybridization assay was evaluated using clinical specimens obtained from Masan National TB Hospital in south Korea. A total of 62 sputum samples with diverse smear test results and MTB confirmed culture positive results were used in this study. In brief, the specificity and the sensitivity of the assay were 100% and 98.4%, respectively. In conclusion, the microplate hybridization assay developed in this study seems to be highly specific and sensitive for detection of MTB. Since the assay was shown to be more sensitive than conventional PCR-agarose gel electrophoresis assay, it may be an alternative new sensitive method for detecting MTB with direct specimens such as sputum.

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Comparison of REBA HPV-ID with DNA Chip Based Assay for HPV Genotyping

S. Kim[1], D. Lee[2], S. Park[1], G. Park[2], T. Kim[1], H. Lee[1]. *[1]College of Health Science, Yonsei University, Wonju, Korea, Republic of, [2]College of Medicine, Yonsei University Wonju Christian Hospital, Wonju, Korea, Republic of,*

Human papillomaviruses(HPV) have been known to cause human cervical cancer which is the second most common cancer in women worldwide. The HPV prevalence classified by the disease severity varies, and more than 100 genotypes of HPV have been identified. For this reason, HPV genotyping rather than mere detection of HPV has been recommended and thus, the test volume for HPV genotyping has been rapidly increasing. Currently, many genotyping tests kits in clinical settings have been commercially available and evaluated to be valuable for clinical diagnosis. This study was set to evaluate a new HPV genotyping kit, REBA HPV-ID from M&D (Wonju, Korea) for its usefulness for genotyping HPV using 356 clinical specimens and the results were compared with that of DNA chip. HPV samples and liquid based cytological smears were obtained from 356 patients attending the Yonsei University Wonju Christian Hospital in South Korea. Cytological diagnoses were interpreted by the pathologists and were classified by 2001 Bethesda System terminology. HPV genotyping using REBA HPV-ID and MyHPV DNA Chip were carried out and sequencing analysis was performed with total seven samples for conformational test. The overall positivity of REBA HPV-ID (80.86%) was higher than which of MyHPV DNA chip (69.75%) using clinical specimens with abnormal cytological interpretation. REBA (64.44%) has about 2 fold higher positivity than DNA chip (34.44%) with normal cytologic diagnosis. To confirm the accuracy and reliability of two methods, sequencing analysis was performed. Five out of seven samples sequenced clearly to interpret data and these results were corrected with REBA HPV-ID. Therefore, these data suggest that REBA HPV-ID has higher sensitivity (72.42%) and accuracy than

MyHPV DNA Chip (28.57%). Recently available HPV genotyping kit, REBA HPV-ID has several advantages over a DNA chip based HPV genotyping method. First of all, REBA does not require expensive equipment such as scanner for detecting DNA chip. Second, it is more suitable for the laboratory with large volume of tests and reducing process time. Sequencing results show that REBA HPV-ID was more reliable and it has 2 fold higher sensitivity.

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Blood transcriptome profile and antiplatelet drug response studies in patients with acute myocardial infarction

A. D. Luchessi[1], V. N. Silbiger[1], R. D. C. Hirata[1], L. G. Lima-Neto[1], A. Carracedo[2], M. Brión[3], A. Iñigues[4], G. Bastos[5], M. S. Bravo[5], A. G. M. R. Souza[6], M. H. Hirata[1]. *[1]Universidade de São Paulo - Faculdade de Ciências Farmacêuticas, São Paulo, Brazil, [2]Grupo de Medicina Xenómica - Universidade de Santiago de Compostela - CIBERER, Santiago de Compostela, Spain, [3]Grupo de Medicina Xenómica - Universidade de Santiago de Compostela - CIBERER; Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain, [4]Hospital do Meixoeiro, Vigo, Spain, [5]Hospital Meixoeiro, Vigo, Spain, [6]Instituto Dante Pazzanese do Estado de São Paulo, São Paulo, Brazil,*

Background: Some patients with acute myocardial infarction (AMI) who undergo coronary angioplasty have low response to antiplatelet drugs such as acetilsalicilic acid (AAS) and clopidogrel. This condition maybe partially explained by differences in drug pharmacokinetics and pharmacodynamics, including the pharmacogenetic profile.
Objetctives: To investigate the relationship between response to antiplatelet drugs and global mRNA gene expression in peripheral blood cells (PBC) in patients with AMI.
Methods: Twenty-six AMI patents who undergo percutaneos coronary intervention (PCI) were recruted from Hospital doMeixoeiro, Vigo, Spain. Patients were treated crhonically with AAS (100 mg/day) and clopidogrel (75 mg/day). Blood samples were drown before PCI to evaluate platelet reactivity and RNA extraction. Platelet reactivity was measured using VerifyNow AAS for AAS and P2Y12 for clopridogrel assays (Accumetrics, USA) kits. RNA was extracted from PBC using PAXgene Blood RNA kit (Qiagen, USA) and the automated Qiacube system (Qiagen, USA). Transcriptome analysis was carried out using the Affymetrix GeneChip Human Exon 1.0 ST array (Affymetrix, USA). Signals were quartile-normalized by the Partek genomic suit software (Partek incorporated, USA). Functional interpretation in terms of biological process, molecular interaction and disease processes were performed by the Ingenuity Systems software v. 8.4 (Ingenuity, USA).
Results: Platelet response to clopridogrel (PRU) and to AAS (ARU) values were categorized in quartiles. For clopidogrel, PRU quartile 1 (Q1) and 4 (Q4) were 121±42 and 301±46, respectively. For AAS, ARU values were 384±6 and 504±34, in the Q1 and Q4, respectively. After normalization, filtering and statistics analysis of transcriptomic data, 29 genes were differentially expressed, with 1.2 fold change, between Q1 and Q4 ($p<0.01$) PRU values. For ARU data, 117 genes were differentially expressed between (Q1 vs Q4 ($p<0.01$). Functional analysis showed that 17 genes were differently expressed (11 upregulated and 6 downregulated) when ARU Q1 and Q4 quartiles values were compared ($p<0.01$). Two genes were upregulated and 7 were downregulated by functional analysis after comparison of the PRU Q1 and Q4 quartile data. **Conclusion:** These results suggest that differences in platelet responses to AAS and clopidogrel influence gene expression in PBC, and transcriptome analysis of antiplatelet drugs may provide evidences for new markers useful for monitoring antiplatelet therapy in AMI individuals undergo angioplasty.

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Evaluation of the performance of the PCR-RFMP HPV genotyping by comparison with direct sequencing

Y. Cha[1], J. Lee[2], T. Um[3], E. Lee[4], H. Chi[5]. *[1]College of Medicine, Chung-Ang University, Seoul, Korea, Democratic People's Republic of, [2]Korea Cancer Center Hospital, Seoul, Korea, Democratic People's Republic of, [3]Inje University College of Medicine and Ilsan-Paik Hospital, Seoul, Korea, Democratic People's Republic of, [4]Green Cross Reference Laboratory, Seoul, Korea, Democratic People's Republic of, [5]University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea, Democratic People's Republic of,*

PCR and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry based HPV genotyping assay, called PCR-RFMP, has been recently developed and used in a clinical laboratory in Korea. We compared the PCR-RFMP with direct sequencing to evaluate its performance and clinical usefulness. Five concentrations of standards (0.25, 2.5, 25, 250, and 2,500 copies/uL) for HPV 16, 18, and 11 were constructed using ATCC DNA for determining the limit of detection (LOD) of both assays. 423 Cervical liquid-based cytology (ThinPrep) specimens were chosen to be able to compare the two HPV genotyping assays, PCR-RFMP and sequencing. Cervical smears from 423 women enrolled in this study were diagnosed as 'normal' in 50, 'ASCUS (atypical cells of undetermined significance)' in 100, 'LSIL (low-grade squamous intraepithelial lesion)' in 157, 'HSIL (high-grade squamous intraepithelial lesion)' in 92, and 'SCC(squamous cell carcinoma)' in 24 smears. Probit assay showed the LOD of PCR-RFMP and sequencing were 18 copies/uL, 109 copies/uL for HPV type 16, 14 copies/uL, 80 copies/uL for HPV type 18, 294 copies/uL, 1987 copies/uL for HPV type 11, respectively. Concordant rate between PCR-RFMP and sequencing were 91.7% (kappa value, k=0.855) in total 423 specimens, 88.0% (k=0.787) in 50 normal specimens, 89.0% (k=0.833) in 100 ASCUS, 91.1% (k=0.838) in 157 LSIL, 94.6% (k=0.646) in 92 HSIL, and 91.7% (k=0.760) in 24 SCC specimens. In summary, PCR-RFMP is more sensitive than sequencing for detection of HPV subtypes and the typing result was concordant with the result by sequencing, especially in higher grade smears such as LSIL, HSIL and SCC. This results show that PCR-RFMP is a sensitive and accurate method for HPV genotyping.

B-123

Molecular Diagnosis using Real-time Nucleic Acid Sequence Based Amplification for detection of Norovirus

Y. Cho[1], J. Kim[1], D. Choi[1], I. Lee[1], H. Jeong[2], D. Chun[2], H. Lee[1], T. Kim[1]. *[1]College of Health Science, Yonsei University, Wonju, Korea, Republic of, [2]National Institute of Health, Seoul, Korea, Republic of,*

Noroviruses (NoroV), members of the *Caliciviridae* family, contain a positive-sense, single stranded RNA genome of approximately 7.6 kb. These viruses are the major cause of nonbacterial gastroenteritis in humans worldwide, resulting in acute diarrhea, nausea and vomiting. The NoroV are genetically diverse and classified into five distinct genogroups(GI, GII, GIII, GIV, GV) based on sequence comparison of the RNA polymerase and capsid region of the genome. It has been reported that G1 and G2 of the genogroups mainly cause the disease which relates with the human. Since NoroV cannot yet be cultured *in vitro* and their diagnosis by electron microscopy requires at least 10^6 viral particles/g of a variety of molecular detection techniques represents an important step towards the detection of NoroV. In the present study, we have developed a simple and rapid detection assay for detection of NoroV using real-time nucleic acid sequence-based amplification (real-time NASBA). In order to develop real-time NASBA assay which can detected NoroV GI and GII, a selective region of the genes encording the capsid protein was used to design PCR primers and genotype-specific molecular beacon probes. The specificity of the real-time NASBA using newly designed PCR primers and genotype-specific molecular beacon probes were confirmed using standard viral RNA of NoroV GI and GII. The cut-off value used was background signal value + 3 standard deviations. The background signal value corresponded to the mean signal value measured in a well that contained specific molecular beacon probes and negative control RNA. Accordingly, the cut-off value was set at 1.6. The results from this study suggested that two specific molecular beacon probes used in this real-time NASBA assay could specifically detect NoroV GI and GII, respectively. To determine the sensitivity of this assay, serial 10-fold dilutions of standard viral RNA of NoroV GI and GII were used for reverse transcription(RT)-PCR and real-time NASBA. The results showed that while agarose gel electrophoresis could detect RT-PCR products with 10 pg of standard viral RNA, the real-time NASBA assay could detect 100 fg of standard viral RNA. These results suggest that the real-time NASBA assay has much higher sensitibity than conventional RT-PCR assay and can detect the specimens which could have been false negative by RT-PCR.

B-124

Genetic variants of transcription nuclear factor (NF)-kappaB and atopic diseases

J. He[1], P. Pare[1], D. Daley[1], A. Becker[2], M. Chan-Yeung[1], B. Tripp[1], D. Zamar[1], A. Kozyrskyj[3], B. Musk[4], A. James[4], C. Laprise[5], A. Sandford[1]. *[1]University of British Columbia, Vancouver, BC, Canada, [2]University of Manitoba, Winnipeg, MB, Canada, [3]University of Alberta, Edmonton, AB, Canada, [4]Sir Charles Gairdner Hospital, West Australia, Australia, [5]Univ du Québec à Chicoutimi, Chicoutimi, QC, Canada,*

Background: Allergic (atopic) asthma is a chronic airway disorder characterized by airway inflammation and airway hyperresponsiveness (AHR). Numerous studies have been performed to investigate potential associations of genetic variants of inflammatory cytokine and chemokine genes with atopic diseases. Expression levels of many of these genes are controlled by nuclear transcription factor (NF)-kappaB (NFB). Studies have demonstrated that inhibition of NFB activation is associated with the attenuation of atopic diseases. However, genetic association of NFB with atopic diseases has not been reported. Objective: The aim of this study was to evaluate the relationship between single nucleotide polymorphisms (SNPs) of NFB genes *NFBIA*, *NFBIB*, and *NFBIE* and asthma and related phenotypes.

Methods: We selected 20 SNPs and genotyped 5,565 individuals from four independent asthma studies and tested for association with asthma, atopy, atopic asthma and AHR. The study group consisted of: 1) the Canadian Asthma Primary Prevention Study (CAPPS), 2) the Study of Asthma Genes and Environment (SAGE), 3) a French Canadian founder population study from the Saguenay-Lac St. Jean and Quebec (SLSJ) and 4) a population based sample from Australia the Busselton Health Study (Busselton study). Genotyping was performed with the Illumina Bead Array System and a genotyping concordance of 99.995% was achieved from a set of 372 replicate samples. A general allelic likelihood ratio test was performed for each cohort as well as a joint analysis of family-based and case-control samples. P values were corrected for the effective number of independent SNPs at each gene as well as the effective number of phenotypes.
Results: In the joint analysis of all four cohorts, rs3138052 in *NFBIA* was associated with atopic asthma (corrected P = 0.034). In analysis of each cohort separately, the following genotype-phenotype associations were observed after correction for multiple comparisons. Three SNP in *NFBIA* rs10782383, rs3138052 and rs2233419 were associated with atopic asthma in the SAGE study (corrected P = 0.012 - 0.030); two SNPs in *NFBIA* rs10782383 and rs7152826 were also associated with asthma (corrected P = 0.013 and 0.050, respectively) in the SAGE study. The *NFBIB* SNP rs3136641 was associated with atopy in the CAPPS study (corrected P = 0.026); *NFBIE* SNP rs520639 was associated with atopy in the Busselton study (corrected P = 0.041).
Conclusions: Genetic variants in *NFBIA*, *NFBIB*, and *NFBIE* were associated with atopy and atopic asthma. This research identified genes potentially involved in susceptibility to allergic disease and these genes might be targets for therapeutics in future studies.

B-125

Association of variant in NNMT with smoking behaviour

C. L. Brasen[1], T. L. Nielsen[1], B. Abrahamsen[2], L. Christiansen[3], C. hagen[1], M. Andersen[1], K. O. Kyvik[3], T. I. A. Sørensen[4], K. Brixen[1], L. bathum[5]. *[1]Odense University Hospital, Odense C, Denmark, [2]Gentofte Hospital, Hellerup, Denmark, [3]University of Southern Denmark, Odense C, Denmark, [4]Copenhagen University Hospital, Copenhagen, Denmark, [5]Slagelse Hospital, Slagelse, Denmark,*

Introduction Smoking behaviour has been shown to be partially genetically determined. Associations have been shown between smoking behaviour and genes involved with the effects of nicotine and a single gene involved in the metabolism of nicotine. We hypothesized, that variations in nicotinamide N-methyltransferase gene (NNMT) could have implications for nicotine metabolism and thereby smoking behaviour. We investigated a polymorphism (dbSNP: rs694539) located in intron 1 in NNMT for association with smoking behaviour.
Methods Using Real-Time PCR we genotyped 783 healthy men aged 20-29 years from the Odense Androgen Study and replicated the findings in 1,206 healthy twins aged 18-65 years from the GEMINAKAR-study, respectively. We then investigated the possible association with smoking behaviour in terms of current smoker status, current cigarette consumption, ever-smokers and number of pack-years in the two cohorts We used logistic regression, first unadjusted and then adjusted for age, alcohol consumption and education level.
Results In Odense Androgen Study we found an odds ratio of 1.37 for smoking for individuals having two G alleles. This was significant in unadjusted (p=0.048; CI: 1.00-1.94) but failed to achieve significance in the adjusted analysis (p=0.075; CI: 0.97-1.95). In GEMINAKAR in the group of women maximum 38 years of age, we found significant association for smoker (p=0.045) and trend association for ever-smoker (p=0.064). Although the young men show a trend for both smoker and ever-smoker, this failed to test significant (p=0.456). Using logistic regression, adjusting for gender, alcohol consumption and education, we found an odds ratio of 1.80 (p=0.045; CI: 1.01-3.19)) for smoking for individuals in the young female group having two G-alleles, but not in the other groups. No associations were found for the old group.
Discussion The results from these two independent population-based cohorts indicate that the investigated NNMT-polymorphism is associated with smoking behaviour in young individuals. The sizes of the OR's in the two cohorts are of significance in genetics of smoking behaviour and the NNMT genotype could be an important factor in determining the best method for smoking cessation for an individual.

B-126

Acetylator phenotype and genotype polymorphisms of N-Acetyltransferase-2 in Indian patients on Anti Tubercular Treatment

R. P. ola, S. V. Rana, s. K. sharma, S. K. sinha, S. K. arora, P. pandhi, K. singh. *PGIMER, chandigarh, India,*

Introduction: Isoniazid is one the important drugs in anti tubercular treatment regime and it cause hepatotoxicity.
Aim: To compare whether genotype pharmacogenetic test N-acetyltransferase-2 (NAT2) and phenotype test of measuring INH in serum are useful to identify acetylator status of patients on anti tubercular treatment.
Materials and Methods: In 251 patients on standard treatment for tuberculosis (TB), isoniazid induced hepatotoxicity was diagnosed. NAT2 genotype was assessed by PCR with restriction fragment length polymorphism (RFLP) and serum INH levels were measured by flourometery.
Results: 251 TB patients (153 males, mean age 44.6 ± 3.7 years) with a body mass index (BMI) of 18.9 ± 3.7 kg/m^2 were studied. Out of 251 patients, 50 (19.9%) developed hepatotoxicity. In hepatotoxicity group, on phenotypic estimation 17 out of 50 (34%) were slow acetylators while 33 fast acetylators. Genotypically 19 out of 50 (38%) were slow acetylators and 31 fast acetylators. In the non-hepatotoxicity group, on phenotypic analysis 46 out of 201 (22.9%) were slow acetylators & 155 fast acetylators, and on genotypic analysis 30 out of 201 (14.9%) were slow acetylators & 171 fast acetylators. Both the methods showed significantly higher slow acetylators in hepatotoxicity group. Slow acetylators (25.1%) measured phenotypically were not significantly different as compared to slow acetylators (19.5%) measured genotypically.
Conclusion: This study suggests that the acetylator status of TB patients can be detected as efficaciously by phenotypic method as by genotypic method. Therefore, phenotypic method may replace genotypic method as it is simple and inexpensive.

B-127

Hepatocyte Growth Factor Gene Polymorphism in Patients with Kidney Stone

N. Ozturk[1], H. Aksoy[1], Y. Aksoy[2], A. Yildirim[1], V. Yanmaz[1], F. Akcay[1]. *[1]Ataturk University, Medical School, Department of Biochemistry, Erzurum, Turkey, [2]Ataturk University, Medical School, Department of Urology, Erzurum, Turkey,*

Nephrolithiasis is a complex and multifactorial disorder. The environmental and genetic factors are important in development of this disease. In a recent study, it was demonstrated that hepatocyte growth factor (HGF) have an anti-apoptotic effect and thus it can reduce of adhesion of calcium oxalate monohydrate crystals to renal epithelial cells. The aim of this study was to evaluate the possible association of HGF polymorphism with nephrolithiasis. One-hundred and five patients with nephrolithiasis and 70 healty volunteers with similar demographic features were included to this study. HGF intron 13 C>A (in 104 stone patients and 68 healthy subjects) and intron 14 T>C (in 99 stone patients and 56 healthy subjects) polymorphisms were determinated using real time-polymerase chain reaction with TaqMan allelic discrimination method. Additionally, we measured serum HGF levels in two groups. There were no significant differences in terms of HGF intron 13 C>A and intron 14 T>C polymorphisms between the control and patient groups (chi-square=1.71 df=2; p=0.42, and chi-square=0.68 df=2; p=0.71, respectively). Mean serum HGF concentrations were significantly lower in the stone disease patients than in control subjects (1045.65±625.60 pg/mL and 1345.04±578.29 pg/mL respectively, p<0.0001). When compared the allele distribution frequency between stone patients and healthy subjects, there were no significant differences in intron 13 and intron 14 allele distributions between two groups (p=0.43 and p=0.44, respectively).
It may be suggested that decrease in HGF levels has a role in renal stone formation independently from these gene polymorphisms.

B-128

Analysis the variations of Dystrophin gene in 39 DMD/BMD patients by multiple ligationprobe amplification(MLPA)

J. Wang, Y. Zhou, B. Ying, X. Nie, L. Zhang, X. Lu, L. Wang, L. Ding, X. Song, B. Dai, J. Zhuang, L. Lin, Y. Ye, X. Liu. *West China hospital of Sichuan university, Chengdu, China,*

Background: Dystrophin-associated muscular dystrophies range from the severe Duchenne muscular dystrophy (DMD;OMIN:310200) to the milder Becker muscular dystrophy (BMD; OMIN:300376). DMD/BMD are an X-linked disorders caused by mutations in the DMD gene which is composed of 79 exons encoding a 14-kb mRNA. Most of DMD/BMD causative variations are contiguous and single exonic deletions and duplications (60%-65%, 5-10% respectively).This study aims to analysis the gene variations in clinic DMD/BMD patients using the novel multiplex ligation-dependent probe amplification (MLPA) method which has been proved accurate and effective.
Method: The 39 clinic patients with DMD/BMD and as well as the 6 normal unrelated male controls were enrolled in this study. Genomic DNA was extracted from the peripheral blood of the patients with DMD/BMD and also normal male controls using QIAamps DNA Blood Mini Kit (Qiagen Inc. Hilden Germany). The MLPA DMD/BMD test kit (SALSA P034/035) was obtained from MRC-Holland,Amsterdam, The Netherlands. The kit contains one probe for each of the 79 DMD exons of the dystrophin gene and

in addition, a probe for the alternative exon 1 (Dp427c). These 80 probes have been divided in two probe mixes P034 and P035. For the individual analysis 100-200 ng DNA was used. Further analysis was done following the manufacturer's protocol. Final PCR products were analyzed on a capillary sequencer (Applied Biosystems 3130). The raw data were analyzed using Coffalyser 9.0 (MRC-Holland).And single exon variation and point mutation confirmed by DNA sequence.
Result: Eight samples of the subjects were found having contiguous deletions (46%), 5 samples having a single exon deletion(13%),5 samples having contiguous duplications(13%), one sample having a non-sense mutation((2%),and the remaining was not found significant variation(26%).The frequency of variations are similar to the previously research. Three of the mutations(exon11-41dup, exon7-9del and NM_004006.1 c.2704 C>T NP_003997.1 p.Q902X,) were first reported ,comparing to an online DMD variations database,http://www.dmd.nl. Furthermore, of all the exonic deletions and duplications found in this study, nine mutations were in frame and twenty mutations were out-of frame(31%,79%,respectively). Most 5' breakpoints of the exonic variations located in the 2 hotspots (major hotspot, exon45-52,16/29,55%; minor hotspot, exon2-19,10/29,34%, respectively),however, the proportions of 3' breakpoints located in the 2 hotspots were found significant decrease(major hotspot, exon45-52,11/29,38%; minor hotspot, exon2-19,4/29,14%, respectively).that is corresponding to other's studies from the globe.
Conclusion: MLPA is an effective and cost effective method to analysis gene dosage, it can be used to detect both deletion and duplication of ~40 particular genome sequences simultaneously . We recommend that MLPA should be applied as the first line for clinical molecular testing for DMD/BMD and other deletion/duplication causative genetic disorders.

B-129

Branched DNA based quantitative detection of plasma Alu DNA in severe acute pancreatitis

L. Zhang[1], L. Yin[2], M. Zhang[2], L. Zhang[2], P. Okunieff[2]. *[1]Department of Laboratory Medicien, West China Hospital of Sichuan University, Chengdu, China, [2]Department of Radiation Oncology, University of Rochester School of Medicine and Dentistry, Rochester, NY,*

Background: Plasma DNA, also called cell free DNA, has been well explored as a biomarker of cell death and damage in numerous physiological and pathological conditions. Severe acute pancreatitis is characterized by pancreatic cellular disruption, necrosis and accelerated apoptosis. Nevertheless, there is relatively paucity of investigation on the plasma DNA in patient with severe acute pancreatitis. Various methods of quantitative detection of plasma DNA have been developed and introduced in pervious studies during the past years. However, this is first report of application of branched DNA signal amplification technology in plasma DNA quantification, which circumvents complicated nucleic acid processing procedures. More importantly, targeting Alu DNA as a structural genetic marker of cell death and damage, as well as a functional early responder to cell or gene stresses in plasma, may allow our assay extreme sensitivity and hence further potential in interpreting the biological disorders.
Methods: A new branched DNA technology-based assay targeting the Alu repetitive elements was established for quantitatively detecting cell free DNA directly from plasma. Method evaluation was conducted concerning precision, accuracy, linearity and sensitivity. 40 patients were enrolled in this study according to Atlanta consensus conference criteria (1992) and EDTA-anticoagulated venous blood samples were collected from the patients for analysis on the day of admission. EDTA-anticoagulated venous blood samples were also collected from 44 healthy control subjects matched for sex and age. Plasma samples were obtained by two-step centrifugation for completely eliminating the contamination of cellular DNA.
Results: In the range of DNA concentrations between 1.6 to 200 ng/ml, excellent linearity was demonstrated in two-fold dilution serials of human genomic DNA (Promega Inc.) prepared in Tris EDTA buffer ($R2=0.998$, $P < .05$). In the experiment, signal saturation was observed at the concentration of 250 ng/ml and loss of linear response at low concentration starts at 0.8 ng/ml. In addition, plasma with a high level of plasma Alu DNA from a patient was serially diluted to detect any disruption of the Alu DNA measurement by the plasma matrix. Good linearity ($R2=0.995$, $P < .05$) indicates there is little or no matrix interference, consistent with the relatively small amount of sample that was loaded into the reaction system. The lowest limit of detection, calculated from background measurements, was 0.4 ng/ml. The average within- and between-assay coefficiencts of variance were 12.0% and 16.5%, respectively. The average recovery rate was 107.8%, of spiked DNA into plasma. The median concentration of plasma Alu DNA for patient and control group were 171.2 and 89.5 ng/ml, respectively ($P<0.0001$). Our result is entirely alien from what has previously been reported by other researchers that a decreased level of plasma DNA was found in patients with severe acute pancreatitis compared with healthy control group.
Conclusions: The branched DNA-based assay is reliable, accurate, and sensitive in detecting plasma Alu DNA quantitatively. The content of plasma Alu DNA was detected significantly elevated in patients with severe acute pancreatitis, suggesting that it may serve as a potential diagnostic indicator and risk factor for further patient stratification.

B-130

MMACHC gene analysis by high-resolution amplicon melting and unlabeled probes

q. huang[1], G. H. Reed[1], L. Zhou[1], Y. Wang[1], J. P. Lerner-Ellis[2], N. Anastasio[2], J. Liu[2], D. S. Rosenblatt[2], C. T. Wittwer[1]. *[1]Department of Pathology, University of Utah Medical School, Salt lake city, UT, [2]Department of Human Genetics, McGill University, Montreal, QC, Canada,*

Background and objective: Methylmalonic aciduria and homocystinuria, *cblC* type, is the most common inherited disorder of vitamin B_{12} (cobalamin) metabolism and is caused by mutations in the *MMACHC* gene. More than 60 mutations are known that result in either early-onset or late-onset disease. To rapidly screen for *MMACHC* mutations without sequencing, we applied high-resolution amplicon melting and unlabeled probe analysis.
Methods: All 4 coding exons and flanking intronic regions of the *MMACHC* gene were amplified by PCR under identical conditions. Common variants in a random control panel of 96 DNA samples were identified in each exon by high-resolution melting on a LightScanner®. Unlabeled probes were designed to specifically genotype one polymorphism (rs2275276) and three common mutations (c.271dupA, c.331C>T, and c.394C>T). A blinded study on 55 samples enriched for disease-causing variants was performed.
Results: Two sequence variants were identified in the 96 control DNA samples. One single base variant (rs2275276) in exon 3 was commonly observed (62% G alleles, 38% A alleles). The only other variant detected (rs35219601) occurred in 1 out of 192 alleles. In the blinded study, we found 6 samples with mutations in exon 1, 28 samples with mutations in exon 2, 17 samples with mutations in exon 3, and 22 samples with mutations in exon 4. All of the heterozygous variants and some of the homozygous variants were directly identified by atypical melting curves of a different shape as compared to that of wild type homozygotes. Additional homozygous variants were identified after mixing the amplicons of the unknowns with wild type amplicons. Detection of variants was improved by first grouping the samples according to common variant genotype as determined by unlabeled probes. Unlabeled probes could also be placed over multiple nearby variants to detect and differentiate them by melting temperature. For example c.388T>C, c.388_390delTAC, c.389A>G, c.391C>T, c.394C>T, and c.398_399delAA are close enough to each other that they were all analyzed with a single unlabeled probe.
Conclusion: High-resolution melting methods provide a simple and rapid alternative to sequencing for mutation scanning of the *MMACHC* gene. Common variants and mutations can be specifically genotyped with unlabeled probes. Combined high-resolution amplicon melting and unlabeled probe analysis vastly reduces the sequencing burden of genetic screening.

B-131

Comparison of peripheral lymphocyte gene expression profiles between individuals with low plasma HDL-C and normal plasma HDL-C

K. A. Ford, B. Steffen, V. L. Arends, M. Y. Tsai. *University of Minnesota, Minneapolis, MN,*

Background: Low levels of high density lipoprotein (HDL), or hypoalphalipoproteinemia (HA), are associated with an increased risk of atherosclerosis and cardiovascular disease. Thus far, studies have been unable to fully elucidate the potential genetic etiologies of HA in the majority of individuals with low HDL and relatively low triglyceride levels. In addition, little is understood regarding the putative anti-inflammatory effects of plasma HDL.
Objective: The aim of this study was to compare the gene expression profiles of peripheral lymphocytes of individuals with low HDL and normal HDL to further understand the genetic etiologies of HA.
Methods: Using microarray gene expression profiling of peripheral lymphocytes, mRNA levels were measured from 44 African-American individuals in the Multi-Ethnic Study of Atherosclerosis (MESA) cohort. Gender, age, triglyceride, and BMI matched individuals were sorted into two groups based on HDL-C concentrations---a low HDL group (n=20, 30±2.9 mg/dL HDL-C) and normal HDL group (n=24, 57±10.0 mg/dL HDL-C).
Results: Paired t-test analysis revealed two genes that were significantly lower in the low HDL group compared to the normal HDL group. Both PRKAB2 (AMP-activated, beta 2 non catalytic subunit) and TGFBR3 (transforming growth factor, beta receptor III) mRNAs showed a fold-change of 0.732 and 0.569, respectively. PRKAB2 is an essential subunit of AMPK, a kinase that inhibits cholesterol and triglyceride synthesis. TGFBR3 plays a role in the anti-inflammatory activities of TGF-β by presenting TGF-β ligand to the TGF-β type II receptor.
Conclusion: A reduced expression of PRKAB2 and TGFBR3 mRNA levels was observed

in individuals with low HDL. Future research is needed to determine whether there is a corresponding decrease in their protein levels or protein activities, as well as the clinical significance in terms of HDL metabolism and the development of atherosclerosis.

B-132

Genotyping of the SDHAF2 gene: Mutation Related to Paraganglioma

D. Milosevic, A. Algeciras-Schimnich, S. K. G. Grebe. *Mayo Clinic and Foundation, Rochester, MN,*

Background: Germline mutations in *SDHB*, *SDHC* and *SDHD* genes are frequently associated with paragangliomas and, to lesser degree pheochromocytomas. However, some apparently familial paragangliomas lack mutations in these genes. Recently, mutations in the succinate dehydrogenase complex assembly factor 2 (SDHAF2) gene have been found in some of these cases. SDHAF2 (aka PGL2 or SDH5) is an evolutionary conserved cofactor of flavine adenine dinucleotide (FAD) and is crucial for the functionally indispensable flavination of SDHA, the only component of the SDH multienzyme complex that has thus far not been shown to be directly associated with familial paraganglioma or pheochromocytoma.
Objective: To develop and validate a method for fast, comprehensive, and accurate *SDHAF2* mutation detection.
Methods: Point mutations and small deletions were detected by DNA sequencing of the entire coding region and flanking introns of all four exons of *SDHAF2* gene. Four primer pairs were used to amplify *SDHAF2*, followed by automated cycle sequencing with nested primers and BigDye Terminators (ABI BigDyeTerminator TM v1.1). The sequencing traces were analyzed semi-automatically with Mutation Surveyor® software.
Results: Reproducibility was assessed by running one sample in triplicate on one run and by running one sample in 10 different runs. Results showed 100% concordance. The average sequencing quality score for bidirectional sequencing of SDHAF2 was 38 (range: 10-55) with average intra- and interassay CVs of 15.6% (range: 2.6-38.4%) and 14.2% (range: 5.6-26.3%), respectively. Twelve normal control samples were tested and shown to be free of any disease-causing mutations or deletions. Blinded analysis of 23 different patient samples suspected to have paraganglioma but no mutations in *SDHB*, *SDHC*, *SDHD* and *VHL* genes. DNA samples were successfully amplified and sequenced. No mutations were found.
Conclusion: We have developed and validated full gene sequencing for detection of mutations in *SDHAF2* gene. Genetic testing of SDHAF2 should be considered in patients with head and neck paraganglioma patient with a clear family history, who lack mutations or deletions of *SDHB*, *SDHC* and *SDHD*. Together with *VHL* and *SDHB*, *SDHC*, *SDHD* genotyping, which we recently developed and implemented as clinical assays, addition of SDHAF2 gene mutation testing will improve diagnostic work-up of pheochromocytoma and paraganglioma.

B-133

BDNF Val66Met polymorphism is not associated with coronary atherosclerosis in Czech patients

M. Petrek[1], J. Petrkova[1], A. Stahelova[2], F. Mrazek[2], M. Taborsky[1]. *[1]Faculty Hospital and Palacky University, Olomouc, Czech Republic, [2]Palacky University, Olomouc, Czech Republic,*

Background: Brain-derived neurotrophic factor (BDNF) has been implicated in the pathogenesis of coronary artery disease (CAD). Recently, human *BDNF* Val66Met polymorphism has been associated with CAD in Chinese population [1].
Objective: To further explore possible role of *BDNF* Val66Met polymorphism as a genetic modifier in CAD we have investigated its association with myocardial infarction in the Czech population.
Methods: *BDNF* Val66Met polymorphism was genotyped by real-time PCR in 180 healthy unrelated Czech control subjects and in 217 Czech patients with myocardial infarction diagnosed according to the international (ESC) consensus.
Results: Genotype and allele frequencies of the *BDNF* Val66Met polymorphism did not differ between patients (frequency of *BDNF* 66Met allele: 18%, Met/Met genotype 4%) and control subjects (17%, 5%, p> 0.05). Two investigated groups also did not differ in carriage rates (phenotype frequencies; patients: 31%, controls: 29%) of the *BDNF* 66Met variant.
Conclusions: The *BDNF* Val66Met polymorphism was not associated with myocardial infarction in our Czech population. We could not, therefore, replicate the observation from China [1], which suggested that *BDNF* Met/Met genotype is a genetic modifier in CAD. Investigations in further centres and/or populations [2] are, therefore, necessary to obtain more information on possible role of *BDNF* as a factor of genetic variability in coronary artery disease.
References: [1] Jiang H et al. Clin Chim Acta 2009; 400:3-7; [2] Little J et al. Genet Epidemiol. 2009; 33:581-598.
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B-134

Comparison of DNA sequencing and Autogenomics Infiniti® assay for *KRAS* and *BRAF* mutation testing in cancer

D. French, A. Smith, M. P. Powers, A. H. B. Wu. *University of California San Francisco, San Francisco, CA,*

Background: Binding of a ligand to the epidermal growth factor receptor (EGFR) stimulates various intracellular signaling pathways resulting in cell cycle progression, proliferation, angiogenesis and apoptosis inhibition. *KRAS* is involved in signaling pathways including *MAPK* and *RAS/RAF* and mutations in this gene result in constitutive activation of these pathways, independent of EGFR activation. Seven mutations in codons 12 and 13 of *KRAS* comprise around 95% of the observed mutations, rendering monoclonal antibodies against EGFR (e.g. cetuximab and panitumumab) useless in treatment of colorectal cancer. *BRAF* is a serine-threonine kinase that is mutated in malignant solid tumors, with highest prevalence in melanomas. It is also involved in *MAPK* signaling and activation of this pathway due to mutations in *BRAF* (>90% are codon 600 T1799A) in thyroid cancer and melanoma is common.
Objectives: To compare *KRAS* and *BRAF* mutation testing by two methodologies on DNA extracted from colorectal and thyroid tumors and melanomas.
Methods: DNA was extracted from the tissue of 20 colorectal and 3 thyroid tumors and 3 melanomas. For DNA sequencing, the DNA was amplified by PCR, M13 tails were added and then the DNA sequenced by capillary electrophoresis (Life Technologies, Foster City, CA) specifically for the detection of any mutations in codons 12 and 13 of *KRAS*. The real time PCR assay used dual hybridization probes and melting curve analysis to detect mutations that were then sequenced specifically for the detection of mutations in *BRAF*. Using the Autogenomics Infiniti® assay (Carlsbad, CA), the DNA was amplified by PCR, fluorescently labeled using analyte specific primer extension, hybridized to a microarray and then scanned. The signal from the microarray was detected and analyzed for 20 *KRAS* mutations in codons 12, 13 and 61 and 7 *BRAF* mutations in codon 600. The results from the two methods were then compared.
Results: Out of 20 colorectal tumor samples tested, 19 were concordant between the two methodologies for the *KRAS* mutations that were detected in both assays. In the discrepant patient, a mutation in codon 13 was found by the Autogenomics Infiniti® assay that was not found by DNA sequencing and we are investigating the cause of this. Mutations in codon 61 of *KRAS* were not included in the DNA sequencing method and with the Autogenomics Infiniti® assay we found codon 61 mutations in 2 of the 18 patients. Of 3 thyroid tumor and 3 melanoma tumor samples tested, all were concordant between the two methodologies for codon 600 *BRAF* mutations detected by both assays. Our studies are ongoing. Conclusions: We observed good concordance between the Autogenomics Infiniti® assay and DNA sequencing methodologies for the detection of *KRAS* mutations in colorectal tumor samples and *BRAF* mutations in thyroid tumor or melanoma samples. This study indicates the utility of the Autogenomics Infiniti® methodology in a clinical laboratory setting where DNA sequencing capabilities do not exist.

B-135

Performance evaluation of the AutoGenomics INFINITI CYP450 2C19+ multiplex pharmacogenetic assay

A. C. Smith, A. H. B. Wu, K. F. Lajom. *University of California San Francisco, San Francisco, CA,*

Background: The cytochrome P450 enzyme 2C19 is a member of the mixed-function oxidase system, which is responsible for the metabolism of several groups of drugs. These include antiepileptics, proton pump inhibitors, and anti-depressants. The 2C19 enzyme also metabolizes the prodrug clopidogrel into its active form. This metabolite inhibits platelet aggregation by binding to the P2Y12 receptor. Clopidogrel (Plavix) is used to prevent thrombosis in patients diagnosed with cardiovascular disease. Genetic polymorphisms exist in the 2C19 gene, which result in phenotypes that are poor metabolizers (*2,*3), and ultra-rapid metabolizers (*17). Patients with *2 and *3 genotype have increased rate of major adverse cardiac events at 1 year relative to the wildtype (*1/*1), when treated with clopidogrel. Patients with *17 genotype show improved clinical outcomes relative to the wildtype. Testing for 2C19 may result in changing to a different anticoagulant, or increasing the dose of clopidogrel.
Methods: We evaluated the performance characteristics of the AutoGenomics INFINITI CYP450 2C19+ assay using a cohort of 96 ethnically diverse subjects collected at UCSF. DNA was extracted from EDTA whole blood using the Qiagen QIAamp DNA kit. INFINITI results were compared to bi-directional sequencing, and to the Roche AmpliChip assay. The AmpliChip method is currently the only FDA approved assay for CYP 2C19. The INFINITI multiplex assay is able to detect *2,*3,*4,*5,*6,*7,*8,*9,*10, and *17 mutations. The AmpliChip assay is able to detect *2 and *3 mutations. We also evaluated reproducibility of the INFINITI assay using three lots of reagent for a total of 168 determinations.

Results: Within the 96 subject cohort, only *2,*3 and *17 mutations were detected. The relative frequency of each was 35.4%, 2.1%, and 28.1% respectively. Of this cohort, 4.2% are considered poor metabolizers, having homozygous mutations for the null *2 and 4.2% were ultra-rapid metabolizers (*17/*17). The concordance of the INFINITI assay as compared to sequencing was 100%. Since the AmpliChip does not detect *17 mutations the agreement was 71.9%. All *17 mutation resulted in *1 calls on AmpliChip. The lot to lot comparison yielded 164 correct calls for 168 samples (97.6%). Four samples were "No Calls" on the first attempt. Upon repeat these four samples resulted in correct calls.
Conclusions: We found that our cohort contained a high frequency of *2 and *17 mutations. These mutations can influence dose requirements of drugs metabolized by the 2C19 enzyme. Knowledge of these genotypes may aid in modification of drug dosing for drugs such as clopidogrel, or lead to a switch to a more appropriate medication. The INFINITI CYP450 2C19+ assay performed with very high precision, and was able to detect all relevant mutations.

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Characterization of the INFINITI® Platform for a CYP450 2C19 Genotyping Application

C. Smith[1], I. Mongrain[2], Y. Fu[1]. *[1]AutoGenomics Inc., Vista, CA, [2]Genome Quebec & Montreal Heart Institute Pharmacogenomics Centre, Quebec, QC, Canada,*

Polymorphisms of CYP2C19 enzyme can affect an individual's ability to metabolize a wide range of clinically relevant drugs such as antidepressants, antithrombotic agents, proton pump inhibitors, and xenobiotics. In addition, other factors such as food or other drugs can interfere and disrupt overall therapeutic effectiveness when used in combination. Genotyping for CYP2C19 provides an indication of an individual's enzyme metabolic rates as a guide for proper dosing, thereby minimizing the risk of adverse drug reactions. Recent studies have indicated that one third of Plavix patients with CYP450 2C19 variations have a 3.58 times greater risk for major adverse cardiovascular events[1]. The CYP2C19 variants *2, *3, *4, *5, *6, *7, or *8 result in no enzymatic activity, while *9 or *10, result in decreased activity, while *17 results in increased activity. Common misclassification of *10 for *2 (homozygosity) is often contributed by technical obstacles due to the fact that these two essential SNPs (for the relevant allelic haplotype) are in close proximity to each other, 19154G>A (*2) and 19153C>T (*10). Degenerate primer design often reduces the power of detecting a particular SNP in the presence of an adjacent one. To accurately identify each SNP, the INFINITI® CYP450 2C19 assay has employed all possible combinations in the design of the detection primer set for *2 and *10 (19153_4: CG, CA, TG, and TA) used in the analyte specific primer extension process. Results from all CYP2C19*2 and/or *10 samples tested with the INFINITI® CYP450 2C19 assay were confirmed in a recent collaboration with the Center of Disease Control and Prevention[2] and were in concordance with bidirectional sequencing data without ambiguity.

[1] Plavix May be Relabeled to Recommend DNA Testing. DNA Today 2010, Issue 9, Volume 1

[2] Victoria M. Pratt, Characterization of 107 genomics DNA reference materials for CYP2D6, CYP2C19, CYP2C9, VKORC1 and UGT1A1: A GeT-RM and Association for Molecular Pathology collaborative project. 2010

B-137

Development of an application for the SimpleProbe CYP2C9/VKORC1 assay on the ABI 7500 Fast instrument

M. Al-Ghoul[1], K. K. Reynolds[2], E. A. Hudson[2], M. W. Linder[1], R. Valdes, Jr[1]. *[1]PGXL Laboratories and University of Louisville School of Medicine, Louisville, KY, [2]PGXL Laboratories, Louisville, KY,*

Background: The combination of CYP2C9*2, CYP2C9*3 and VKORC1 genotypes together with patient specific physical characteristics can provide guidance for estimating individualized maintenance doses of warfarin. Recently, Idaho Technology, Inc (IT) developed high precision melting-curve methods based on allele specific SimpleProbe technology for determination of CYP2C9*2, CYP2C9*3 and VKOR C1 -1639G>A alleles. SimpleProbe technology relies on the quenching of fluorescence signal when the allele specific probe is free in solution, thus fluorescence intensity increases proportionally with heteroduplex melting. These reagents have not been optimized or validated for use on the Applied Biosystems, Inc (ABI) 7500 Fast platform.
Objective: Our objective was to identify optimal assay parameters for application on the ABI 7500 Fast instrument and then evaluate these reagents by assessing accuracy and reproducibility.
Methods: Optimization: Assay parameters including, DNA template concentration, total assay volume, temperature cycling parameters and melting rate were initially derived from manufactures recommended procedures for application on the LightScanner. Evaluation: Twenty-two anonymous human DNA samples previously genotyped using the Luminex xTAG CYP2C9/VKOR C1 assay were used as known samples for the evaluation.
Results: Of the assay parameters listed above, only modification of the melting rate analysis was required to yield optimal assay performance. The manufacturer recommended rate of lower than 0.3 degrees/ sec was modified to 0.1 degrees per second (1% ramp), resulting in better separation of allele specific melting curves. Modification of melting temperature ramp rate yielded excellent separation of melting peaks for each gene variant of interest. Melting temperatures for CYP2C9 430 C, 50.8± 0.50 vs 430 T 58.26 ± 0.15; CYP2C9 1075 A, 49.3 ± 0.78 ºC vs CYP2C9 1075 C 60.03 ± 0.8 ºC; VKORC1 -1639G 54.07 ± 0.54 vs VKORC1 -1639A, 64.26 ±0.40. Reproducibility in melting point was evaluated for each gene variant of interest. There was a difference of 7.4 ºC ± 1% between CYP2C9 C vs T, 10.73 ºC ± 0.6 % between CYP2C9 1075 A vs C, and 10.19 ºC ± 1% between VKORC1-1639G vs A. There was 100% concordance between the CYP2C9 and VKORC1 genotypes determined by the IT reagents on the ABI 7500 Fast and Luminex xTAG warfarin assay. In order to evaluate possible interference by other known variants, two of the samples analyzed possessed rare CYP2C9 SNPs. One sample was heterozygous for the CYP2C9 429C>T which is known to interfere with the detection of CYP2C9*2 in some assays. The second sample was a compound heterozygote with the CYP2C9*3/*6 genotype. Neither of these samples generated peaks in the CYP2C9*2 IT assay and the *3/*6 only resulted in a peak at CYP2C9*3, as expected. The IT reagents on the ABI 7500 Fast platform take approximately 2 h for 33 patient DNA samples to be genotyped.
Conclusion: Minor modifications of the IT (CYP2C9/VKORC1) assay were required for running on the ABI 7500 Fast platform. This method demonstrated excellent allele discrimination, precise allele specific melting temperatures and 100% concordance with the existing technology currently in use in our laboratory.

Tuesday PM, July 27

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

B-138

Establishment of a differential biomarker signature for muscle diseases

K. Ohlendieck, L. Staunton, C. Lewis, E. Mullen, S. Carberry. *National University of Ireland, Maynooth, County Kildare, Ireland,*

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

B-139

Analytical validation of the Abbott Architect urine NGAL.

E. Cavalier[1], D. Saarbach[1], A. Bekaert[1], A. Carlisi[1], J. Chapelle[1], P. Delanaye[2]. *[1]Department of Clinical Chemsitry, University Hospital of Liège, University of Liège, Liège, Belgium, [2]Department of Nephrology, University Hospital of Liège, Liège, Belgium,*

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

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

B-140

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

N. Q. Muhamadiev[1], B. B. Mahmudov[2], S. M. Sayitkulov[1]. *[1]Samarkand State University, Samarkand, Uzbekistan, [2]Samarkand State Medical Institute, Samarkand, Uzbekistan,*

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

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

B-141

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

Y. Itoh[1], I. Kawabata[1], N. Kubota[2], K. Sakabe[2], T. Notomi[2], H. Enomoto[2].
[1]Asahikawa Medical College, Asahikawa, Hokkaido, Japan, [2]Eiken Co. Ltd., Tokyo, Japan,

Urine albumin is a well-established marker for diabetic nephropathy. Recent clinical interests are focused on a mortality and morbidity maker for cardiovascular and neurovascular events. Elevation of minute change of albumin may be of clinical significance for early detection and prevention of all above pathophysiologic changes.
We newly developed a novel latex-enhanced turbidimetric immunoassay for low-grade albuminuria. 4 different clones of mouse monoclonal antibody were used. All the procedure for value assignment for HSA in calibrator was performed by weighing. The assigned value was confirmed to be very close to that assigned from ERM DA-470. 0.006 ml of samples was reacted with optimal concentration of antibody-attached latex solution incubating at 20°C for 122 seconds. The detection limit was 0.16 mg/L. An actual analytical range was 0.16-25 mg/L. By setting parameter, it can be extended to approximately 100 mg/L. Three different concentrations of urines were measured for 10 consecutive times until days 14. Intra-assay CVs were 0.5, 0.5, and 0.4 %, respectively, while inter-assay CVs were 1.2, 0.3, and 0.3%, respectively. Recovery tests were satisfactory without effects of 7 different substances. Although varied between urines, the protein was stable at 4°C and -80°C. However, it was unstable at -20°C beyond at day two. More than two cycles of freeze and thawing gave a gradual decrease of the measured value. Vibrating urine vigorously for more than one hour at room temperature resulted in varied value in the range between -94% and 107% beyond 48 hours. Non-specific adsorption of urine albumin was observed on hydrophobic and hydrophilic but not super-hydrophilic plastic tube. Degree of adsorption was much varied depending on urine sample. The lowest recovery rate was 65.6%, that is, approximately 5 mg/l loss when hydrophobic tube is used. Good recovery rate was obtained with hydrophilic tube, but not hydrophobic tube by using belonging sample dilution buffer. Reference intervals were 5.61 ± 3.28 mg/g•Cr (mean ± 1SD) ranging from 1.14 to 17.28 mg/g•Cr in 155 healthy individuals.
The present established assay system was functionally reliable for accurate and precise measurement of low -grade albuminuria. Non-specific adsorption can, however, affect the measured value which was unpredictable. Use of hydrophilic tube in optimized sample buffer solution cleared the problem. The present assay may be a harbinger as an early, sensitive, diagnostic and preventive marker for renal and cardiovascular disorders.
The present style was supported by a Grand-in-aid the Sapporo Biocluster "Bio-S", Ministry of Education, Culture, Sports, Science and Technology. (2009)

B-142

Evaluation of a Cystatin C Urine application on the Abbott Aeroset

T. Liponis, P. Halloran, T. Pisani. *Genzyme Corporation, Framingham, MA,*

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

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Validation of the ImmunoCAP® ISAC.

R. Gadisseur[1], A. Blanco Catafal[2], J. Chapelle[1], E. Cavalier[1]. *[1]University of Liège, Department of Clinical Chemistry, Liège, Belgium, [2]University of Barcelona, Department of Pharmacy, Barcelona, Spain,*

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

B-144

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

T. B. Ledue, M. F. Collins. *Foundation for Blood Research, Scarborough, ME,*

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

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

Method Comparison Studies (Deming regression)

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

B-145

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

L. N. Kamel[1], M. Akl[1], N. El Badrawy[1], A. Anass[1], T. Abou Shousha[1], I. Mostafa[1], S. Hunter[2]. *[1]Theodor Bilharz Research Institute, Giza, Egypt, [2]Kasr El Aini, Cairo, Egypt,*

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

B-146

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

Q. Chen, M. Yan, F. Zheng, Y. Liu, G. Song. *Zhongnan Hospital of Wuhan University, Wuhan, China,*

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

B-147

Quantitative electrophoretic profiling for diagnostic prediction of major serum constituents.

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

H. Kataoka[1], T. Hisahara[1], T. Sakaki[2], K. Ichihara[3], Y. Hatakeyama[1], Y. Okuhara[1], T. Sugiura[1]. *[1]Kochi Medical School, Nankoku, Kochi, Japan, [2]A&T Corporation, Yokohama, Japan, [3]Yamaguchi University, Yamaguchi Ube, Japan,*

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

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

B-148

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

P. McGivern, P. Kelly, P. Armstrong, J. Campbell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

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

B-149

Preliminary commutability study on a candidate reference material for cystatin C

I. Zegers[1], S. Blirup-Jensen[2], H. Althaus[3], C. Schmidt[2], V. Lindström[2], H. Schimmel[1], A. Grubb[2]. *[1]IRMM, Geel, Belgium, [2]University Hospital Lund, Lund, Sweden, [3]Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany,*

Background: Plasma (or serum) cystatin C has been proposed as a marker for the glomerular filtration rate, a measure of the capacity of the kidneys to filter plasma (GFR) [1]. Several studies, as well as one meta-analysis [2], have suggested that it is superior to serum creatinine for estimation of GFR. Particularly, cystatin C could be used for tests for children, elderly, patients with low muscle mass, and the early stages of kidney problems, where creatinine measurements do not perform well. Additionally cystatin C is used as a marker for cardiovascular risk and pre-eclampsia.
The IFCC working group for the standardisation of cystatin C has, in collaboration with the IRMM, prepared a candidate reference material (RM) for cystatin C. Homogeneity and stability studies have been completed successfully. The characterisation of the RM was performed using immunoassays, calibrated with a pure protein preparation to which values had been assigned by dry mass determination. Here the results from a preliminary commutability study, performed in order to optimise the design of a planned large-scale commutability study, are presented.
Methods: Commutability studies have been performed using four assays, all employing antibodies from different sources. Thirty serum samples were measured together with dilutions of the candidate RM (mass fractions of 0.1, 0.2, 0.25, 0,3, 0.6 in assay diluent) as well as the undiluted material, in one analytical run, in triplicate. The methods used were the Siemens N Latex Cystatin C Test Kit run on a BN ProSpec®, the Sentinel CH assay run on an Architect c16000, the Roche tina quant cystatin C assay run on a Cobas c501 and the Gentian cystatin C immunoassay run on an Architect.
In order to assess the commutability of the material it was investigated whether the measurement results of the dilutions and the candidate reference material were within the 95 % prediction interval of the linear regressions.
Results: The data were first analysed by performing linear and polynomial regressions on the means of the measurement results of the patient samples for each combination of assays. For comparisons of measurements performed with the Siemens, Sentinel and Gentian assays the results showed a good linear correlation. For comparisons of the results of these three methods with the Roche assay a better R^2 was obtained with a polynomial regression as there was a deviation from linearity at high concentrations. The candidate RM and its dilutions are commutable for combinations of the Siemens, Gentian and Sentinel assays. Dilutions with a mass fraction equal to or below 0.6 are commutable for combinations of these three assays and the Roche tina quant assay.
Conclusions: The candidate reference material is commutable for combinations of methods tested, although sometimes only for dilutions. The planned large scale commutability study should include appropriate dilutions of the RM, and test plasma as well as serum.
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B-150

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

S. Shaw, T. Sparks, P. Hollis, H. Sparke, A. DeFrance, B. Combs. *Abbott Laboratories, Irving, TX,*

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

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

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

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

K. Makris, S. Potamitis, I. Merianos, K. Tsopelas, I. Drakopoulos. *Clinical Biochemistry Department, KAT General Hospital, Athens, Greece,*

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

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

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

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

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

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

B-152

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

C. R. Garlipp, P. V. Bottini, R. R. Queiroz. *University of Campinas, Campinas, SP, Brazil,*

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

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

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

B-153

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

S. W. Graves[1], D. K. Crockett[2], K. Merrell[1], M. Esplin[3], M. T. Bench-Alvarez[1], R. Grow[1]. *[1]Brigham Young University, Provo, UT, [2]ARUP Institute for Clinical and Experimental Pathology®, Salt Lake City, UT, [3]University of Utah School of Medicine, Salt Lake City, UT,*

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

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

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

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

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

B-154

Clinical Utility of Cystatin C in screening of Kidney Dysfunction

Y. Minakawa[1], Y. Meguro[1], K. Aoki[2], T. Hotta[2], Y. Kayamori[2], D. Kang[2]. *[1]Denka Seiken Co.,Ltd., Tokyo, Japan, [2]Kyushu University, Fukuoka, Japan,*

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

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

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

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

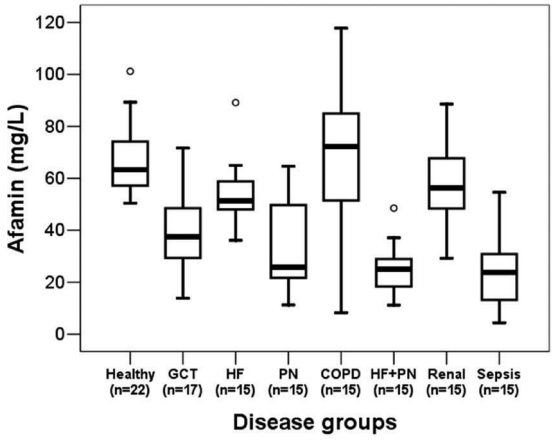
B-155

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

B. Dieplinger[1], T. Mueller[1], M. Steinmair[1], C. Gabriel[2], W. Poelz[3], M. Haltmayer[1], H. Dieplinger[4]. *[1]Department of Laboratory Medicine, Konventhospital Barmherzige Brueder Linz, Linz, Austria, [2]Red Cross Transfusion Service of Upper Austria, Linz, Austria, [3]Institute for Applied System Sciences and Statistics, University of Linz, Linz, Austria, [4]Division of Genetic Epidemiology, Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria,*

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

Fig. 1. Afamin plasma concentrations in various diseases



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

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

F. Derlet[1], J. Allaeys[1], G. Vranken[2], C. Fillée[1], P. Wallemacq[1], D. Gruson[1]. *[1]Cliniques Universitaires Saint-Luc, Brussels, Belgium, [2]Analis, Gent, Belgium,*

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

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

L. Korol, L. Migal, I. Dudar. *Institute of nephrology of AMS, Kyiv, Ukraine,*

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

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

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Polyethylene Glycol Precipitation of Eight Enzymes for Macroenzyme Detection

S. P. Wyness[1], J. J. H. Hunsaker[1], S. L. La'ulu[1], L. V. Rao[2], W. L. Roberts[3].
[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]UMASS Memorial Medical Center, Worcester, MA, [3]Department of Pathology, University of Utah, Salt Lake City, UT,

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

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

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

S. v. Freytag-Loringhoven, P. Schu, M. Loeber-Blecher, E. Metzmann, T. Hektor. *DiaSys Diagnostic Systems GmbH, Holzheim, Germany,*

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

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Serological markers of gastric mucosa

A. Caleffi[1], R. Merli[1], M. Mercadanti[1], G. Salvagno[2], F. Di Mario[3], G. Lippi[1].
[1]U.O. di Diagnostica Ematochimica, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy, [2]Sez. Chimica Clinica, Dip. Scienze Morfologico-Biomediche, Verona, Italy, [3]U.O. Gastroenterologia ed Endoscopia Digestiva, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy,

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

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

C. A. McCusker, A. L. Considine, A. M. Cahill, M. D. McCusker. *Beckman Coulter Inc., Co. Clare, Ireland,*

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

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

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

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

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

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

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

K. B. Sikder[1], R. Vannest[1], A. DeFrance[1], C. Baum[2], R. Verdun[2], B. Shull[2], D. Petty[1], L. Hoskins[1]. *[1]Abbott Laboratories, Irving, TX, [2]Thermo Fisher Scientific, Indianapolis, IN,*

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

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

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

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

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

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

B. Korzun, S. Helm, A. Hutcherson. *Virginia Commonwealth University, Richmond, VA,*

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

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

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

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

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

S. A. Dambinova[1], C. Foerch[2]. *[1]Kennesaw University, Kennesaw, GA, [2]Goethe University, Frankfurt am Main, Germany,*

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

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

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

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

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

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

U. Danilenko[1], A. Bagumyan[1], A. Shikuev[2], G. Khunteev[1], G. A. Izykenova[2]. *[1]CIS Biotech, Inc., Atlanta, GA, [2]GRACE Laboratories, LLC, Decatur, GA,*

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

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

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

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

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

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

J. Ordonez-Llanos, J. Merce, J. Freixa, K. Nunez, J. Baldira, L. Zapata, A. Roglan, A. J. Betbese. *Hospital de Sant Pau, Barcelona, Spain,*

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

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

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

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

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

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Evaluation of a fully automated NGAL particle-enhanced turbidimetric assay

K. Bangert, L. Uttenthal. *BioPorto Diagnostics A/S, Gentofte, Denmark,*

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

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

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

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

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

B-168

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

A. Barassi[1], R. Pezzilli[2], R. Capra[1], A. Mariani[3], A. Gabrielli[4], A. M. Morselli-Labate[2], R. Pacciolla[1], G. V. Melzi d'Eril[1]. *[1]Department of Medicine, Surgery and Dentistry, University of Milan, San Paolo Hospital, Milan, Milan, Italy, [2]Department of Digestive Diseases and Internal Medicine, S.Orsola-Malpighi Hospital, Bologna, Italy, [3]Gastroenterology and Digestive Endoscopy Unit, San Raffaele Hospital, Milan, Milan, Italy, [4]Digestive Endoscopy Unit, Campus Biomedico, Rome, Italy,*

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

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

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

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

B-169

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

J. Li, W. Dunn, A. Breaud, D. Elliott, L. J. Sokoll, W. Clarke. *Johns Hopkins Medical Institutions, Baltimore, MD,*

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

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

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

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

B-170

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

A. Kaur[1], T. Snelus[1], F. Mitchell[1], P. J. Showell[1], M. T. Drayson[2], A. R. Bradwell[2], S. J. Harding[1]. *[1]The Binding Site Ltd, Birmingham, United Kingdom, [2]Division of Immunity and Infection, The Medical School, University of Birmingham, Birmingham, United Kingdom,*

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

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

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

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

F. Rota, E. Marelli, A. Cugini, F. Vespasiani, M. Valdambrini. *Sentinel CH. SpA, milano, Italy,*

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

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

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

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

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

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

B-172

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

E. Baldassarre, A. Odero, S. Valaperta, E. Corsi, M. Monari, A. Montanelli. *Istituto Clinico Humanitas, IRCCS, Milan, Italy,*

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

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

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

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

B-173

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

C. Bystrom, S. Sheng, N. Clarke. *Quest Diagnostics, San Juan Capistrano, CA,*

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

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

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

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

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

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

J. C. Lieske, C. Grafft, M. Semret, S. Wagner, K. Kashani, T. Larson, T. Borland. *Mayo Clinic, Rochester, MN,*

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

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

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

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

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

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

L. A. Helms, J. H. Herod, J. D. Nagle. *Maine Standards Company, Windham, ME,*

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

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

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

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

O. A. Mojiminiyi[1], F. Safar[1], H. Al Rumaih[2], T. Al Rammah[1], M. Diejomaoh[1]. *[1]Faculty of Medicine, Kuwait University, Kuwait, Kuwait, [2]In Vitro Fertilisation Unit, Maternity Hospital, Kuwait, Kuwait,*

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

Wednesday AM, July 28

Poster Session: 10:00 am – 12:30 pm
Clinical Studies/Outcomes

C-01

Do Coumadin Clinics (CCs) Work?

C. Sylvester[1], C. Puza[2], E. Pearlman[1]. *[1]Boston Medical Center, Boston, MA, [2]South boston Community Health Center, Boston, MA,*

CCs attempt to enhance the efficacy of oral anticoagulant therapy (OAT) through patient education, increased patient responsibility for his/her own health care and intensification of monitoring using the INR. Assessments of the effectiveness of this approach however, are difficult to locate. A CC was begun at SBCHC in late November, 2008. Allowing a four month recruitment and learning curve period data from 51 patients who participated in the CC between April 1 and July 31 2009 [Group 2] was analyzed Of these 22 had data from the entire period, 17 from 3 months and the remainder from 2 months. Comparison was made to 80 patients who were either home draws or declined participation in the CC [Group 3] and to results from 111 patients collected from the 4 months prior to establishment of the CC [Group 1]. All patients had more than 1 INR result in the electronic medical record for each time period. Prior to the start of the CC, INR determinations were done at a referral lab [Quest Diagnostics; Cambridge, MA] but were brought in-house shortly before initiation of the CC using a Sysmex 530 coagulation analyzer [Siemens; Newark, DE] according to manufacturer's instructions. Group 2 patients were 56.9% female with a median age of 67 YO while Group 3 patients were 55 percent female with a median age of 70 YO [p=NS for both age (median test) and gender differences (z score)] The median number of INRs per patient was 7 in both Groups 2 [range: 2-17] and 3 [range: 2-19] as compared to 5 in group 1 [range: 2-12]. There were 84 patients in common between groups 1 and groups 2 and 3 combined and 55 of these patients had an increase and 18 a decrease in testing in the post-CC as compared to the pre-CC period [p<.001; McNemar test]. The proportion of INR results in the therapeutic range [2-3.5] was .543 in group 2 and .585 in group 3 (z score = 1.296; p>.15). For the 84 patients in common between groups 1 and the combined groups 2 and 3 the analogous results were .585 for the pre and .569 for the post-CC period [odds ratio (95% confidence interval) = .94 (.725, 1.18); p=NS]. The current study was small and used data generated early in the development of the CC. The information does suggest that startup of a CC is associated with increased frequency of INR monitoring. The lack of significant difference between groups 2 and 3 suggest either an effect of bringing INR determination in-house or the possibility of an increased awareness among healthcare providers in general of the importance of INR testing in patients on OAT that was secondary to the establishment of a CC. The lack of significant difference in the proportion of INR results within the therapeutic range could reflect the youth of the CC but also suggests that the efficacy of OAT may be greatly influenced by patient factors e.g. co-morbidities that are difficult to modify.

C-02

Sepsis Vs Systemic Inflamatory Syndrome-Role Of Procalcitonin In Differential Diagnosis:

K. A. George. *M.I.M.S.,Calicut., Trivandrum, India,*

SCOPE; Sepsis is a serious concern in critically ill patients. There are reports that Procalcitonin is increased in various conditions of systemic infections.**AIM :** Hence this study was conducted to evaluate whether PCT, being one of the early markers, can facilitate early prompt differential diagnosis, so that the patient management become easy and financially more viable (by avoiding additional tests and unwanted antimicrobial treatment).

METHODS& SUBJECTS: Patients for the present study were selected and included as per the guidelines given in ACCP/SSCM norms. A total of 102 patients (45 Males and 57 Females) and 34 normal subjects (15 Males and 19 Females) were identified for the study. The ratio between patients and normal controls were also maintained in matching the age and sex (ie. Male 64.7± 14.6 and Female 54.3 ± 18.1). Patients were classified into 4 groups namely SIRS - 24 (27%), Sepsis - 34 (52%), Severe Sepsis 9(8%) and MODS - 15(14%). (according to ACCP/SSCM criteria). In all the above patients and normals, Temp, Heart Rate and Respiratory Rate were measured by the clinicians using standard techniques. WBC was measured on Cell Dyne Ruby. PCT was measured on Elecsys E41. Statistical Analysis of the data was done by students 't' test and variance test. Patient below the age of 18 years, pregnant women, patients having severe single or multiple organ failure were excluded from the study.

RESULTS DISCUSSION: Results are given in table -1. On statistical Analysis it was observed that there is no significant difference in the PCT values of SIRS and Sepsis P>0.05)where as SIRS vs Severe Sepsis and SIRS vs MOD showed significant difference (P<0.05). At the same time, measurement of PCT in a second sample after six Hrs of first measurement showed a significant difference (Mean 0.86 ± 0.35; (P<0.05) with respect SIRS in 50% of the patients classified as sepsis group. Further, study of the body fluids of the patients for Microbiological identifications showed that, 46% of the Sepsis groups had positive cultures, of which 94% was gram negative, 3% gram positive and 6% mixed.

CONCLUSION : From the findings it can be concluded that ; I. Single measurement PCT values in the ICU patients is not enough to differentiate Sepsis from SIRS. II. A repeated assay can be of better help in the process of differentiation and patient management. III. In more severe form of Sepsis, PCT can be a good prognostic tool. IV. Even after considering the specificity and sensitivity variability at different concentration, the analyte is of use in a more viable financial management of the disease status.

TABLE: Values of Blood and Body parameters for various Groups.

C-03

Comparison of Glycated Hemoglobin and Adenosine Deaminase levels before and after management in Type 2 Diabetes.

V. K. Buabeng[1], P. Ofori - Mensah[2], F. A. Botchway[3], S. V. Rana[2], S. D. Amankwah[2]. *[1]Attibie Government Hospital, Attibie, Eastern Region, Ghana, [2]University Of Ghana Medical School, Accra, Ghana, [3]Department Of Child Health, Korle Bu Teaching Hospital, Accra, Ghana,*

BACKGROUND: Diabetes mellitus is a heterogenous group of syndrome, which is characterized by an elevation of fasting plasma glucose caused by a relative or absolute insulin deficiency. Type 2 diabetes may produce a measurable amount of insulin but there is decreased effectiveness of insulin due to insulin resistance and hence tissue failure to respond normally to insulin. It is believed that Adenosine Deaminase enzyme may be an important enzyme for modulating the bioactivity of insulin but its clinical significance in diabetes mellitus has not yet been characterized.

AIM: The aim was to compare total Adenosine Deaminase (ADA) activity with Glycated Hemoglobin (GHB) levels in newly diagnosed Type 2 diabetes.

METHODS: A total of 33 newly diagnosed Type 2 diabetes between the ages of 19 and 70 years attending the Diabetic Research Centre were recruited as the base line subjects for the study. 22(66.7%) were females, non was pregnant and 11(33.3%) were males. 33 apparently healthy non diabetic individuals were recruited as controls. They were age and sex matched. A structured questionnaire was used for demographic information, health, life style, and medications taken. Fasting blood samples were taken from patients into tubes containing EDTA, Flouride and gell separators for the initial determination of glycated hemoglobin (GHB), fasting plasma glucose (FPG) and adenosine deaminase (ADA) respectively. Patients were asked to come for follow up after 2 months of medication/exercise management. Samples were taken again for the determination of glycated hemoglobin, fasting plasma glucose and adenosine deaminase after 2 month of initiation of diabetic therapy. Plasma glucose was determined by Glucose oxidase method, glycated hemoglobin was determined by affinity chromathography method, while ADA was determined by Giusti method. Results were expressed as the mean +/- 1SD. Statistical analysis were done by simple parametric methods, simple regression analysis and Wilcoxon's sign rank test with a probability of less than 5% (p<0.05).

RESULTS: The mean FPG in control subjects was 4.6 +/-1.4 mmol/L. The mean FPG in Type 2 diabetes before diabetic management was 13.3+/-4.1mmol/L . After 8 weeks of medication/ exercise management, the FPG was 8.7+/- 4.1 mmol/L .

The mean ADA in control subjects was 16.4+/-3.7 IU/L. The mean ADA in Type 2 diabetes before management was 41.7+/-18.8 IU/L and after 8 weeks of diabetic management, the mean ADA was 29.5+/-11.8 IU/L. The mean GHB in the control subjects was 5.6 +/-0-6 %. The mean GHB in Type 2 diabetes before management was 14.6 +/- 3.8% and after 8 weeks of diabetic management, it was 11.5 +/-4.0%.

CONCLUSION: There was a good correlation between the FPG and ADA before diabetic management (r =0.78, p<0.001) as well as after management ;(r = 0.60, p<0.01) The after management correlation between GHB and FPG; GHB and ADA were r =0.56 , p<0.02 and r= 0.70, p<0.005 respectively. Lowering or normalization of plasma glucose level after management was associated with significant decrease in ADA and GHB activity in all subjects. Measurement of total ADA activity may be an alternate important tool for monitoring Type 2 Diabetes.

C-04

Diagnostic Accuracy Of An Automated Urine Flow Cytometer Analyzer In The Detection Of Urinary Tract Infection

B. A. Goicochea Razuri[1], D. G. Ordinola Goicochea[2], L. P. Vasquez Hinostroza[1], S. M. Flores Toledo[1], J. C. Jara Aguirre[1]. *[1]School of Medical Technology, Faculty of Medicine Alberto Hurtado, Universidad Peruana Cayetano Heredia., Lima, Peru, [2]Clinical Laboratory Service, Hospital Nacional Cayetano Heredia, Lima, Peru,*

Background: Urinary tract infections (UTI) are a serious health problem affecting people each year and are the second most common type of infection in humans. A urine bacterial culture is essential for the final diagnosis of UTI, which is a very time-consuming and expensive procedure, so it is necessary evaluate the diagnostic accuracy and clinical usefulness of the automated urine flow cytometer analyzer Sysmex UF-100 in the detection of urinary tract infection.

Materials and Methods: We conducted a prospective study at the Hospital Nacional Cayetano Heredia, a university general care hospital in Lima, Perú between January and May 2007. 384 urine samples submitted for diagnostic urinalysis to the laboratory from consecutive male (n=111) and female (n=273) patients between 18 and 60 years old with clinically suspected of UTI from adult outpatient services were included. The inclusion criteria for urine samples obtained were fresh midstream clean-catch, without antibiotic treatment and analyze it until two hours after of urine sample was obtained. Urine Samples first were analyzed for urine bacterial culture as a reference method with cutoff $\geq 10^5$ CFU/mL, then microscopic urinalyses of sediment (MUS) for leukocyte counts and bacterial in a high-power field (HPF) was realized with a cutoff ≥ 06/HPF for leukocytes and 2+ for bacterial count, reagent strips (RS) for nitrites and leukocyte esterase (Combur-10 Test M®, Roche) and an automated urine flow cytometer analyzer UF-100 (Sysmex) with a cutoff ≥11/HPF (≥ 57 leukocytes/uL) of leukocyte counts and ≥3000 per uL (2+) for bacterial count in the UF-100 analyzer, were performed. The clinical sensitivity and specificity, positive and negative predictive values, likelihood ratios, areas under receiver operating characteristic (ROC) curves, were calculated using data analysis and statistic software STATA version 9.

Results: 28.13% (n=108) urine samples were positive for urine bacterial culture. For MUS, the leukocyte count and bacterial count have 77.8%, 66.67% of sensitivity, 75.7%,87.68 specificity, positive and negative predictive values were 55.6%,67.92% and 89.7%,87.05% respectively, likelihood positive ratios LR(+) 3.2, 5.41 and areas under ROC curves were 0.8109 (IC 95% 0.76-0.84), 0.8466 IC 95% 0.80-0.88), respectively. For RS, the leukocyte esterase and nitrite have 70.37%, 37.96% of sensitivity, 65.57%, 96.37% specificity, positive and negative predictive values were 44.4%, 80.39% and 84.97%, 79.87% respectively, likelihood positive ratios LR(+) 2.04, 3.2 and areas under ROC curves were 0.7118 (IC 95% 0.66-0.75) for leukocyte esterase. For UF-100 analyzer, the leukocyte and bacterial count have 68.51%, 76.84% of sensitivity, 77.53%, 68.47% specificity, positive and negative predictive values were 55.41%, 48.82% and 86.29%, 88.31%, respectively, likelihood positive ratios LR(+) 3.05, 2.44 and areas under ROC curves were 0.7955 (IC 95% 0.75-0.83), 0.8109 (IC 95% 0.76-0.84), respectively.

Conclusions: UF-100 analyzer showed advantages over MUS, displaying higher specificity and similar negative predictive value (NPV) as compared to the leukocyte count of MUS, and higher sensitivity with high NPV for the bacterial count.

Keywords: Urinary tract infection, urinary sediment, reagent strip, flow cytometry, diagnostic accuracy.

C-05

Assessment of microalbuminuria in septic acute kidney injury

S. Basu[1], M. Bhattacharyya[1], T. K. Chatterjee[2], S. Chaudhuri[1], S. K. Todi[1], A. Majumdar[1]. *[1]Advanced Medicare and Research Institute Hospitals, Kolkata, India, [2]Jadavpur University, Kolkata, India,*

Introduction: In sepsis, a generalised inflammatory onslaught causes systemic endothelial dysfunction; the glomerular manifestation being microalbuminuria. Recent histo-pathological evidence of extensive glomerular capillary infiltration by leucocytes in septic AKI suggests that endothelial dysfunction may be an important contributor. Markers of endothelial dysfunction such as microalbuminuria may have a potential diagnostic role in septic AKI.

Methods: Prospective observational study in a 20 bedded Intensive Care Unit (ICU) in a tertiary care hospital. Microalbuminuria estimated as spot urine albumin-creatinine ratio (ACR, mg/g) was measured on ICU admission. 266 patients were recruited between Jan 2007 and Dec 2008. Patients with ICU stay of less than 24 hours, pregnancy, menstruation, anuria, hematuria, proteinuria due to renal and post- renal structural diseases, were excluded. Sepsis was diagnosed by the ACCP/SCCM criteria; AKI was identified using the RIFLE criteria.

Results: For the entire cohort, median age was 61.5 yrs, 61% were male, median APACHE II score was 15. Twenty six patients (9.8 %) received RRT while 56 (21%) of patients died on the ICU. ACR showed significant correlation with serum creatinine values (p=0.0049, Spearman Rank correlation(r) = 0.173) and was negatively associated with estimated GFR (p=0.0002, r= -0.225). The median level of ACR [204.1 mg/g, (IQR 119.7 - 402.0)] of patients with septic AKI (n=66) on admission to ICU was significantly greater (p<0.0001) than the median ACR level of 67.1 mg/g (IQR 32.99 - 129.9) of patients who had AKI from other causes (n=70), and also in patients who had sepsis but no AKI (n=51) [ACR 119.3 mg/g (IQR 37.02 - 354.3)] (p=0.0137).

Conclusions: The combined presence of sepsis and AKI were associated with significant levels of microalbuminuria on ICU admission. This is suggestive of glomerular endothelial dysfunction as an important event of septic AKI.

C-06

Markers of Bone Cells Activity in Periodontal Disease

C. S. Vlad[1], V. Dumitrascu[2], D. C. Vlad[3], C. V. Gurban[4], A. Cheveresan[2], A. A. Matusz[5]. *[1]Dentissimo, Timisoara, Romania, [2]Victor Babes University of Medicine and Pharmacy, Pharmacology Department, Timisoara, Romania, [3]Smart Lab Diagnostics, Timisoara, Romania, [4]Victor Babes University of Medicine and Pharmacy, Biochemistry Department, Timisoara, Romania, [5]Private Family Health Care Unit, Timisoara, Romania,*

Introduction: BAP (bone-specific alkaline phosphatase) is a magnesium-dependent enzyme released by osteoblasts, involved in bone mineralization in periodontal disease.

Objectives: To assess the evaluation of the serum BAP and Mg(2+) in the crevicular gingival fluid (GCF) and serum, as an expression of bone remodeling response in periodontitis.

Material and method: A cohort of patients with periodontitis (n=14, study group), in comparison with a control group (n=6, healthy subjects) was performed. The serum levels of BAP were measured by ELISA (enzyme-linked immunoasorbent assay) technique; the serum levels of Mg(2+) were measured by a Vitros 250 dry biochemistry analyzer, and the GCF levels of the Mg(2+) were measured by the FAAS (bone flame atomic absorption spectrometry).

Results and discussions: The serum BAP concentration was significantly decreased in the periodontal disease group (14.24±3.25µg/ml; p<0.02) comparing to the control group (16.72±2.61µg/ml). The serum Mg(2+) concentration was decreased in patients with periodontitis (1.41±0.05mg/dl; p<0.05) comparing to the control group (1.86±0.25mg/dl), and the GCF Mg(2+) concentration was significantly decreased in the study group (5.72±2.17µg/ml; p<0.001) comparing to the healthy subjects (9.33±1.15µg/ml).

Conclusions: The levels of BAP and Mg(2+) were significantly decreased which demonstrates a decreased proliferation and differentiation of the osteoblasts in periodontal disease.

C-07

RANKL/OPG and Zinc Ions - Markers of Bone Demineralization in Gonarthrosis

V. Dumitrascu[1], D. C. Vlad[2], D. V. Poenaru[3], C. V. Gurban[4], B. G. Barac[1], S. Giju[5]. *[1]Victor Babes University of Medicine and Pharmacy, Pharmacology Department, Timisoara, Romania, [2]Smart Lab Diagnostics, Clinical Laboratory, Timisoara, Romania, [3]Victor Babes University of Medicine and Pharmacy, Orthopedic and Trauma Surgery Department, Timisoara, Romania, [4]Victor Babes University of Medicine and Pharmacy, Biochemistry Department, Timisoara, Romania, [5]County Emergency Hospital, Clinical Laboratory Department, Timisoara, Romania,*

Background: RANKL/OPG (receptor activator of nuclear factor-kappa B ligand/osteoprotegerin) system and Zn2+ (zinc ions) play an important role in the bone demineralization process in patients with gonarthrosis.

Objectives: to determine values of RANKL, OPG and Zn2+ in synovial fluid in patients with gonarhrosis (n=21) versus control group (n=5).

Material and Methods: The synovial fluid levels of RANKL and OPG were measured by ELISA (enzyme-linked immunoasorbent assay) technique and the synovial fluid levels of Zn2+ were measured by the FAAS (bone flame atomic absorption spectrometry).

Results: In the study group (n=21) RANKL synovial fluid levels were 71.35±6.43 pg/ml (p<0.002), those of OPG were 39.17±2.75 pg/ml (p<0.001) and Zn2+ synovial fluid values were 0.369±0.012 µg/ml (p<0.001).

In the control group (n=5) we foud RANKL synovial fluid values of 24.75±4.16 pg/ml, those of OPG were 42.37±5.15 pg/ml and Zn2+ synovial fluid values of 0.618±0.25 µg/ml.

Conclusions: The increased synovial fluid values of RANKL demonstrates osteoclasts activation, the lower levels of OPG suggests decreased osteoblastic multiplication capacity and the significantly decreased levels of Zn2+ demonstrates a decreased proliferation and

differentiation of the osteoblasts. The resorbtion rate exceeded the osteoblastic synthesis and induces bone demineralization process in gonarthrosis.

C-08

Non Alcoholic Fatty Liver Disease- An Emerging Risk Factor For Coronary Artery Disease In A Local Indian Population- A Preliminary Study

D. S. Sheriff[1], M. T. Priya[2]. *[1]Al Arab Medical University, Benghazi, Libyan Arab Jamahiriya, [2]University of Calicut, calicut, India,*

Background: Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of disease ranging from hepatocellular steatosis through steatohepatitis to fibrosis and irreversible cirrhosis. The prevalence of NAFLD has risen rapidly in parallel with the dramatic rise in obesity and diabetes, and is rapidly becoming the most common cause of liver disease in Western countries. In the great majority of patients NAFLD develops in association with features of IR and the metabolic syndrome. Additionally, insulin resistance has been implicated in the development of nonalcoholic fatty liver disease (NAFLD), a common cause of chronic liver disease in the West and independent risk factor for cardiovascular events in diabetics. NAFLD appears to be common in Indian populations also and increases in prevalence with worsening glucose intolerance. NAFLD is therefore considered as one of the risk factors for coronary artery disease(CAD). In the present study in known CAD patients the prevalence of NAFLD was evaluated along with parameters known to be associated with NAFLD to understand the link between NAFLD and CAD..

Aim-Materials and Methods: The present study was undertaken in proven coronary artery disease patients (CAD) (N;633; males:468; female:165)to identify the presence of NAFLD in these patients based on insulin resistance (IR). 290 patients had hyperinsulinemia (Males n:191; females:N:99). Age, gender, Boby Mass Index (BMI), were mesaued.Lipid profile, inflammatory markers high sensitive C-reactive proein (hsCRP), tumor necrosis factor alpha(TNF-α) and plasminogen activator inhibitor ((PAI-I) were analysed with routine standardized methods. NAFLD was identified with serum transaminases levels and ultrasonographic studies. (Total:145; Males: 69; females).

Results: Patients with NAFLD(NASH)had a mean AST to ALT ratio of 0.9 (range 0.3-2.8, median 0.7) The mean AST levels were 66 U/L and the mean ALT levels 91 U/L , in NAFLD patients with CAD taken for the study. The NAFLD patients had low HDL levels, high serum triglycerides and high LDL-cholesterol. apoB100/apoA ratio is increased in the patients. They had visceral adiposity as measured by Waist to hip ratio.

The levels TNF-α and PAI-I were increased in the patients compared to controls subjects. The levels of adiponectin was lower both in female and male patients with NAFLD associated with CAD, the levels being decreased more in the females.

Conclusion: The preliminary study in the local Indian population bring out the presence of IR, NAFLD, in CAD patients. The female CAD patients had higher incidence of NAFLD compared to males. These patients age was >50 years and were post-menopausal.

C-09

Measurement of the urine advanced glycation endproduct, methylglyoxal-derived hydroimidazolone in patients with microalbuminuria and early protein losing nephropathy.

M. Curran, E. Randell, S. Vasdev, Y. Han. *Memorial University, St. John's, NL, Canada,*

Objectives: Advanced glycation endproducts (AGEs) are implicated in a number of pathophysiologies including nephropathy. Methylglyoxal-derived hydroimidazolone (MG-H) is one of the most abundant AGEs *in vivo* in body fluids and cellular proteins. The purpose of this work was to develop a novel method for measurement of MG-H in urine samples to examine the levels in patients with normal and abnormal urinary protein excretion.

Design and Methods: A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to measure urine MG-H levels. The method involved solid phase extraction on Oasis® MCX mixed mode ion exchange columns. The bound extract was eluted using 5% ammonium hydroxide in methanol and analyzed by LC-MS/MS. MG-H was chromatographed on an Atlantis® HILIC Silica column using a water gradient (10% to 55%) against acetonitrile and with formic acid concentration maintained at 200 mM and measured by selective ion monitoring using a Quattro Ultima Mass Spectrometer (Waters). Possible changes in MG-H excretion with sex and age was assessed by examining urinary MG-H levels in 114 individuals without urinalysis abnormalities ranging in age from 22 to 91 years. The relationship between MG-H levels and urinary protein loss were measured in 48 controls and 49 patients (age and sex matched) with evidence of microalbuminuria (Albumin to Creatinine ratio ACR > 2 mg/mmol) and urinary protein loss.

Results: The LC-MS/MS method showed excellent sensitivity (<0.1 µmol/L) and dynamic range (<0.1 to >100 µmol/L) for measuring urine MG-H in a short run time of 6 minutes. There were no significant differences in MG-H levels or MG-H normalized for creatinine with age or sex among non-proteinuric individuals. The upper 95th %ile values of MG-H with and without normalization for urine creatinine were 4.7 µmol/mmol creatinine and 69 µmol/L, respectively. A separate group of patients with urinary protein loss had mean ACR (40 ± 133 mg/mmol) and PCR (39 ± 66 mg/mmol) that were higher than the control group (ACR 0.8 ± 0.4; PCR 6.7 ± 4.5). The mean creatinine normalized MG-H levels, however, were not significantly different in controls (2.09 ± 1.78 µmol/mmol creatinine) compared to the protein loss group (2.01 ± 1.27 µmol/mmol creatinine). Urinary MG-H levels were significantly correlated with urinary creatinine ($r = 0.43$; $p < 0.001$) and not with ACR ($r = -0.08$; $p = 0.430$) and PCR ($r = -0.169$; $p = 0.111$) for the whole group.

Conclusions: We adapted a LC-MS/MS method for measurement of MG-H in human urine. Urine MG-H levels were similar in males and females and did not change with age. Urine MG-H levels correlated with urinary creatinine levels but were not affected by presence of early nephropathy and mild proteinuria.

C-10

RELATIONSHIP BETWEEN SERUM LIPOCALIN-2 LEVELS AND METABOLIC SYNDROME PARAMETERS

R. Uzun, B. Arslan Senturk, f. ustuner, N. Goren, G. Oru. *Atatürk Training Hospital, Izmir, Turkey,*

Background: Metabolic syndrome is a combination of medical disorders like insuline resistance, dyslipidemia, santral obesity and high blood pressure that increase the risk of developing cardiovascular disease and diabetes. Adipose tissue produces hormones and cytokines that are imlicated in the pathogenesis of MetS. Lipocalin-2 is a novel adipokine with elevated levels in obesity and diabetes. The purpose of this study was to determine the relationship between lipocalin-2 and the MetS parameters and the importance of the lipocalin-2 in diagnosis and treatment of MetS.

Methods: We assessed 79 individuals (47 patients with MetS and 32 healty subjects) who admitted Izmir Atatürk Education and Training Hospital endokrinology department between may and july 2009. The diagnosis of MetS based on the IDF definition**.** Serum lipocalin-2 levels were measured by ELISA. All data obtained were proccessed statistically using the sofware SPSS 15.0 for windows. The differences between groups were determined The differences between groups were determined with Student's t test. The Pearson's correlation is used to find a correlation between parameters.

Results: Serum lipocalin-2 levels were significantly higher in patients with MetS than control group (respectively 63,7 ± 18,7 ng/ml and 45,2 ±16,7 ng/ml; p<0,001). There was statistically significant relationship between serum lipocalin-2 levels and fasting glucose, fasting insulin and CRP levels, waist/hip ratio, HOMA-IR.

Conclusion: Lipocalin-2 might be useful marker related to metabolic syndrome and it's complications. But further studies are needed to fully elucidate the physiology of lipocalin-2 in human and its role in diagnosis, follow up and treatment.

C-11

THE COMPARISION OF IgG INDEX AND MS OLIGOCLONAL BANDS WITH MRI AND EVOKED POTENTIALS

H. Ellidağ, B. Arslan Şentürk, F. ustuner, S. Türe. *Atatürk Training Hospital, Izmir, Turkey,*

Background: Multiple Sclerosis (MS) is the most frequent demyelinating disease of the central nervous system (CNS). MS diagnosis is based on neurological history, examination findings and exclusion of other disorders. Recently, in the diagnosis of MS, magnetic resonance imaging, neurophysiological tests and oligoclonal bands (OCB) are in use. Other than magnetic resonance imaging, most consistent laboratory abnormality in MS patients is OCB in cerebrospinal fluid (CSF) and increased IgG index.

The pathogenesis of OCB and intratechal IgG syntesis in MS is uncertain. In this study, we compared IgG index and OCB with cranial and cervical magnetic resonance imaging (MRI) findings, evoked potentials, other CSF tests (CSF protein, CSF LDH and CSF cell count), biochemical tests (Total protein, Cholesterol, HDL, LDL, Trigliseride, Total Bilirubin, Direct Billirubin, Uric acid, Vitamin B12, Folic acid, CRP, RF) to understand the OCB finding better and to search the pathogenesis of OCB.

Methods: In this study 27 certain MS, 11 possible MS and 18 patients with other neurological diseases who admitted to Izmir Atatürk Education and Training Hospital and had the diagnosis according to Mc Donald's criteria, were included searched retrospectively.

In 27 certain MS patients, 17(%62,96) were OCB (+), 10 (%37,04). were OCB (-). In 11 possible MS patients 5 (%45,45) were OCB (+), 6 (%54,55) were OCB (-).

Results: We compared OCB and IgG index with cranial and cervical MRI (atrophy, oedema, contrast enhancement, plaque number and plaque maximum size). In all MS groups when OCB and IgG index were compared with MRI, contrast enhancement in cervical MRI was statistically significant only in OCB (-) patients. We found no significant

association with the other MRI findings.
Furthermore there was no significant association in CSF protein, CSF LDH and IgG index compared with cranial and cervical MRI lesion size. There was no significant association between CSF leucocyte count and cranial, cervical MRI lesion size.
In our study we found no statistical significance between visual evoked potentials (VEP) and sensory evoked potentials (SEP) and OCB, CSF LDH and IgG index. We found significant association only between CSF protein and SEP.
We found significant relationship between CSF LDH and CRP (r=0,58 p=0,01) though the correlation is not strong. We found significant relationship between CSF LDH and CSF protein (r=0,44 p=0,02 ve r=0,54 p=0,01 respectively) though the correlation is not strong.
Conclusion: Finally when OCB and IgG index are compared with MRI and evoked potentials, we found that all these diagnostic tools are unique on their own. The results in this study suggest that the laboratory findings occur from independent mechanisms. Although OCB and IgG index is important in MS pathogenesis , the exact role is not clearly understood.
Furthermore, the OCB characteristics of MS can differ from geographic regions. Although numerous studies have been performed in this regard in other countries, there has not been any systematic and validated study in our country. For this reason, the studies with broad participation must be done and OCB characteristics of MS must be searched with details.

C-12

The ability of serum cystatin C in the prediction of kidney dysfunction in type 2 diabetic patients

Y. Sun[1], P. Chang[1], J. Hwang[2], E. Chan[3], C. Sun[1]. *[1]Department of Laboratory Medicine, Chang Gung Memorial Hospital, Taipei, Taiwan, [2]Department of Metabolism and Endocrinology, Chang Gung Memorial Hospital, Taipei, Taiwan, [3]Department of Medical Biotechnology and Laboratory Medicine, Chang Gung University, Taipei, Taiwan,*

The accuracy in detecting early stage of chronic kidney disease is important for intervention and treatment for diabetes patients. In this study, we evaluated the ability of cystatin C in predicting kidney dysfunction in type 2 diabetic patients. We measured serum cystatin C and creatinine from baseline samples of 159 healthy individuals, 419 diabetes patients without kidney dysfunction (estimated GFRserum creatinine≥60 mL/min per 1.73 m^2) and 100 diabetes patients with kidney dysfunction (estimated GFRserum creatinine < 60 mL/min per 1.73 m^2 lasting over three months). Significantly higher serum cystatin C levels were found in diabetes patients with kidney dysfunction (1.6±0.6 ng/mL) and diabetes patients without kidney dysfunction (0.8±0.3 ng/mL) compared with healthy individuals (0.7±0.1 ng/mL). Among 419 diabetes patients without kidney dysfunction, 120 patients had elevated cystatin C (≥0.92 ng/mL, refer to the 95th percentile of 159 healthy individuals) and 299 patients had low cystatin C (<0.92 ng/mL). The 419 diabetes patients without kidney dysfunction had been followed over 3 years. During follow-up, 34 kidney dysfunction had occurred. More incidents (21.7%) occurred in patients with elevated cystatin C than patients with low cystatin C (2.7%). Higher cystatin C levels were significantly associated with kidney dysfunction (adjusted hazard ratio 1.13, 95% confidence interval 1.09-1.17 for per 0.1 ng/mL increase). Serum creatinine levels had less association (adjusted hazard ratio 1.06, 95% confidence interval 1.03-1.09 for per 0.1 mg/dL increase) with kidney dysfunction than cystatin C had. These results indicate serum cystatin C is a better alternative to serum creatinine and estimated GFR to predict kidney dysfunction in type 2 diabetic patients.

C-13

Use of clinical biology techniques in clinical practice: injections of platelet-rich plasma to heal tendon

J. Kaux[1], C. Le Goff[2], P. Drion[1], F. Pascon[1], V. Libertiaux[1], A. Gothot[1], S. Cescotto[1], D. Jean-Olivier[1], M. Rickert[3], J. Crielaard[1]. *[1]University of Liège, Liège, Belgium, [2]University Hospital of Liège, Liège, Belgium, [3]University of Heidelberg, Heidelberg, Germany,*

Background: A tendon is a tissue which does not heal easily. For example, tendinopathy is a condition which often becomes chronic in the case of bad or overdue management. Several studies, essentially in vitro and, more recently, a few in clinical practice, have demonstrated the positive effects of platelets on the healing process of tendons. A local injection of platelet-rich plasma (PRP), which releases many growth factors, has the potentiality to enhance the tendon healing process. The aim of our experiment was to ascertain whether the use of PRP could accelerate the healing process of an Achilles tendon after a surgically induced lesion.
Methods (*): PRP was obtained from the blood of 12 Sprague Dawley rats by cardiac puncture under general anaesthesia until the heart stopped beating. Quantities of 1mL of anticoagulant, adenosine-citrate-dextrose-acid (ACD-A), were added immediately to each 4,5mL of blood. The blood was then centrifuged at 180g for 10 minutes. To improve platelet concentration of the PRP, the supernatant was centrifuged for a second time at 1000g for 10 minutes. The platelets were then collected using a gauge pipette. Cell and platelet counts were made by an auto-analyser. Platelet concentration was around 2.2 to 2.9 x10^6/mm^3.
A 5mm defect was surgically induced in the Achilles tendon of 60 rats. Rats were divided into 2 groups of 30: A: a control group (no injection) and B: with a PRP injection. The ats of group B received a PRP injection in situ 1 hour after the surgery on the site of the lesion of the Achilles tendon. Fifty micro-litres of PRP were injected in each rat of the PRP group. Platelets were activated by the local presence of collagen in the wound. Afterwards, the rats of both groups were placed in their cages without immobilization. After 5, 15 and 30 days, 10 rats of eac group were euthanized. The traumatized Achilles tendon of each rat was dissected and removed. Immediately after sampling, tendons were submitted to a biomechanical tensile test up to rupture, using a tensile machine with a "Cryo-jaw".
Results: We demonstrated that the force necessary to induce tendon rupture during biomechanical tensile testing was greater for tendons which had been submitted to an injection of PRP. These results were observed and significant ($p<0.05$) from day 5 onwards.
Discussion: This experimentation showed that PRP injections could accelerate the tendon healing process and increase the force needed to break tendons in their healing process. This "accelerating" process can be observed and is significant ($p<0.05$) as early as day 5.
Conclusion: PRP, by the local release of growth factors, would be a new therapeutic tool to accelerate tendon healing.
Acknowledgement: This experimentation was partially financed by "Standard de Liège" and "Lejeune-Lechien" grants.
(*) All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Liège.

C-14

New use of VEGF in therapeutics: application in tendon lesions

J. Kaux[1], L. Caroline[2], P. Drion[1], V. Libertiaux[1], F. Pascon[1], A. Colige[1], L. Charles[1], B. Nusgens[1], S. Cescotto[1], J. Defraigne[1], M. Rickert[3], J. Crielaard[1]. *[1]University of Liège, Liège, Belgium, [2]University Hospital of Liège, Liège, Belgium, [3]University of Heidelberg, Heidelberg, Germany,*

Background: As demonstrated in previous studies, mechanical overload, injury and inflammation, hypoxic condition or any combination of the above could lead to increased expression of VEGF in the tendon. Thus, VEGF could participate in the healing of pathological tendons. Indeed, some authors are convinced that this neovascularization is the sign of a chronic tendinopathy while others plead in favour of it being a sign of healing processes. The VEGF111, which is a biologically active and proteolysis-resistant VEGF-A isoform, was recently identified. It is induced by ultraviolet B and genotoxic drugs. Experimentation shows that, in nude mice, tumors formed by HEK293 cells expressing VEGF111 develop a more widespread peritumoral neovascularisation than those expressing other VEGF isoforms. Good angiogenic activity and resistance to proteolysis makes VEGF111 a potential beneficial therapeutic option for ischemic diseases. The aim of our study was to determine whether if VEGF111 could have a therapeutic interest in the framework of tendinous pathology.
Methods (*): A 5mm defect was surgically induced in Achilles tendon of 60 rats. Rats were divided into 2 groups of 30: A: a control group (no injection) and B: with a VEGF111 injection. The rats of group B received an injection of 100 ng of VEGF111 in situ 1 hour after surgery on the site of the tendon lesion. Afterwards, rats of both groups were placed in their cages without immobilization. After 5, 15 and 30 days, 10 rats of each group were euthanized. The traumatized Achilles tendon of each rat was dissected and removed. Immediately after sampling, tendons were submitted to a biomechanical tensile test up to rupture, using a tensile machine with "Cryo-jaw". Statistical analyses were made with an ANOVA.
Results: A significant increase over time of the force necessary to induce tendon rupture was observed for tendons which had been submitted to an injection of VEGF111 ($p=0.016$). The force required to break the tendon is always greater for the VEGF111 group ($p<0.05$).
Discussion: We demonstrated that the force necessary to induce the rupture of a rat's Achilles tendon during biomechanical tensile testing was greater for tendons which had been submitted to an injection of VEGF111. Thus, this experimentation showed that VEGF111 injections could accelerate the tendon healing process and increase the force needed to break tendons in their healing process.
Conclusion: VEGF111 could be a new therapy for tendon lesions. However, other experimentation using a rat model with different concentrations of VEGF111 should be made to ascertain the best concentration for this healing process.
Acknowledgement: This experimentation was partially financed by "Standard de Liège" and "Lejeune-Lechien" grants.
(*) All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Liège.

C-15

The Importance of Oxidative Stress in Patients with Chronic Renal Failure whose Hypertension is Treated with Peritoneal Dialysis.

S. Demirci[1], M. R. Sekeroglu[1], T. Noyan[1], R. Koceroglu[1], Y. U. Soyoral[2], H. Dulger[1], R. Erkoc[2]. *[1]Yuzuncu Yil University, Medical School, Department of Biochemistry, VAN, Turkey, [2]Yuzuncu Yil University, Medical School, Department of Internal Medicine, Division of Nephrology, VAN, Turkey,*

Background: It has been reported that there is a relationship between oxidative stress and the progression of chronic renal failure. The present study aimed to investigate if hypertension had an effect on oxidative stress.
Methods: A total of 50 patients treated with peritoneal dialysis were divided into two groups: The patients with blood pressure levels of 135/90 mmHg and above were considered hypertensive, the patients with lower blood pressure were considered normotensive. The control group included 25 healthy individuals. Serum malondialdehyde (MDA), advanced oxidation protein product (AOPP), myeloperoxidase (MPO), catalase (CAT) and glutathione peroxidase (GSH-Px) levels were measured in all groups.
Results: In this study, MDA level, an indicator of lipid peroxidation, was significantly higher in the hypertensive group compared to the control group ($p<0.001$), while the increase in the normotensive group was not statistically significant ($p> 0.05$). However, the difference between the hypertensive and normotensive groups was statistically significant ($p<0.001$). The levels of AOPP, an indicator of protein oxidation level, and MPO, an indicator of neutrophil activation, were not statistically significant between the groups, while the activities of antioxidant CAT and GSH-Px decreased in both normotensive and hypertensive groups compared to the control group ($p<0.001$), and there was not a significant difference between the patient groups ($p>0.05$).
Conclusions: The results of this study show that peritoneal dialysis patients have increased-oxidative stress and decreased antioxidant levels and hypertension might have an additional effect in increasing oxidative stress in peritoneal dialysis patients.

C-16

***Vibrio vulnificus* DNA Load and Mortality**

D. Kim[1], S. Lee[2]. *[1]Chosun University College of Medicine, Gwang-Ju, Korea, Republic of, [2]Seonam University, Namwon, Korea, Republic of,*

Introduction: *Vibrio vulnifcus* (*V. vulnificus*) is a halophilic gram-negative bacillus that can cause fulminant sepsis in patients with chronic liver disease or immunocompromised conditions. The aim of this study was to examine the association between serum tumor necrosis factor- α concentration and mortality in patients with *V. vulnificus* infection.
Methods: This study included 25 adult patients aged ≥18 years who were diagnosed with skin and soft tissue infections at 4 university hospitals between 2006 and 2008. Serum TNF-α concentration was measured with blood samples taken at presentation. Thirteen healthy adults were used as controls.
Results: The median TNF-α level was 261.0 pg/mL (IQR, 101.0-376.0 pg/mL) in the non-survivor group and 69.5 pg/mL (IQR, 17.5-103.5 pg/mL) in the survivor group, and the difference was statistically significant (P=0.001). The serum TNF-α level measured 6 to 48 hours after antibiotics administration was also significantly higher in non-survivors than in survivors (P=0.044). The median TNF-α level was significantly higher in patients with Vibrio infection than in control subjects (P<0.001).
Conclusion: From the results of this study, it is concluded that serum TNF-α concentration at presentation may be a useful early predictor for mortality of patients with *Vibrio* septicemia.

C-17

Serum Hepcidin-25 in Comparison to Biochemical Markers and Hematological Indices for the Differentiation of Iron-Deficient Erythropoiesis

R. Roeddiger[1], L. Thomas[2], U. Kobold[3], C. Thomas[4]. *[1]Roche Diagnostics GmbH, Clinical Trials, Mannheim, Germany, [2]Laboratory Medicine, Hospital Northwest, Frankfurt, Germany, [3]Roche Diagnostics GmbH, Instrumental Analytics, Penzberg, Germany, [4]Klinik und Poliklinik für Urologie, Universitätsklinikum, Mainz, Germany,*

Objective Biochemical markers hardly allow the ability to distinguish iron-deficiency anemia (IDA) from anemia of chronic disease (ACD) and the combined state of ACD/IDA in patients with inflammation. Serum hepcidin-25 might be a marker resolving this problem. The primary objective of this prospective study was to evaluate the extent to which IDA can be differentiated from ACD and ACD/IDA based on hepcidin-25 alone or on its combination with other biochemical markers or hematological indices. The second objective was to investigate the association of hepcidin-25 with biochemical markers and hematological indices of iron status.
Materials and Methods: In 155 anemic patients (ACD 67, IDA 52, ACD/IDA 36) serum hepcidin-25 and a panel of biochemical markers and hematological indices of iron metabolism were determined. The panel included the CBC, reticulocyte count, CHr, HYPO, ferritin, sTfR, ferritin index, TSAT, CRP, haptoglobin, exclusion of thalassemia trait, and an RBC scatter diagram. Hepcidin-25 was determined using an isotope-dilution micro-HPLC-tandem mass spectrometry method.
Results: Hepcidin-25 significantly correlated with ferritin, particularly for ferritin levels below 200µg/L ($y=0.0478x - 0.2230$, N=81, $r^2=0.323$, $p<0.001$) and TSAT ($y=0.86x - 12.1$, N=81, $r^2=0.387$, $p<0.001$). Based on linear regression analyses, hepcidin-25 concentrations were below the detection limit of 0.2 nmol/L at ferritin concentrations ≤9 µg/L and TSAT ≤14.3%. Correlations of hepcidin-25 with sTfR, FI, and CRP were weaker but still significant (sTfR: $y= -0.3371x + 5.224$, N=80, $r^2=0.067$, $p=0.020$; FI: $y= -0.3815x + 4.844$, N=80, $r^2=0.102$, $p=0.006$; CRP: $y=0.092x + 1.13$, N=155, $r^2=0.281$, $p=0.02$). No significant relationship of hepcidin-25 with hematological indices was found. With respect to hepcidin-25, median concentrations were lowest in IDA patients and high in ACD and ACD/IDA. Based on a ROC analysis, hepcidin-25 enabled differentiation of IDA from patients with ACD (AUC_{ROC}, 0.968; 95% CI 0.915- 0.992; sensitivity 98.1%, specificity 84.5%; p=0.0001) and IDA/ACD (AUC_{ROC} 0.995; 95% CI 0.948-1.000; sensitivity 98.1%, specificity 97.1%; p=0.0001). However, it was not possible to differentiate between ACD and ACD/IDA (AUC_{ROC} 0.569; 95% CI 0.461-0.671; sensitivity 42.3%, specificity 75.0%; p=0.2590) in patients with inflammation. The optimal hepcidin-25 cutoff value from ROC curves for the diagnosis of IDA was ≤4.0 nmol/L. As the ROC analysis indicated weak discriminatory power of hepcidin-25 alone to differentiate ACD from ACD/IDA , we described the relationship between hepcidin-25 and the CHr (hemoglobin content of reticulocytes) in a diagnostic plot divided into quadrants that corresponded to the four states ACD, ACD/IDA, IDA (classic) and ACD/IDA (latent state). Data points in the hepcidin-25 range ≤4 nmol/L reflected iron-deficient states whereas data points in the range >4 nmol/L were consistent with ACD. Patients with data points in the CHr range ≥28 pg (normal CHr) did not have IDA, whereas those with a CHr <28 pg (reduced CHr) had IDA or ACD/IDA.
Conclusion: The results show that erythropoiesis can be iron deficient even in the presence of high hepcidin-25 levels. The combination of hepcidin-25 with CHr, a potent marker of iron-deficient erythropoiesis in a diagnostic plot, appears to be useful for the differentiation of ACD/IDA from ACD and IDA.

C-18

Comparison of Two Methods for Malondialdehyde (MDA) Measurement

S. Al-Fawaeir, E. O. Akgul, T. Cayci, H. Demirin, Y. Gulcan Kurt, I. Aydin, M. Agilli, E. Ozkan, H. Yaman, E. Cakir, K. M. Erbil. *Gulhane Military Medical Academy, Ankara, Turkey,*

Background: Reactive oxygen species (ROS) and particularly free radicals induced lipid peroxidative tissue damage have been implicated in the pathogenesis of various diseases. Lipid peroxidation is assessed indirectly by the measurement of the secondary products such as malondialdehyde (MDA). MDA has three-carbon atoms and low molecular weight and spontaneous breakdown product of peroxides that can be produced from free radicals attack on polyunsaturated fatty acids (PUFAs).
Methods: The analysis of MDA by the thiobarbituric acid (TBA) assay has been widely employed over the many years in biological systems for the assessment of lipid peroxidation. It is a spectrophotometric assay, based upon heating of the sample under acidic conditions to form the adduct of MDA-TBA. This reaction, although simple and reproducible, is unfortunately rather non-specific as TBA reacts with many other carbonyl-containing compounds. Plasma fatty acids can also be oxidized during the 95 -100 °C heating step with TBA, generating artificially high results. In an attempt to over come these difficulties, in a direct chromatographic assay suitable to be applied for accurate and specific quantification of MDA have been developed. However, TBA reacts not only MDA but also with many other compounds. Therefore, derivatization of MDA with 2,4 dinitrophenylhydrazine (DNPH) and conversion into pyrazol and hydrazine derivatives has since been found to allow a more specific estimation of these compounds, especially if combined with their separation using high pressure liquid chromatography (HPLC) in the biological samples. In this study, our purpose is to measure the levels of MDA with TBA test and HPLC-DAD after derivatisation of MDA with DNPH in human serum. Furthermore, we compared HPLC and spectrophotometric methods.
Results: When the results of HPLC (1.85±0.09) (µmol/L) and spectrophotometry (2.47±0.18) (µmol/L) measurements are compared, a significant difference has been found ($p<0.001$). Cohesion between measurements made by two different methods have been evaluated and the interclass correlation coefficient has been found as 0.365 ($p=0.042$). Statistically significant weak, mild degree positive correlation has been found between measurements ($r=0.284$, $p=0.028$).
Discussion: In all of the measurements, spectrophotometric MDA levels have been found higher than HPLC MDA levels. There was a difference between two measurements ranging from 8% to 61%.

C-19

Evaluation of urinary stone risk profile

K. Park, S. Lee. *Samsung medical center, Seoul, Korea, Republic of,*

Background : Effective medical treatment and prevention of the recurrent urinary stones can be based on the appropriate metabolic evaluation in stone formers. The purpose of the present study is to comprehensively evaluate the clinical and biochemical risk factors in urinary stone disease

Materials and Methods : We retrospectively reviewed 191 cases of stone risk profiles in 187 patients. We evaluated biochemical components (sodium, calcium, oxalate, magnecium, chloride, potassium, uric acid, citrate, and ammonium) and calculated relative supersaturation (RSS) in 24-hour urine. Nitroprusside-cyanide test and urinalysis in spot urine and serum biochemical tests (calcium, phosphate, uric acid, ALP, and PTH) were also evaluated. Physical analysis of urinary stone was performed using FT-IR system 2000.

Results : Male gender, mid-age, obesity, DM and HTN were important clinical risk factors. 189 cases among 191 cases had abnormal urinary biochemistry with or without increased RSS with respect to their stone components. Low urine volume (66%), hypercalciuria (48%), hypocitraturia (41%), natriuresis (38%), hyperoxalaturia (33%), and hyperuricosuria (31%) were commonly found. Especially, hypercalciuria was counted up to 61% of the patients with calcium oxalate (CaOx) stones and 17% of the uric acid (UA) stone formers. Increased RSS with respect to the CaOx and/or UA was commonly found in CaOx stone (77 cases among 88 cases) and most of UA stone formers showed increased RSS with respect to UA (21 cases among 24 cases). Stone analysis was not performed in 48 cases, and all but one of them showed increased RSS regarding some of the stone components.

Conclusions : Low urine volume, hypercalciuria, hypocitraturia, and increased RSS regarding any component were the important findings in association with the high risk of stone formation. Increased RSS were complementary to biochemical abnormalities and may be good predictor of stone components especially when stone analysis was not available. Comprehensive interpretation of urinary stone risk profile would help to understand the pathophysiology of stone formation and to determine appropriate preventive strategy in stone formers.

C-20

Multi-Center Outcomes-Based Clinical Evaluation of the VITROS® Immunodiagnostic Products HBeAg Assay (VITROS HBeAg Assay) for Detection of Hepatitis B e Antigen (HBeAg) in Subjects at High Risk for Hepatitis, or with Chronic Hepatitis B Virus (HBV) Infection

L. Bergmeyer[1], E. R. Schiff[2], M. de Medina[2], M. A. Janzen[3], J. B. Gorlin[3], D. Uettwiller-Geiger[4], B. Attar[5], H. F. Yee, Jr.[6], N. Kasahara[6], M. Q. Ansari[7], A. T. Banks[8], S. A. Alvey[1], S. Wang[1], J. V. Calcagno[1]. *[1]Ortho Clinical Diagnostics, Raritan, NJ, [2]Univ. of Miami School of Medicine, Center for Liver Diseases, Miami, FL, [3]Memorial Blood Centers, St. Paul, MN, [4]The John T. Mather Memorial Hospital, Port Jefferson, NY, [5]Cook County Hospital, Chicago, IL, [6]UCLA Dept. of Medicine, Los Angeles, CA, [7]Parkland Memorial Hospital, Dallas, TX, [8]Banks Hepatology Institute, PC, College Park, MD,*

This multi-center clinical study was conducted to establish the performance characteristics of the VITROS HBeAg assay (an enhanced chemiluminescence immunometric assay) versus an FDA-approved HBeAg comparator enzyme immunoassay among subjects without known or previously determined HBeAg results. Samples from 1922 U.S. subjects at high risk for hepatitis prospectively enrolled at four geographically separated collection sites (Miami, FL, College Park, MD, Dallas, TX and Chicago, IL) were tested at three clinical laboratories located in Miami, FL, St. Paul, MN and Port Jefferson, NY. Samples prospectively collected from 311 subjects from India with signs and symptoms of viral hepatitis, from an area with a high prevalence of hepatitis, were tested at the same three laboratories. Also tested were samples from 76 subjects with chronic HBV infection, from 245 pregnant women at high risk for hepatitis, and from 165 pediatric subjects at high risk for hepatitis. Because the comparator assay is a non-reference standard method, positive and negative agreement (rather than sensitivity and specificity) were calculated as the percentage of comparator assay positive or negative samples testing VITROS reactive or negative, respectively.

Positive agreement between the VITROS and comparator HBeAg assays was 58.54% (24/41; 95% exact confidence interval (CI) = 42.11% to 73.68%) and negative agreement was 99.47% (1871/1881; CI = 99.02% to 99.74%) among the 1922 U.S. subjects. Positive agreement between the assays among the 311 subjects from India was 77.78% (49/63; CI = 66.54% to 87.28%). Negative agreement was 89.92% (223/248; CI = 85.48% to 93.37%). Statistical testing yielded no homogeneity issues among the three testing sites with either population.

Positive agreement between the VITROS and comparator assays among 76 subjects with clinically documented chronic HBV infection was 81.82% (18/22; CI = 59.72% to 94.81%). Negative agreement was 94.44% (51/54; CI = 84.61% to 98.84%). Positive and negative agreement among 245 samples from pregnant women were 25.00% (1/4; CI = not meaningful when calculated on small numbers) and 100% (241/241; CI = 98.48% to 100%), respectively. Negative agreement of the VITROS and comparator assays was 100% (165/165; CI = 97.79% to 100%) among 165 pediatric subjects. Neither assay was reactive in this group.

The two assays were reactive in the same bleed in parallel testing of six of 11 seroconversion panels. The VITROS assay was reactive one bleed (two to five days) later in five panels. Total precision estimates for a six-member panel with site, lot and day introduced as sources of variation fell between 7.3% and 10.9% for the VITROS assay.

We conclude that the VITROS HBeAg assay is safe and effective for the *in vitro* qualitative detection of HBeAg in human adult and pediatric serum using the VITROS ECi/ECiQ Immunodiagnostic System. The assay is indicated, in conjunction with other serological and clinical information, for the laboratory diagnosis of individuals with acute or chronic hepatitis B, or recovery from hepatitis B infection. This assay is pending FDA approval.

C-21

Usefulness of cystatin C in patients with borderline eGFR

M. Faryna, D. Bobilewicz, B. Sosnowska, M. Iwanowska, E. Przybyszewska-Kazulak, H. Zborowska. *Medical University, Warsaw, Poland,*

Background: Serum creatinine is accepted for assessment of kidney function. It is used to calculate estimated glomerular filtration rate (eGFR) according to MDRD formula (Modification of Diet in Renal Disease Study Group). As many factors like age, sex, muscle mass and diet have influence on creatinine level so the utility of other agents like cystatin C are considered to become a tool for diagnosis of chronic kidney disease. Earlier studies suggested that cystatin C could be useful in the detection of early decline of GFR. The aim of the study was to examine the usefulness of serum cystatin C for assessment of kidney function in unselected population of hospital patients with modestly reduced eGFR (50-59 ml/min/1,73 m^2).

Material and methods: The subjects were 247 patients (130 women and 117 men) with eGFR 50-59 ml/min/1,73 m^2. Creatinine was measured by compensated Jaffe method (Roche) and cystatin C by immunonephelometric method (Dade Behring). References ranges for creatinine 0,5-1,1 mg/dl and for cystatin C 0,53-0,95 mg/dl were used. For statistical analyses U Mann-Whitney test and Spearman's rank correlation were applied.

Results: Mean serum creatinine was 1,18±0,17 and cystatin C 0,95±0,29 mg/dl . The values showed significant correlation ($r = 0,14$, $p< 0,05$). Serum creatinine and cystatin C above reference values were observed in 53% and 43% of patients respectively. Serum creatinine was lower in women than in men (1,03 ± 0,06 and 1,34 ± 0,08 mg/dl respectively, $p<0,05$), but difference between concentration of cystatin C in women and men was not significant. In 115 patients with normal level of creatinine 44 (38%) patients had cystatin C above reference values. In 142 patients with normal cystatin C level 71 (50%) patients had higher concentration of creatinine.

Conclusion: Our results suggest, that cystatin C doesn't improve assessment of kidney function in unselected hospital patients with borderline eGFR.

C-22

Diagnostic Utility of Plasma Procalcitonin for Nosocomial Pneumonia in the ICU Setting

S. M. Brown, J. Dallas, M. G. Scott, M. H. Kollef. *Washington University in St. Louis School of Medicine, St. Louis, MO,*

Background: Nosocomial pneumonia is a common infection in the intensive care unit (ICU) setting and includes patients with healthcare-associated pneumonia (HCAP), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP). However, noninfectious conditions can mimic the clinical and radiographic features of nosocomial pneumonia making an accurate diagnosis problematic in many cases. Procalcitonin has been shown to be a specific and sensitive biomarker of some bacterial infections, yet its diagnostic utility in nosocomial pneumonia has yet to be established. This prospective, single-center study was designed to determine the accuracy of plasma procalcitonin concentration for the diagnosis of nosocomial pneumonia in the ICU setting.

Methods: The study population included 104 patients in surgical and medical ICUs with clinically suspected nosocomial pneumonia. Plasma measurement of procalcitonin was obtained using the Bio-Merieux BRAHMS PCT enzyme-linked fluorescent assay on a VIDAS analyzer. Measurements were obtained at 0 and 48 hours after clinical suspicion of pneumonia.

Results: Though the mean procalcitonin concentration was greater in patients with definite nosocomial pneumonia (n=67; 18.3 ± 99.1 ng/mL) than those with definite absence of nosocomial pneumonia (n=12; 1.7 ± 2.0 mg/mL), this difference was not statistically significant (p=0.662). A cut-off value of procalcitonin > 1 ng/mL yielded a diagnostic

sensitivity of 50.0% and a specificity of 48.6% for definite nosocomial pneumonia. Receiver operating curve and multivariate logistic regression analyses demonstrated that measurement of procalcitonin was inferior to clinical parameters for the diagnosis of nosocomial pneumonia. However, compared to patients with an initial procalcitonin value >1 ng/mL, those with lower procalcitonin values had fewer total antibiotic days (13 ± 10.3 days vs. 19.7 ± 12.0 days; p<0.001) and fewer antibiotic days for treatment of nosocomial pneumonia (10.0 ± 5.9 days vs. 14.7 ± 7.4 days; p<0.001).
Conclusion: These results indicate that while measurement of plasma procalcitonin has minimal diagnostic value for nosocomial pneumonia, it does have prognostic value for prediction of prolonged antibiotic therapy.

C-23

A prospective study of the use of HLA-B*1502 genotyping in preventing carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis

P. Chen[1], C. Shen[1], J. Lin[2], C. Ong[3], S. Wu[4], P. Tsai[1], W. Chen[1], W. Chung[5], S. Hung[6], C. Chang[7], C. Chen[1], S. Lin[1], H. Chuan[1], L. Huang[1], Y. Chen[1], L. Chen[7], Y. Chen[1], -. Taiwan SJS Consortium[8]. *[1]Institution of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [2]Chu Shang Show Chwan Hospital, Nan-tou, Taiwan, [3]Chia-Yi Christian Hospital, Chia-Yi, Taiwan, [4]Changhua Christian Hospital, Chang-Hua, Taiwan, [5]Department of Dermatology, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, Taipei, Taiwan, [6]Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, [7]Pharmigene, Palo Alto, CA, [8]Taiwan SJS Consortium, Taipei, Taiwan,*

Background: Carbamazepine (CBZ)-induced Stevens-Johnson syndrome (SJS) and its related disease, toxic epidermal necrolysis (TEN), are strongly associated with the human leukocyte antigen (HLA) B*1502. This study aimed at preventing CBZ-induced SJS/TEN by identifying individuals at risk using HLA-B*1502 genotyping and evaluating the effectiveness of prospective HLA-B*1502 screening.
Methods: Patients, recruited from 26 hospitals, who had not previously received CBZ and would nomrally have been treated with CBZ, underwent prospective HLA-B*1502 screening and HLA-B*1502-positive patients were excluded from CBZ treatment. For ethical considerations, the study was designed as a nonrandomized trial and historical incidence was used as the control. The incidence of CBZ-induced SJS/TEN in the period of CBZ use without HLA-B*1502 screening was compared to that seen with prospective screening.
Results: Between January 2007 and December 2009, 4473 subjects were recruited from 26 participating hospitals in Taiwan. Screening showed that 7.8% of the patients carried the HLA-B*1502 allele and no CBZ was prescribed; instead, they were either given alternative medication or were left on their before-study medication. Of all the enrolled patients, 4.9% developed mild, transient skin rashes, but six had more wide-spread rashes and were hospitalized (final diagnosis: 3 maculopapular eruption, 2 hypersensitivity syndrome, 1 urticaria). None of the 2946 participating patients who were given CBZ developed SJS/TEN, in contrast to the estimated 7 cases of CBZ-SJS/TEN in the historical control (0.23% of CBZ users) (p value=0.0046; Fisher's two-tailed exact test).
Conclusion: Our data suggest that application of HLA-B*1502 genotyping as a screening tool for patients taking CBZ can effectively reduce the incidence of these life-threatening adverse drug reactions.

C-24

Estimation of bone turnover marker by laboratory screening test results

T. Hisahara, H. Kataoka, K. Ogura, T. Sugiura. *Kochi Medical School, Nankoku, Japan,*

Background Osteoporosis is a social problem in aging society with fewer children. The number of patients with osteoporosis in Japan is estimated to be more than 1000 million. It is important to evaluate the vulnerability of the bone at an early stage to decrease the risk of fracture due to osteoporosis. However, bone turnover markers are tested after the symptom actually appears. Accordingly we performed a multiple linear regression analysis using routine screening laboratory tests to determine the important laboratory indices predicting bone turnover.
Methods The database of this study was obtained from routine laboratory test results stored in our laboratory information system. SQL (Structured Query Language) was used to extract the laboratory data from 2945 patients. Bone turnover markers, BAP (bone specific alkaline phosphatase), OC (osterocalcin), ucOC (undercarboxylated osteocalcin), U-DPD (urinary excretion of deoxypyridinoline), or U-NTx (urinary excretion of cross-linked N-telopeptide of type I collagen), were used as objective variables, and screening laboratory test items, TP (total protein), ALB (albumin), ALP (alkaline phosphatase), Cre (creatinine), UN (urea nitrogen), UA (uric acid), T-Cho (total cholesterol), Ca (calcium), Mg (magnesium) and P (inorganic phosphorus), were used as explanatory variables in the multiple linear regression analysis.
Results When U-NTx was used as an objective variable and ALP and Ca, P and Age as explanatory variables, a good correlation was obtained between bone turnover marker and routine laboratory test results : U-NTx = 0.1907 +$(ALP)^{-0.521}$×2.808 + Ca×(-0.00885)+P×(-0.02323) + Age × $0.04906)^{-1/0.377}$ (Multiple correlation coefficient R = 0.79, Squared multiple correlation coefficient adjusted for the degrees of freedom= 0.62, Number of study data= 284). Other bone turnover markers could not be predicted by routine laboratory screening tests.
Conclusion U-NTx, a bone turnover maker, could be predicted by laboratory screening test results.

C-25

Adiponectin as a risk assessment marker for metabolic syndrome in Japanese men

S. Yatsuzuka[1], K. Nakajima[2], T. Nakano[2], Y. Tokita[2], H. Sekine[2], H. Kikuchi[2], T. Nagamine[2], K. Kikkawa[1], M. Akuzawa[1], M. Takahashi[1], Y. Shimomura[1], I. Kobayashi[3]. *[1]Diabetes and Metabolic Disease Research Center, Hidaka Hospital, Takasaki, Japan, [2]Gunma University, Maebashi, Japan, [3]Gumma Paz College, Takasaki, Japan,*

Introduction: We have studied 1,219 Japanese subjects (man 810, woman 408; aged 39-70) for the diagnostic purpose of metabolic syndrome at health-check up in Hidaka Hospital, Takasaki, located near Tokyo. Together with the diagnostic criteria such as abdominal circumference, blood pressure, blood sugar and hyprelipidemia (TG and HDL-C) for the detection of metabolic syndrome, serum adiponectin levels have been recommended to measure for the future risk prediction of cardiovascular disease from large clinical studies. Here we report the serum levels of adiponectin detected by latex assay in metabolic syndrome cases diagnosed by the above Japanese criteria and compared with other diagnostic markers.
Methods: We have classified the 810 cases of men to the three categories. 1. Normal controls without significant diseases (N=382). 2, Non-metabolic syndrome cases with abdominal circumference above 85 cm but less than one metabolic syndrome diagnostic criteria (N=305). 3, Metabolic syndrome with abdominal circumference above 85 cm and more than two other diagnostic criteria (N=123). As the frequency of woman with metabolic syndrome (2.2%; 9/408) was too low to compare the statistical differences among the three groups because of the Japanese criteria (woman abdominal circumference above 90 cm), we have investigated the cases in man only for this study. All the studies were performed under the informed consent and the approval of ethical committee at Hidaka Hospital and Gunma University School of Medicine. Adiponectin and Lp(a) were measured by latex method (Otsuka and Denka, respectively). RLP-C and RLP-TG were measured by immunoseparation method (Otsuka). TC, TG, LDL-C and HDL-C were measured by Denka reagents at Toshiba-Abbott auto-analyzer.
Results: Adiponectin levels in men with metabolic syndrome (median; 5.8 μg/ml) were significantly lower when compared with the cases in non-metabolic syndrome (median; 6.2μg/ml), and normal controls (median; 7.7μg/ml). The frequency of less than 4 μg/ml in metabolic syndrome cases (risk cut off value proposed by Dr. Matsuzawa) was 17.1% (21/123), while non-metabolic syndrome cases were 12.2% (15/123) and normal controls 7.3% (9/123), respectively. Serum RLP-C and RLP-TG levels were significantly higher in metabolic syndrome (median; 8.1 and 26 mg/dL) compared with non-metabolic syndrome (median; 4.7 and 11 mg/dL) and controls (median; 4.3 and 7.6mg/dL), while Lp(a) levels were median 10, 11, 10 mg/dL, respectively and did not show such differences among the groups.
Conclusions: Not all the metabolic syndrome cases showed significantly lower adiponectin levels (less than 4 μg/ml), which has been generally accepted as a high risk group in Japan. We have found that approximately one fifth of metabolic syndrome cases showed significantly low adiponectin levels in Japanese men. These metabolic syndrome cases with significantly low adiponectin levels may be the inevitable target to follow up the risk assessment of cardiovascular disease.

C-26

The prognostic value of the Elecsys S100 assay in patients with suspected cerebral trauma.

D. C. Gaze, N. Leeks, P. O. Collinson. *St George's Healthcare NHS Trust, London, United Kingdom,*

Objective: To evaluate the diagnostic and prognostic value of S100 in patients presenting with suspected cerebral trauma.
Clinical relevance: The early diagnosis or exclusion of cerebral trauma is closely related to survival. Imaging technology to aid diagnosis may not always be available within a short turn around time. A blood borne marker is an attractive cost-effective alternative to imaging for the early diagnosis of cerebral injury. S100 is released into the cerebrospinal fluid and blood from brain tissue following tissue damage.
Methods: Serum from 74 healthy individuals with no prior history of cerebral disease acted as a control group. Serum was obtained daily over the course of 7 days in patients presenting to the Emergency Department with suspected brain injury. Patients were followed up by electronic case note review for a median of 1.67 (95%CI 1.03-2.30)years. All serum samples were stored frozen at -70°C until batched analysis was performed. S100 was determined by electrochemiliuminesence using the Elecsys 2010(Roche Diagnostics). The detection limit of the assay is 0.005µg/L with an upper limit of 39µg/L. The total assay precision is 1.7-2.8% at 0.08 to 3.33µg/L. Parametric and non-parametric statistical analysis was performed using Analyse-it for Microsoft Excel and Kaplan-Meier survival analysis was performed using SPSS.
Results:74 (46 males, 28 females, median age 35years, interquartile range 17-53years) healthy controls had a median S100 concentration of 0.039,(95%CI=0.032-0.046)µg/L. There was no significant difference(p=0.1853) between male (0.033,(0.019-0.042) µg/L) and female (0.038,(0.031-0.044)µg/L) S100 concentrations. The upper 95th centile reference interval was 0.087µg/L. 76 patients (44 male, 32 female, median age 75years interquartile range 55-94years) with suspected brain injury were studied. At day 0, the median S100 concentration was 0.079,(0.068-0.102)µg/L. At follow up, there were 20 (26%) deaths. Serum S100 was significantly higher(p=0.0032) in those who died (0.115,(0.07-0.164)µg/L) compared to those alive (0.075(0.062-0.089)µg/L at follow up. The peak S100 concentration was 0.111,(0.083-0.145)µg/L. although significantly different between patients who were alive or dead at follow up, both S100 at day 0 and the peak S100 concentration did not predict death (Chi-Square=2.626,p=0.105 and 0.001,p=0.978 respectively).
Conclusions: Serum S100 is significantly higher in patients who present with acute cerebral injury however S100 determination alone is not a prognostic risk marker. Further studies are required to evaluate the usefulness of S100 in relation to cerebral imaging data.

C-27

Nuclear factor-kappa B activity in the peripheral blood lymphocytes correlates with metabolic syndrome-related biomarkers in patients with essential hypertension

K. Harada, F. Tomoda, S. Kagitani, T. Koike, H. Inoue, I. Kitajima. *University of Toyama, Toyama, Japan,*

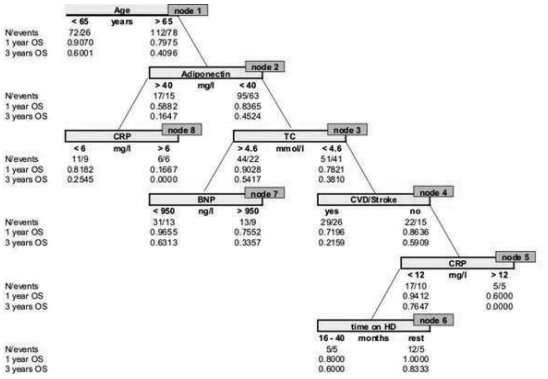
Background Vascular inflammation plays critical roles in the pathogenesis of atherosclerosis, which contributes to the elevation of blood pressure. Nuclear factor-kappa B (NF-κB) is a transcription factor and is known as a key regulator of many inflammatory processes. The expression of various inflammatory cytokines is regulated by NF-κB. We hypothesized that NF-κB activity could be the useful indicator of systemic and/or vascular inflammation in patients with hypertension. However, convenient assay systems to measure the NF-κB activity quantitatively in a timely manner have not been available in the setting of a hospital laboratory. Therefore, we have established a novel measurement system for the NF-κB activity using fluorescence correlation spectroscopy (FCS). We used this method to evaluate NF-κB activity and examined the correlation of NF-κB activity with other clinical parameters in 45 untreated patients with essential hypertension.
Methods FCS was a methodology to examine the size and number of fluorescence-labeled molecules in a confocal area by direct observation of their Brownian motion in solutions. The principle of the measurement was to detect the different of molecular weights of fluorescent-labeled DNA probe and DNA probe-NF-κB complex and to count the number of these molecules separately. We collected peripheral blood lymphocytes from 45 patients (mean age±SD: 51.8±13.1) with hypertension, who have no sign of renal dysfunction (eGFR > 60mL/min/1.73m^2), and examined the NF-κB activity in the nuclear extracts of lymphocytes. We assessed the correlation between NF-κB activity and other biomarkers by multiple stepwise regression analysis and logistic regression analysis.
Results NF-κB activity in patients with essential hypertension (mean±SD: 0.19±0.15 ng/µg of nuclear protein) was positively correlated with serum triglyceride levels (r=0.405, p<0.05), serum total cholesterol levels (r=0.384, p<0.05), serum lactate levels (r=0.374, p<0.05), body mass index (r=0.301, p<0.05), and daytime diastolic blood pressure (r=0.320, p<0.05). On the other hand, it was inversely associated with creatinine clearance (r=-0.295, p=0.052). Multiple stepwise regression analysis revealed that serum total cholesterol levels, serum triglyceride levels, and serum lactate levels were independently correlated with NF-κB activity (R^2=0.3270). Eighteen out of 45 patients were diagnosed with metabolic syndrome. NF-κB activity in patients with or without metabolic syndrome was 0.25±0.17 and 0.14±0.12 ng/µg of nuclear protein, respectively. In logistic regression analysis, NF-κB activity was significantly correlated with patients with metabolic syndrome (p<0.0001).
Conclusions These results suggest that the elevation of NF-κB activity reflects the systemic and/or vascular inflammation caused by dyslipidemia in the patients with hypertension. The development of this assay system using the FCS technique enables us to rapidly measure the NF-κB activity with high sensitivity in nuclear extracts of lymphocytes. Measuring the NF-κB activity in the patients with essential hypertension would be useful as an inflammatory marker to evaluate patients with metabolic syndrome-associated hypertension.

C-28

Adiponectin levels are useful in stratification of hemodialysis (HD) patients

J. Racek[1], D. Rajdl[1], M. Vostrý[1], J. Eiselt[1], L. Malánová[2], L. Trefil[1]. [1]*Charles University Hospital, Pilsen, Czech Republic,* [2]*Hemodialysis Center B. Braun Avitum, Pilsen, Czech Republic,*

Background: HD patients suffer from highly increased overall mortality. Simple algorithms for differentiating high-risk individuals are useful for improving HD patients survival rates. Adiponectin (ADPN) levels are associated with both positive and negative outcomes. Natriuretic peptide type B (BNP) is a marker of heart failure - the leading cause of death in HD patients. We aimed to prove, whether adiponectin and BNP, in addition to traditional markers, can be useful in selecting high-risk HD patients.
Methods: We analyzed total ADPN (ELISA), BNP (chemiluminescent immunoassay), C-reactive protein (CRP), albumin, hemoglobin, total cholesterol (TC), HDL cholesterol by routine methods in blood samples of 184 HD patients (123 males); median (interquartile range) of age 68 (59-74) years. For multivariate statistical analysis, classification and regression tree (CART) was used. Predictors in each node of CART were found by stepwise multivariate Cox regression model, only predictors with p<0.05 were included. In addition to above mentioned analytes, age, sex, time on HD, BMI, smoking, presence of diabetes and history of cardiovascular disease or stroke (CVD/Stroke) were tested for inclusion in the final model.
Results: ADPN levels with cut-off value 40 mg/l were the second most important factor in differentiating high-risk patients. Furthermore, BNP was the seventh most important factor. From traditional predictors, age, TC, CVD/Stroke, CRP and time on HD proved their usefulness. The resulting CART is depicted in the picture (N = number of patients; 1 or 3 year OS = 1 or 3 year overall survival; in simple words, reading from top to bottom is algorithm of subsequent questions that leads to the most efficient selection of high-risk patients).
Conclusions: ADPN and BNP levels in HD patients can be useful in differentiating high risk patients. Surprisingly, some of more traditional survival factors (albumin, hemoglobin) did not prove their value in this setting.



C-29

*CYP2C19*2* allele carriers have longer time to treatment failure on tamoxifen therapy in advanced breast cancer treatment

R. H. N. van Schaik[1], M. Kok[2], F. C. J. G. Sweep[3], M. van Vliet[1], M. Look[4], M. van Fessem[1], M. Meijer-van Gelder[4], C. Seynaeve[1], J. Lindemans[1], J. Wesseling[2], L. van 't Veer[2], P. Span[3], H. van Laarhoven[3], S. Sleijfer[1], J. Foekens[4], S. Linn[2], E. M. J. J. Berns[4]. *[1]Erasmus University Medical Center, Rotterdam, Netherlands, [2]Netherlands Cancer Institute, Amsterdam, Netherlands, [3]St Radboud University Hospital, Nijmegen, Netherlands, [4]Josephine Nefkens Institute, Rotterdam, Netherlands,*

Background: The anti-estrogen tamoxifen is frequently used in the treatment of breast cancer, either as adjuvant or for treatment of advanced disease. Tamoxifen is metabolized by cytochrome-P450 enzymes, with endoxifen as most active metabolite. *CYP2D6* poor metabolizer genotypes have been associated with worse therapy outcome of adjuvant tamoxifen for breast cancer, although contradictory results have been published. In addition, *CYP2C19*17* was associated with a better outcome on adjuvant tamoxifen. However, results on *CYP2D6* and *CYP2C19* genotype on outcome for tamoxifen used in the advanced disease are currently lacking.

Objective: to investigate the effect of *CYP2C19* polymorphisms on the effectivity of tamoxifen treatment in advanced breast cancer.

Methodology: We performed a retrospective multicenter study on *CYP2C19* and *CYP2D6* genetic polymorphisms of 499 estrogen receptor (ER)-positive primary breast tumor specimens of patients treated with tamoxifen as first-line therapy for advanced disease. Primary endpoint was the Time to Treatment Failure (TTF).

Results: A significantly longer TTF was found for *CYP2C19*2* carriers compared to non-carriers (HR (95% CI): 0.72 (0.57-0.90), p=0.004). *CYP2C19*17* carriers had a shorter TTF. The *CYP2C19*2* results remained significant in a pooled multivariable analysis, stratified for center and time between primary diagnosis and occurrence of metastases, corrected for menopausal status at start of treatment, adjuvant chemotherapy and *CYP2D6*4* status. The *CYP2D6*4* status itself was not associated with TTF. To investigate whether the effect was indeed treatment related, we genotyped an independent breast cancer patients cohort, not treated with tamoxifen after surgery. *CYP2C19*2* carrier status was not related to disease free survival. Surprisingly, *CYP2C19*17* carriers had a significant longer disease free survival compared to non-carriers (HR=0.66 (0.46-0.95), p=0.025).

Conclusion: *CYP2C19*2* is associated with a longer TTF in hormone-sensitive breast cancer patients treated with tamoxifen for advanced disease. In contrast, *CYP2C19*17* carriers have a significantly longer disease free survival, and is thus identified as a prognostic factor. These findings shed new light on the involvement of CYP2C19 in breast cancer progression and breast cancer treatment with tamoxifen.

C-30

Serum leptin is able to distinguish autoimmune pancreatitis from both chronic pancreatitis and pancreatic neoplasms

A. Barassi[1], R. Pezzilli[2], M. M. Corsi[3], A. M. Morselli-Labate[2], D. Campana[2], G. Dogliotti[3], R. Casadei[4], D. Santini[5], R. Pacciolla[1], R. Corinaldesi[2], G. V. Melzi d'Eril[1]. *[1]Department of Medicine, Surgery and Dentistry, University of Milan, San Paolo Hospital, Milan, Italy, [2]S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy, [3]Institute of General Pathology, University of Milan, Milan, Italy, [4]Departmentsof Emergency, General and Transplant Surgery, University of Bologna, Bologna, Italy, [5]Department of pathology, Sant'Orsola-Malpighi, Hospital, University of Bologna, Bologna, Italy,*

Background. Serum leptin and adiponectin determinations have been proposed as markers for distinguishing pancreatic cancer and chronic pancreatitis from autoimmune pancreatitis; however, no studies exist in patients with autoimmune pancreatitis and in those with intraductal papillary mucinous tumors of the pancreas. The aim of this study was to evaluate the circulating concentrations of receptor for advanced glycation end products (RAGE), leptin and adiponectin in patients with chronic pancreatic diseases.

Material and methods. Seventy-five consecutive patients with chronic pancreatic diseases (47 males, 28 females; mean age 67.0 ± 13.2 years; range 37-97 years) were studied: six (8.0%) had autoimmune pancreatitis, 23 (30.7%) had chronic pancreatitis, 34 (45.3%) had pancreatic cancer and the remaining 12 (16.0%) had intraductal papillary mucinous tumors of the pancreas. Serum leptin, adiponectin and RAGE were determined using commercially available kits (R&D Systems, Minneapolis, MN). The intra-assay coefficients of variation (CVs) were < 3.9% and the inter-assay CVs were < 7.9%. The normal reference limits of our laboratory are 2205-11 149 pg/ml in males and 3877-77 273 pg/ml in females for leptin, 865-21 424 ng/ml for adiponectin and 368-4354 pg/ml for RAGE. The distribution of data was assessed using the Kolmogorov-Smirnov test. Normalized leptin showed significant non-normal distributions and had a positive skewness. Log transformation normalized its distribution.

Results. Normalized leptin concentrations were significantly lower in chronic pancreatitis patients (0.53 ± 1.28; p = 0.008) and in those with pancreatic cancer (0.12 ± 0.33; p < 0.001) compared to the overall population (0.58 ± 1.23), whereas autoimmune pancreatitis patients had significantly higher concentrations of this protein (2.18 ± 2.56; p = 0.004) compared to the overall population. RAGE and adiponectin concentrations were similar among the four groups of patients studied. Among the clinical variables considered, only pain was significantly related to leptin concentrations (patients with pain 0.18 ± 0.54, patients without pain 1.07 ± 1.64; p = 0.001).

Conclusion. Serum leptin seems to be a good serum marker for differentiating autoimmune pancreatitis patients from those with chronic pancreatitis and pancreatic cancer.

C-31

2003 vs 2010 American Diabetes Association criteria for the diagnosis of diabetes mellitus: comparing the prevalence of type 2 diabetes and its categories.

K. Santos-Rey, P. Fernández-Riejos, J. Mateo, V. Sánchez-Margalet, R. Goberna. *HUVM, Sevilla, Spain,*

Background HbA_{1c}, traditionally used as a marker for chronic glycemic exposure, has recently been proposed as a new criteria for the diagnosis of type 2 diabetes. Nevertheless, the proposed cutoff for the diagnosis, held in 6.5 %, has triggered a large debate due to the potential differences in patients classification. The aim of this study was to compare both glycemia-based 2003 American Diabetes Association (ADA) criteria and the HbA_{1c}-based 2010 ADA recently proposed criteria in a cohort of individuals at high risk of developing type 2 diabetes.

Methods A total of 781consecutive individuals at high risk of developing type 2 diabetes were included in this study. Basal glycemia and HbA_{1c} were analysed in every individual. An oral glucose tolerance test (OGTT) was performed to everyone who had a basal glycemia under 7.0 mmol/L. HbA_{1c} was measured using an HPLC method. Our laboratory follows the NGSP recommendations for HbA_{1c} measurement, with a Level I Certification.

Results Using the 2003 ADA criteria for the diagnosis of type 2 diabetes, 260 individuals were normoglicemic, 214 presented impaired fasting glycemia, 128 impaired glucose tolerance and 179 type 2 diabetes. On the other hand, using the ADA 2010 diagnosis HbA_{1c} criteria, the three resulting categories were the following: 512 individuals inside the low risk categorie (HbA_{1c} < 5.7%), 204 inside the high risk categorie (HbA_{1c} 5.7-6.4%) and 37 inside the diabetes categorie ($HbA_{1c} \geq 6.5\%$).

Conclusion Comparing both diagnosis panels, the new HbA_{1c} - based criteria seems to underdiagnose both diabetes and prediabetes.

C-32

High Sensitivity C-Reactive Protein Concentrations and Metabolic Syndrome in Thai Population

S. Vanavanan[1], A. Chittamma[2], P. Suriyawongpaisal[3]. *[1]Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand, [2]Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol Universit,Ratchatevi, Thailand, [3]Center of Community Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand,*

Background: Many studies have shown that inflammation marker, high sensitivity C-reactive protein (hsCRP), is associated with metabolic syndrome (MetSyn) and both are risk factors for cardiovascular disease. The purpose of this study is to examine hsCRP levels in Thai population aged range 35 to 74 years old with and without MetSyn.

Methods: Serum hsCRP were determined in 3,679 sera obtained from participants of the International Collaborative Study on Atherosclerosis and Stroke in Asia (InterASIA) in 2000 using nephelometric method on BN 100 analyzer. MetSyn was characterized by modification of the Thrid Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP/ATP III).

Results: MetSyn was diagnosed in 38.9% of the participants. The geometric mean with 95% CI of hsCRP was significantly higher in participants with MetSyn (2.03 mg/L; 1.94 to 2.12 mg/L) than those participants without MetSyn (1.10 mg/L; 1.07 to 1.14 mg/L), $p<0.05$ (Fig. 1). In participants with hsCRP > 3.00 mg/L, we found 56.7% was diagnosed in MetSyn and a relation between hsCRP concentrations and MetSyn was statistical significant ($p<0.05$). Using the logistic linear regression analysis, we found all MetSyn criteria components were significantly associated with hsCRP concentrations ($p>0.05$).

Conclusion: HsCRP was elevated in cases of MetSyn in the Thai population. According to its ability on prediction of future cardiovascular disease, these findings is useful for providing information to guide health planners to develop strategies plans for reducing MetSyn in Thailand, especially in population with high concentrations of hsCRP.

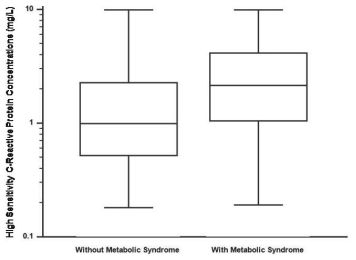


Figure 1 High Sensitivity C-Reactive Protein Concentrations (mg/L) in Thai Adults without and with Metabolic Syndrome

C-33

Urinary and plasma NGAL levels reflect the severity of acute kidney injury

K. Bangert, L. O. Uttenthal. *BioPorto Diagnostics A/S, Gentofte, Denmark,*

Background NGAL levels are dramatically increased in urine and plasma from patients with acute kidney injury (AKI). In this study we tested the clinical utility of NGAL in critically ill patients with many confounding conditions including sepsis and various adenocarcinomas.

Methods Urinary and plasma NGAL were monitored (daily to alternate days) in 135 consecutive patients admitted to intensive care. AKI was classified according to the RIFLE criteria. Three patients were excluded because of incomplete data, e.g. due to early transfer. NGAL was determined with a commercially available ELISA kit (BioPorto Diagnostics). Differences between maximal NGAL levels in the patient were analyzed non-parametrically by the Kruskal-Wallis test. Data are reported as median (interquartile range) in ng/mL.

Results Sixty two patients did not have AKI; 15 were classified as "risk" (R), 11 as "injury" (I) and 44 as "failure" (F). The urinary NGAL was significantly ($p<0.0001$) higher in patients with AKI [R 377 (102-830), I 519 (321-1845) and F 2747 (559-7621)] than in patients without AKI [51 (29-213)]. Plasma NGAL levels were significantly ($p<0.0001$) higher in patients with AKI [R 354 (259-511), I 546 (331-1106) and F 1062 (584-1817)] than in patients without AKI [199 (128-308)].

ConclusionsIt is concluded that NGAL is dramatically increased in the urine and plasma of unselected critically ill patients with AKI and the degree of injury is reflected by the observed levels despite several confounding conditions. NGAL determination may therefore be useful for the diagnosis of renal injury and monitoring the management of patients admitted to intensive care.

Wednesday AM, July 28

Poster Session: 10:00 am – 12:30 pm
Cardiac Markers

C-34

Myeloperoxidase - an evaluation

S. Ong, S. Lim, B. Saw, S. Saw, S. Sethi. *National University Hospital, Singapore, Singapore,*

Introduction: Myeloperoxidase(MPO), a haemoprotein is released by leukocytes on activation and has been associated with plaque instability. At our Emergency Medicine Department(ED), patients presented with chest pain are triaged into: a)ECG changes with ST elevation, b)ECG changes with non-ST elevation, and c)no ECG changes. Troponin T(TnT, Cobas 411Roche, Germany) is performed in the latter 2 groups of patients. In this study we evaluated MPO using acridinium-labeled chemiluminescent microparticle immunoassay on ARCHITECT-i System 1000SR™(Abbott USA) and correlated MPO levels with TnT levels measured on sera from ED samples.
Methods: We used EDTA plasma samples from ED patients with known TnT levels. Precision studies were conducted over ten days. Interference studies were conducted on triglycerides and hemoglobin spiked samples, and recovery studies using high and low pooled plasma samples dilution. Volunteers consented using the DSRB protocol submitted for the study.
Results: Precision studies data performed on 4 levels of MPO(69.3-3732.3pmol/L) shows intra-day(n=40) %CV mean=4.8%(range 2.6-5.2%) and inter-day(n=40) %CV mean=8.8%(range 3.8-15.1%). Recovery range =100-107% (131.6- 4738.6pmol/L) with regression y(measured MPO)=1.01(calculated MPO)+40.51. Triglycerides and haemoglobin interfered at 1182mg/dL and 6825 mg/dL respectively. Reference interval validation (n=21) is median 57.3 pmol/L (range 14.2-335.6). In negative TnT group (<0.03ug/L, n=18), MPO median 1016.7 pmol/L(range 265.9-6808.5) and positive TnT group (0.03-7.9ug/L, n=22), MPO median 1147.1pmol/L (range 114.9-4307.0).
Limitation: Clinical details were not available for the EDTA samples used.
Conclusion: The ARCHITECT-i MPO is precise and available readily on STAT(assay 15 minutes). Higher levels of MPO in samples with TnT<0.03ug/L compared with lower MPO levels from positive TnT, could suggest myocardium activity upstream in the acute coronary syndrome continuum. Further study in collaboration with cardiologists and ED consultants is in progress.

C-35

Evaluation of test sensitivity of troponin I - clinical correlation.

C. E. Ferreira, J. B. Rabelo, A. C. L. Faulhaber, L. M. A. Forlenza, C. L. P. Mangueira, J. Pasternak. *Hospital Albert Einstein, São Paulo, Brazil,*

Introduction: Several sensitive tests for troponin are available at the diagnostic market. The loss of specificity for acute coronary syndrome, high specificity for cardiomyocytes lesion and high prognostic value allows the test to be applied in other areas: Surgery, Oncology and Infectious Diseases, among others.
Objectives: To evaluate the routine troponin I in a general hospital of 500 beds and correlate it with clinical situations.
Methods: We evaluated all doses of troponin I that were positive in the period from May 09 to November 09 and correlated with the main differential diagnosis.
Results: During this period 4559 assays were done and 1540 tests had values above the cutoff point (0.034 ng /mL) - 33.7%. These are the main diagnosis of patients with tests with results over the cut off value: Acute Coronary Syndrome (25%), cardiac surgery (7.8%), heart failure (7.7%), chest pain (7%), arrhythmia (5.7%), respiratory failure (5.7%), angioplasty (5.2%), noncardiac surgery (5%), sepsis (4.3%), other infectious processes (3.4%), acute pulmonary edema (2.7 %), cardiac catheterization (2.4%), hypertension / pulmonary thromboembolism / Kidney failure / Death (0-2% each) and other diagnoses (13%) - Cancer, Transplantation, Diabetes and other disease situations..
Conclusion: The increased sensitivity of the assay of troponin I allowed a high positive (33.7%) of the test, with a low specificity for acute coronary syndrome, high specificity to cardiomyocytes and the high prognostic value of troponin I test gaining ground in clinical practice for other diseases.

C-36

Effect of Unsupervised Exercise on Autonomic System Regulation and Biochemical Markers in Patients with Chronic Heart Failure.

K. Tsarouhas[1], C. Tsitsimpikou[2], E. Rentoukas[1], S. Adamopoulos[3], H. Tsafaras[4], A. Haliassos[4]. *[1]Amalia Fleming General Hospital, Athens, Greece, [2]Department of Narcotics, 3rd Chemical Service of Athens, General Chemical State Laboratory of Greece, Athens, Greece, [3]Department of Cardiovascular Medicine, Onassis Cardiac Surgery Center, Athens, Greece, [4]Diamedica SA, Athens, Greece,*

Few studies have addressed the impact of moderate unsupervised everyday physical activity in chronic heart failure (CHF) patients. We investigated the effects of a 12-week walking programme on heart rate recovery (HRR), index of the autonomic system equilibrium, on common biochemical markers (cholesterol, LDL, HDL, triglycerides, glucose) and on serum modulators of endothelial function (i.e., asymmetric dimethylarginine - ADMA and homocysteine) in CHF patients. Twenty-four stabilized CHF patients of NHYA class II and III volunteered to participate either in the exercise (n=14) or in the non-exercise (n=10) groups. The exercise programme consisted of 5 days/week unsupervised 40-min walking sessions. All patients underwent blood collection, an echocardiographic evaluation and a Bruce-modified treadmill exercise stress test before and just after the 12-weeks intervention exercise programme. Non-fasting venous blood samples were drawn in the morning and after a supine rest of at least 30 min, centrifugated within 45 min and stored at -20°C. Serum samples were then analysed for ADMA and homocysteine using ADMA-ELISA kit by DLD and FPIA IMx Homocysteine assay by Abbott, respectively. Repeated measures tested by ANOVA analysis revealed significant improvements in HRR ($p<0.001$) in the exercise patients. HDL values were statistically higher ($p<0.03$) and glucose levels statistically lower ($p=0.052$) at the end of the intervention. The non-statistical decrease in all other markers observed could be attributed to the fact that our patients were under statin treatment. ADMA levels in HF patients at baseline were found statistically higher than reference values ($p<0.03$), while decrease in ADMA levels after walking was associated with HRR changes ($r=0.79$, $p=0.007$). Homocysteine levels both at baseline and at the end of the walking intervention, while, non-significantly, decreased in the exercise group, were still higher than reference values. Decrease in ADMA and homocysteine were positively correlated with decrease in LDL ($p<0.02$), while increase of HDL was associated with decrease in TG ($p=0.052$). Average walking distance positively correlated with homocysteine decrease ($p<0.052$). In conclusion, a 12-week unsupervised walking exhibits a pronounced amelioration of HRR, exerts a positive impact on all biochemical markers monitored in this study and possibly attenuates endothelial damage in CHF patients.

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Determinants of blood levels of putative thrombogenic factors in healthy Arab adolescent subjects.

A. O. Akanji, L. Thalib, A. N. Al-Isa. *Kuwait University Faculty of Medicine, Kuwait, Kuwait,*

Background: Clinical presentation with atherosclerotic disease (AD) is linked to plaque rupture and thrombosis possibly related to altered homeostasis of thrombogenic factors. It is unclear if this change can be predicted from blood levels of these factors as observed in children and adolescents at increased risk. This study aims to examine blood levels of lipoprotein(a) and the thrombogenic factors - fibrinogen and plasminogen activator inhibitor -1 (PAI-1) - in healthy adolescents stratified according to gender, pubertal status, body mass and insulin sensitivity.
Methods: There were a total of 774 (316m, 458f) subjects aged 10-19yr. Anthropometry and biochemical tests were carried out on each to determine insulin sensitivity (HOMA-IR) and blood levels of lipids (TG, total cholesterol, LDL, HDL, apo B, lipoprotein(a), PAI-1 and fibrinogen.
Results: HOMA-IR, PAI-1 and fibrinogen levels were higher in the boys than in the girls (all $p < 0.01$).. Additionally, for both sexes, these parameters were greater in the overweight/obese in comparison to the normal weight subjects, suggesting an increased atherogenic and thrombogenic risk. Lp(a) levels were not affected by gender and body mass. Findings in relation to pubertal age differed somewhat between the boys and girls, with a tendency in the girls towards more deleterious levels when post-pubertal, less so with the boys. On simple correlative analyses, the important determinants of these variables are for: Lp(a) - (LDL, apo B and HOMA-IR); PAI-1 - (LDL, apo B, HOMA-IR); fibrinogen (age, BMI, LDL, apo B, HOMA-IR. These determinants remained independent on multiple regression analyses.
Conclusion: Levels of novel putative thrombogenic variables in blood of healthy Kuwaiti adolescent subjects are influenced by age, gender, body mass and insulin sensitivity. It is important to assess these factors in assigning future global atherosclerotic disease risk.
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Folate, vitamin B12 and homocysteine levels in Arab adolescent subjects: potential implications for cardiovascular disease risk in later life.

A. O. Akanji, A. N. Al-Isa, L. Thalib. *Kuwait University Faculty of Medicine, Kuwait, Kuwait,*

Background: Elevated circulating fasting total homocysteine (tHcy) concentration is associated with an increased risk of occlusive vascular disease in adults. Important determinants of tHcy levels are folate and vitamin B12. This study attempts to investigate age, gender, and body mass as determinants of folate, vitamin B12 and tHcy levels in Kuwaiti Arab adolescents and to propose population, gender & age-specific reference ranges for these biomarkers.

Methods: A total of 774 (316 boys, 458 girls) apparently healthy 10-19 yr olds attending various secondary schools in Kuwait were assessed for anthropometry, blood pressure and fasting blood levels of Hcy, folate and vitamin B12, after informed voluntary parental/ guardian consent.

Results: Boys had significantly higher waist/hip ratio, tHcy and folate concentration than girls, although BMI was similar for both groups. Vitamin B12 was greater in the girls. In all, tHcy had positive correlations with both markers of adiposity (BMI and WHR) and with systolic blood pressure. Additionally, tHcy was negatively correlated with folate and vitamin B12. These relationships persisted on multiple regression. Folate and vitamin B12 levels decreased significantly with age. Correspondingly, Hcy levels increased, with mean values (umol/L) for boys (6.6; 7.9) and girls (4.5; 6.2) aged 10-14yr and 14-19 yr respectively.

Conclusion: There is an age-related increase in tHcy in adolescents reflecting decreased levels of folate and vitamin B12. It is suggested that age-related reference ranges for tHcy and associated vitamins be used in adolescents, to assess and prevent future cardiovascular disease risk.

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The Study on the Correlation and the Preanalytical Variables of BNP and NT-proBNP

W. Dai, Y. Li, M. Wang. *Renmin Hospital of Wuhan University, Wuhan, China,*

Background: We aimed at studying the correlation between plasma BNP and NT-proBNP among the patients with suspected chronic heart failure and evaluating the preanalytical variables of plasma BNP and NT-proBNP.

Methods: Plasma BNP and NT-proBNP were detected in 35 individuals with suspected chronic heart failure (17 males and 18 females). Blood samples were collected in plastic EDTA-containing tubes at room temperature (25 °C) and the plasma levels of BNP and NT-proBNP were determined to evaluate the stability of the two biomarkers. Detection was performed at time points 0h, 2h, 4h, 8h, 16h, 24h. Blood samples were collected in plastic tubes containing coagulant, EDTA-K_2 anticoagulation and heparin sodium to evaluate find out which type of sample was most suitable for test.

Results: There was a significantly positive correlation between Plasma BNP and NT-proBNP (r=0.949). At room temperature, after 24h, the concentration of BNP decreased 37.1%, while the concentration of NT-proBNP almost unchanged. One-way ANOVA result showed the detection of BNP had significant difference between serum, EDTA-K_2 anticoagulation sample and heparin sodium anticoagulation sample (p<0.05). However, there was no significant difference in the detection of NT-proBNP.

Conclusion: There was a significantly positive correlation between Plasma BNP and NT-proBNP. At room temperature, Plasma NT-proBNP is more stable than BNP. Among the different blood collection tubes of the detection of BNP, only EDTA plastic tubes were acceptable, but NT-proBNP can be detected in serum, EDTA-K_2 anticoagulation sample and heparin sodium anticoagulation sample. In conclusion, Plasma NT-proBNP was a more stable diagnostic biochemical marker of heart failure and appeared to have fewer effects of preanalytical variables than BNP.

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Distribution of Trible1 gene polymorphisms and the association of Trible1 gene polymorphisms with coronary artery disease in Han population of Hubei province

G. Song, S. Guo, F. Zheng, N. Yang, Z. Liu. *Zhongnan Hospital of Wuhan University, Wuhan, China,*

Background: To investigate the distribution of two TagSNPs (one in intron region and one in 3'untranslated region) in *TRIB1* gene in Han population in Hubei province and compare the distributions of the genotypes and alleles in controls and coronary artery disease group.

Methods: TagSNPs were chosen from hapmap database, then specific primers were designed to amplify regions containing the selected TagSNPs. Using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, the distributions of the two TagSNPs were typed and further verified by sequencing in 139 coronary artery disease sufferers and 236 normal subjects of Han population. The level of plasma triglyceride and cholesterol were detected, and all the datas were analyzed by SPSS14.0.

Results: The genotyping results were coincided with the sequencing results. There is significant difference for triglyceride in the subtypes of rs2235110 in coronary artery disease group. All the alleles in every locus of normal controls were in Hardy-Weinberg balance(rs2235110:χ^2=0.481,p=0.488;rs2235108:χ^2=0.570,p=0.450). No significant difference was found for the allele and genotype distributions of two TagSNPs between controls and patients. There was moderate polymorphism information content (PIC) in the chosen TagSNP rs2235110 (PIC=0.3687,H=0.4875), almost to the maximum theory value of PIC.

Conclusions: Though no significant difference in the distributions of the polymorphisms between the controls and the patients was found, the distributions of the genotypes and alleles for each TagSNP in this study represented the variants of the Trible 1 gene in Han population of Hubei province, those two TagSNPs combined with other TagSNPs in HapMap could be used as an ideal genetic mark.

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The detection for promoters' methylation status in atherosclerosis related genes by methylation array chip

S. Guo, F. Zheng, N. Yang, X. Zhou, Y. Xie. *Zhongnan Hospital of Wuhan University, Wuhan, China,*

Background: Recently, it is reported that epigenetic changes are closely associated with the development and progress of atherosclerosis. One of the best known epigenetic factors is DNA methylation. This research aims to explore the relationship between atherosclerosis and the methylation of promoter regions.

Methods: The surgical resection specimens of two patients with atherosclerosis were collected. Endothelial tissues without lesion surrounding artery plaque were defined as the control tissues. Roche's NimbleGen HG18 promoter methylation array chip was used to screen the differentially methylated genes between atherosclerotic tissue and normal tissue in genome-wide. Genomic DNA of tissues were extracted using proteinase k method and randomly sheared by sonication to generate fragments between 400 and 500 bp, and denatured into single-stranded DNA samples by heat. Single-strand DNAs of two patients atherosclerosis tissues were mixed equally and labled as the disease group, the single-strand DNA of the two patient control tissues as normal group. The DNA sample of each group was divided into two parts. One part was left untreated to serve as Input DNA control; the other part of single-stranded DNA sample was immunoprecipitated with an monoclonal antibody against 5-methylcytidine (5mC) and purified as MeDIP DNA. Input DNA (IN) and MeDIP DNA (M) were linearly amplified and differentially labeled with Cy5 (red) and Cy3 (green). Then the two parts were mixed, denatured and co-hybridized on the methylation microarray chip. The methylation level is measured as the intensity ratio of MeDIP to input DNA. Two representative differentially methylated genes (SOD2 and INSIG1) promoters methylation status were validated by sequencing of bisulfite-converted DNA. Specific primers for for SOD2 and INSIG1 were designed to amplify the methylation different regions. The PCR products were subcloned into T vector and the positive clones were sequenced. At least five positive clones were picked for each sample.

Results: There were totally 2611 differentially methylated genes between the two-parallel chips. The extremely differentially methylated genes contained 26 hypermethylated genes and 29 hypomethylated genes. The moderately differentially methylated genes included 2178 hypomethylated genes and 378 hypermethylated genes. The chip results showed that the differentially methylated genes mainly pertained to genes associated with glucose and lipid metabolism, DNA synthesis and transcription factor genes, cellular signal transduction protein and inflammation related genes. The sequencing results of SOD2 and INSIG1 promoters were in according with those of the methylation array chip.

Conclusions: The screening results of this study not only provided clues to further study the exact molecular mechanism of gene promoter methylation in atherosclerosis disease, but also supply new targets for the treatment of atherosclerosis disease and drug development.

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Biochemical Study Of Emerging Risk Markers In Coronary Heart Disease: our experience

H. V. Singh[1], N. Singh[2], S. Singh[2], A. Raizada[3], N. Trehan[4]. *[1]Hindu Rao Hospital, Delhi-110007, India, [2]G.R.Medical College, Gwalior-474001 MP, India, [3]Medanta-The Medicity, Gurgaon-122001 Haryana, India, [4]Director and Senior Cardiothoracic and Vascular Surgeon, Medanta-The Medicity, Gurgaon-122001 Haryana, India,*

Introduction To provide the best treatment for coronary heart disease (CHD) patients, one must go beyond LDL cholesterol to detect factors contributing to CHD risk or existing CHD. These emerging risk factors appear to identify individuals at increased CHD risk and the assessment of these can modulate clinical judgment when making therapeutic decisions. The objective of the study was to access the five risk markers namely lipoprotein (a) (Lp(a)), high sensitive c-reactive protein (hs-CRP), Total antioxidant status (TAS), homocysteine and Fibrinogen.

Methods Study was carried out by taking 396 subjects, out of which 127 control cases were selected by screening with lipid profile, treadmill test (TMT) and fasting blood sugar, to ensure that they are not suffering from hyperlipidemias, dyslipidemia or diabetes. 269 angiographically proven CAD cases were selected for the present work. Lp(a), hs-CRP, TAS was estimated using Hitachi 912 analyser. The levels of Homocysteine were estimated by EIA method using Bio-Rad kit in Bio-Rad CODA analyzer. The levels of fibrinogen were estimated by Clauss method in Sysmax Autoanalyzer.

Results Emerging markers Lp(a), hs-CRP, homocysteine and fibrinogen levels were significantly increased in CAD cases. The tHcy level was also significantly increased ($p<0.001$) where as oxidative stress indices like TAS was significantly decreased ($p<0.001$) in the CAD group and which is supported by the elevated lipid peroxidation product MDA in both groups.

Conclusions Our study shows that these markers are more closely associated with atherosclerosis. Recent cardiac markers i.e. homocysteine and Lp (a) showed no significant correlation in our study with other parameters therefore they may be considered as independent markers. Lp (a) is closely associated with LDL and low values of HDL-C so it could also be considered as independent marker for CAD assessment. The inflammatory markers hs-CRP and fibrinogen emerged as a marker of choice to diagnose atherosclerosis. Many previous studies also showed that persistent hyperglycemia can cause oxidative stress followed by release of inflammatory markers and then formation of atheroma in vessels. The screening with the emerging risk markers is helpful in risk assessment of coronary Heart disease. This additional diagnostic information can assist in the prevention or early diagnosis and treatment of atherosclerotic cardiovascular disease.

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Prognostic markers for atherosclerotic vascular lesions in patients with chronic renal disease

A. A. Abdel Hady, M. Shehata, D. El Abd, F. El Shanawani, E. Abd Allah, D. H, M. Moghazy. *Theodor Bilharz Research Insitute, Giza, Egypt,*

Background: Endothelial dysfunction has been regarded to as an early stage in the atherosclerotic process and could be evaluated by invasive methods (e.g. catheterization) and or non invasive methods (e.g. circulating endothelial microparticles , EMP).

Aim of the work: This study tries to identify circulating endothelial MPs (such as Cadherin 5 and Annexin V) as potential new risk factors in the occurance of cardiovascular events in patients with chronic renal diseases.

Subjects and Methods: Cadherin 5 and Annexin V were measured in 20 healthy control, 25 patients of chronic renal disease without ischemic cardiovascular complications (Group I) and 35 patients of renal kidney disease with ischemic cardiovascular complications (Group II) by ELISA technique.

Results: Serum **Cadherin 5** was 31.69 ± 11.23 ng/ml in group I and 86.99 ± 21.51 in group II with **highly statistical significant** difference to control group (2.63 ± 1.47) {$p < 0.01$}.Also group II showed **highly** statistical significant difference when compared to group I { $p < 0.01$ } . **Cadherin 5** recorded a high specificity (99.96%) and sensitivity (97%) at cut off 46.8 and area under the curve was 0.998. Serum **Annexin V** was 27.26 ± 11.87 ng/ml in group I and 83.73 ± 22.64 in group II with **highly statistical significant** difference to control group (0.47 ± 0.36) {$p < 0.01$}. Also group II showed **highly** statistical significant difference when compared to group I { $p < 0.01$ } . **Annexin V** recorded a high specificity (99.88%) and sensitivity (94.3 %) at cut off 39.15 and area under the curve was 0.993. Cadherin 5 and Annexin V are more sensitive than C-reactive protein (at cut off 36.0 sensetivity was 71.4 and specificity was 99.6) . A significant direct correlation was found between levels of Cadherin 5 and Annexin V .An inverse correlation was found between Cadherin 5 and Annexin V in one hand and ejection fraction in the other hand in patient group. A significant direct correlation was found between levels of Cadherin 5 and Annexin V in one hand and CRP & ESR in the other hand .

Conclusion: Serum Cadherin 5 and Annexin V is elevated in patients with chronic kidney disease and is considered nontraditional risk factors for prediction of cardiovascular complications especially atherosclerotic ischemic heart disease thus permitting a new therapeutic strategies of cardiovascular complications in patients with chronic kidney disease .

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Evaluation of the Presage™ ST2 ELISA

J. Lu[1], D. G. Grenache[2]. *[1]ARUP Laboratories, Salt Lake City, UT, [2]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT,*

Background: Soluble ST2 (sST2) is a protein in the interleukin-1 receptor family secreted by myocytes in response to mechanical strain. Like NT-proBNP, elevated serum concentrations of sST2 are strongly prognostic in patients with heart failure. Although analogous for prognosis the two markers have been shown to be independent and complementary.

Objective: To perform an analytical validation of the Presage ST2 enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of sST2 in human serum or plasma.

Methods: sST2 was measured using the Presage ST2 (Critical Diagnostics, New York, NY) ELISA. Performance characteristics including imprecision, linearity, recovery, analytical sensitivity, limit of quantification, stability, and sample type comparisons were established using residual specimens sent to ARUP Laboratories. Gender-specific reference intervals were established by non-parametric analysis using serum specimens obtained from 120 healthy males and 120 healthy females. The project was approved by the University of Utah's Institutional Review Board.

Results: Within day and total precision were determined by testing three specimens in triplicate each day for five days. At sST2 concentrations of 11.6, 26.9, and 88.0 U/mL, the within-day CV was 7.6, 2.4, and 3.8%, respectively and the total CV was 11.5, 14.0, and 6.3%, respectively. Linearity of sST2 was determined by serially diluting a specimen with an sST2 concentration of 214.8 U/mL and testing each sample in duplicate. The assay was linear over a concentration range of 2.8-161.1 U/mL ($y=0.95x+1.37$; $r=1.0$). Recovery was evaluated by combining two specimens with sST2 concentrations of 107.4 (high) and 29.8 U/mL (low) in ratios of 2:3 and 3:2 (high:low). Measured concentrations were 57.8 and 73.2 U/mL, which were 95.0 and 95.9%, respectively of the expected concentrations. Analytical sensitivity was determined to be 1.6 U/mL by measuring 10 replicates of the zero calibrator, calculating the mean, and adding 3 standard deviations. The limit of quantification, defined as the concentration that produced a CV ≤20%, was determined to be 3.3 U/mL. sST2 stability was determined using pooled patient specimens with an sST2 concentration of 12.3 U/mL. After storing aliquots for 2 days at room temperature, 10 days at 4°C, and 30 days at -20°C, sST2 concentrations were 94.3, 97.7, and 96.0% of the original concentration, respectively. The mean (SD) concentration of sST2 determined in serum and plasma obtained from each of 13 patients was 141.9 (194.7) and 155.6 (217.2) U/mL, respectively and were not significantly different ($p=0.43$) and showed excellent agreement ($y=1.07x+3.2$; $r=0.96$). Concentrations of sST2 were significantly greater in males compared to females (24.2 vs. 17.5 U/mL, respectively; $p<0.0001$) but were not correlated by age in either gender ($r=-0.09$; $p=0.31$). Reference intervals for sST2 assay were determined to be 10.4-52.1 U/mL for males and 8.4-33.6 U/mL for females.

Conclusions: The Presage ST2 ELISA demonstrates excellent performance characteristics for quantifying sST2 in serum or plasma. The assay is linear over a wide sST2 concentration range and can precisely measure low concentrations of sST2. Concentrations of sST2 are unaffected by age but are greater in males compared to females.

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Performance evaluation of proBNP testing for the diagnosis of destabilized heart failure in patients admitted to emergency department.

D. GRUSON, F. Verschuren, A. Penaloza, F. Thys. *Cliniques Universitaires St Luc, bruxelles, Belgium,*

Background: B-type natriuretic peptide (BNP) and Nt-proBNP are recognized biomarkers to assist in the diagnosis of heart failure (HF) in patients admitted to the emergency department (ED). Both peptide derived from a common precursor, the proBNP$_{1-108}$ (proBNP), synthesized by cardiomyocytes. Different studies have demonstrated that proBNP is also encountered in the circulation and cross-react with a broad spectrum of assays used for measurement of BNP and Nt-proBNP. The aim of this study was to determine proBNP plasma concentrations in patients admitted to ED and suspected of destabilized HF.

Methods: Plasma concentrations of natriuretic peptides were measured in 190 consecutive patients admitted to ED of the Cliniques Universitaires St-Luc in Brussels. Blood samples were collecting at patient ED admission on EDTA plasma. ProBNP levels were determined

with the specific Bioplex® automated immunoassay (BioRad) and plasma BNP levels were also measured with the Access® automated immunoassay (Beckman Coulter).

Results : In this ED population, assays for proBNP and BNP were significantly correlated (r = 0.95, p<0.0001). Median plasma concentrations of proBNP and BNP were higher in patients admitted to ED for destabilised HF (1318.0 and 637.5 pg/mL, respectively; n = 26) than in patients admitted to ED other reasons (83.0 and 66.5 pg/mL, respectively; n = 164; p<0.001). In the HF group, one patient was discordant with a positive proBNP result (124pg/mL) and a negative BNP value (78 pg/mL). Applying receiver operating characteristic curve (ROC) analysis, areas under the curve (AUCs) were 0.889 for proBNP and 0.886 for BNP.

Conclusions: Our results have demonstrated that proBNP, the precursor of BNP and Nt-proBNP, is significantly higher in ED patients with HF in comparison to ¨patients presenting with other diseases. Furthermore, the proBNP assay appears as a relevant tool to assist the diagnosis of HF in patients admitted to ED.

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Patients with congestive heart failure display elevated plasma concentrations of chromogranin A.

D. GRUSON, S. Ahn, M. Rousseau. *Cliniques Universitaires St Luc, Bruxelles, Belgium,*

Background: Neurohormonal activation is a hallmark of heart failure. Intracellular Chromogranin A (CgA) is essential for the formation of secretory granules and sequestration of hormones in neuroendocrine cells. Measurement of CgA levels is included in the diagnostic procedure of neuroendocrine tumors and pheochromocytoma. In recent reports, a role for CgA in the physiopathology of cardiac diseases has been suggested. The study objective was to measure CgA plasma concentrations in patients with congestive heart failure (CHF) and its relationship with other neurohormones involved in the diagnosis and prognosis of CHF.

Methods: Seventy eight fully treated CHF patients (mean age: 68±12 years; ejection fraction < 40%) were included in this study. Plasma concentrations of CgA were measured with a simplified double antibody sandwich assay using rabbit antibodies to a 23 kDa C-terminal fragment of human CgA (Dako, Glostrup, DK). Circulating levels of Nt-proBNP, Nt-proANP and Big Endothelin-1 (Big ET-1) were also determined. Reference values for biomarkers were obtained from 30 healthy age- and sex-matched subjects.

Results: In comparison with controls, CgA plasma concentrations (geometric mean [95% CI]) were significantly increased in CHF patients (67.4 UI/L [4.1-422.0] vs 11.1 UI/L [4.5-22.6], p<0.001). CgA levels were also significantly correlated to Nt-proBNP (r=0.36, p<0.001) and Nt-proANP (r=0.38, p=0.0006). No significant relation was found with Big ET-1 levels. As previously observed for natriuretic peptides, CgA levels were positively correlated with age.

Conclusions: Our results demonstrate that circulating concentrations of CgA are significantly elevated in patients with CHF and are correlated with plasma concentrations of natriuretic peptides; and therefore that CgA should be involved in the neurohumoral response of CHF. The ability of CgA determination to improve diagnosis and risk stratification of CHF patients will have to be confirmed.

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Analytical Performance of the ARCHITECT® *STAT* High Sensitive Troponin-I Immunoassay

M. Matias[1], J. Prostko[1], M. Palafox[1], E. Frias[1], Z. Kogan[1], C. Müller[2], J. Searle[2], F. Holert[2], M. Möckel[2], G. Williams[1]. *[1]Abbott Laboratories, Abbott Park, IL, [2]Charité-Universitätsmedizin, Campus Virchow-Klinikum, Berlin, Germany,*

Introduction: Troponin-I concentrations are used to assist in the diagnosis of myocardial infarction (MI) and in the risk stratification of patients with acute coronary syndromes (including unstable angina and non-ST elevation MI) with respect to relative risk of mortality, myocardial infarction, or increased probability of ischemic events. The newer generation of assays for cardiac troponin have improved analytical sensitivity and improved precision, with the functional sensitivity (at 10% CV) below the 99th percentile of a normal population. This meets the recommendations from the Universal Definition of MI. More sensitive TnI assays are clinically desirable to aid in the earlier diagnosis of MI, and in the risk stratification analysis of acute coronary syndrome. The purpose of this study was to evaluate the performance of the High Sensitive Troponin-I automated chemiluminescent immunoassay (CMIA) on the ARCHITECT® $i2000_{SR}$ and $i1000_{SR}$ instrument platforms.

Methods: The ARCHITECT *STAT* High Sensitive Troponin-I assay is a 2-step assay in which the Troponin-I in the patient sample is bound by anti-Troponin-I antibody-coated paramagnetic microparticles and then sandwiched with an acridinium-labeled anti-Troponin-I antibody. The time to first result is <16 minutes. The assay is designed to have a calibration curve spanning 0-50,000 pg/mL and uses a 10-fold automated dilution to cover samples up to 500,000 pg/mL of Troponin-I. The performance of this assay (*in development*) was compared to that of the Abbott on-market *STAT* Troponin-I immunoassay, List #2K41. Total precision, LOB, LOD and LOQ were determined following recommendations from CLSI documents EP17-A and EP5-A2, and functional sensitivity following recommendation from IFCC cSMCD. Comparative testing was done using TnI-positive samples and also from apparently healthy blood donors. In addition, HAMA, heterophilic antibody and RF panels were tested.

Results: The LOB, LOD and LOQ were 1.8, 2.9, and 6.2 pg/mL respectively. The mean±SD of an apparently healthy population (n=300) was 1.58±3.07 pg/mL for the High Sensitive assay and 2.32±4.01 pg/mL for the on-market assay. The 99th percentile was determined to be 16.8 pg/mL for the High Sensitive assay, and 29.5 pg/mL for the on-market assay. The High Sensitive assay achieved a 10% Functional Sensitivity at a mean concentration of 3.0 pg/mL (range of 2.4-4.8 pg/mL). Within run, between run, between day and total precision ranged respectively from 1.5-6.2%, 0.0-4.2%, 0.0-3.2% and 2.5-8.1%. The overall mean spike recovery in HAMA and RF samples was 97.2% and 100.9% respectively. Comparison between the High Sensitive and on-market assays with elevated random samples (n=109) demonstrated a slope of 0.89 and a correlation coefficient of 0.92. Comparison of the High Sensitive assay between the ARCHITECT $i2000_{SR}$ and the $i1000_{SR}$ demonstrated a slope of 0.95 and a correlation coefficient of 0.95.

Conclusion: The ARCHITECT *STAT* High Sensitive Troponin-I assay was more sensitive in its performance (10% CV below the 99th percentile) compared to the on-market *STAT* Troponin-I assay with no sacrifice in assay time. The *STAT* High Sensitive Troponin-I assay demonstrated comparable performance to the on-market *STAT* Troponin-I assay as well as between ARCHITECT instrument platforms.

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Correlates of serum homocysteine in a Sri lankan population

S. Chackrewarthy[1], Y. S. Wijayasinghe[1], D. Gunasekera[1], R. Wickremasinghe[1], N. Kato[2]. *[1]University of Kelaniya, Ragama, Sri Lanka, [2]International Medical Center, Tokoyo, Japan,*

Correlates of total serum homocysteine concentration in a Sri Lankan population

Background: Hyperhomocysteinemia, a possible risk factor for vascular disease occurs at a higher prevalence in South Asian countries. Serum homocysteine concentrations are influenced by genetic, nutritional and lifestyle factors. Correlates of total serum homocysteine concentration (tHcy) are not well characterized in the Sri Lankan population. Such information is important in developing therapeutic and preventative strategies.

Objective: To investigate the factors potentially associated with fasting levels of serum tHcy in a Sri Lankan population.

Methods: In a cross sectional study, 177 apparently healthy volunteers (91 men and 86 women) aged 38-65 years were selected from residents in an urban health administrative area. Individuals with a history of chronic disease and with any pharmacological treatment were excluded from the study. Information on diet, lifestyle factors and medical history were recorded. Anthropometric indices and blood pressure were measured according standard protocols. Fasting serum levels of tHcy, insulin, creatinine, folate and lipids were estimated using standard protocols.

Results: Fasting serum tHcy levels were higher in males than in females (geometric mean ± SD, 13.75 µmol/l ± 1.41 Vs. 9.58 µmol/l ± 1.43, p<0.001) and were positively associated with age (r=0.204, p< 0.01) in both sexes. 32.3% of males and 10.3% of females had mild hyperhomocysteinemia (tHcy>15µmol/l). tHcy levels were significantly higher in smokers than in non-smokers (geometric mean ± SD, 14.58 µmol/l ± 1.44 Vs.12.71 µmol/l ± 1.37, p<0.05) and in alcohol consumers than in non-consumers (geometric mean ± SD, 14.53 µmol/l ± 1.43 Vs.12.14 µmol/l ± 1.32, p< 0.02). In males, tHcy levels were negatively related to serum insulin (r= -0.397, p<0.001) and BMI (r= -0.244, p <0.02) and positively related to serum creatinine (r=0.235, p<0.02). In females, there was a positive relationship between tHcy and systolic blood pressure (r= 0.239, p<0.02) but there was no significant correlation with serum insulin. In both sexes, serum tHcy levels strongly correlated with serum folate (r= -0.412, p<0.001). There were no significant associations between tHcy and serum lipids. Stepwise regression analysis confirmed the associations between tHcy and folate (p<0.001 in both sexes), insulin (p=0.026 in males) and creatinine (p=0.036 in males).

Conclusion: Low intake of folate, alcohol consumption and smoking were associated with increased tHcy concentrations. Serum insulin and creatinine were independent correlates of tHcy in males, but not in females. Difference in tHcy levels between sexes may partly be attributed to differences in lean muscle mass and to a metabolic link between creatinine synthesis and homocysteine production. Insulin may regulate serum tHcy concentrations by homocysteine remethylation or by increasing homocysteine clearance.

C-49

The effect of population selection on the patient characteristics and the 99th percentile reference limit for cardiac troponin.

P. O. Collinson[1], Y. Heung[1], O. Clifford-Mobley[1], D. Gaze[1], F. Boa[1], R. Senior[2]. *[1]St George's Hospital, London, United Kingdom, [2]Northwick Park Hospital, London, United Kingdom,*

Objective: To determine the effect patient selection on the population distribution and the 99th percentile of two high sensitivity troponin assays using a well-defined subject population.

Methods: Subjects >45 years old were randomly selected from seven representative local community practices. Details were collected by questionnaires and heart rate and blood pressure measurement (the average of two readings), spirometry, electrocardiography (ECG) and echocardiography performed and they were venesected for fasting serum glucose, and creatinine. Estimated glomerular filtration rate (eGFR) was calculated by the diet modification of renal disease equation corrected to a reference creatinine method. Samples not analysed immediately were frozen to -20°C then transferred to -70°C and subsequently analysed for cardiac troponin I (cTnI), cardiac troponin T (cTnT) and N terminal pro B type natriuretic peptide (NTproBNP).

Analyses for cTnI (cTnI ultra) were performed using the ADVIA centaur (Siemens Medical Solutions, Diagnostics) and for cTnT (cTnT hs) using the Elecsys 2010 (Roche diagnostics) using the manufacturers recommended methods. For cTnI the detection limit of the instrument is 6 ng/L and upper limit 50,000 ng/L with 10% CV is 30 ng/L with a 99th percentile of 40 ng/L. For cTnT, the detection limit of the assay is 3 ng/L with an upper limit of 10000ng/L, 10% CV 13ng/L, 99th percentile of 14 ng/L.

Population analysis was performed using the following criteria: all of those attending; excluding those with any recorded history of any cardiovascular diseases or diabetes mellitus or taking any cardioactive drugs. Thereafter the latter group was filtered for one of: hypertension (diastolic <90 and systolic <160 mmHg); NT-proBNP <125 ng/L; eGFR >60; completely normal ECG or ejection fraction >50%. Completely normal subjects were defined as all attending general population subjects with no history of vascular disease, hypertension, or heavy alcohol intake, receiving no cardiac medication; whose blood pressure was <160/90 mmHg as the mean of two readings; whose fasting blood glucose was <6 mmol/l, whose estimated creatinine clearance was >60 mL/min/1.73 m2; and normal cardiac function.

All data analysis was performed using the Analyse it add-in Excel (Analyse-it for Microsoft Excel (version 2.12), www.analyse-it.com) by non-parametric methods.

Results: Data was available from 616/1392 of those invited (295 male) median age 58.0 years, interquartile range 51-67years. There was no difference between males and females for the cTnI data but cTnT showed significantly higher values in males except when screened by history plus ECG or as completely normal. Values for the 99th percentile were as follows. Whole population: (n = 616) cTnI 39.0 ng/L cTnT 23.4 ng/L , no history of CVD, DM or drug therapy (n = 400) cTnI 40.0 ng/L cTnT 21.5 ng/L. Completely normal (n = 248) cTnI 40.0 ng/L cTnT 15.5 ng/L.

Conclusion: The 99th percentile of cTnT but not cTnI is dependent on patient population selection. Patients with demonstrably good cardiac function are required for reference populations.

C-50

The assay performance and 99th percentile reference limit for cardiac troponin T using a new high sensitivity assay in a fully characterised reference population.

P. O. Collinson[1], Y. Heung[1], D. Gaze[1], F. Boa[1], R. Senior[2]. *[1]St George's Hospital, London, United Kingdom, [2]Northwick Park Hospital, London, United Kingdom,*

Objective: To determine the assay performance and 99th percentile of a high sensitivity troponin T (cTnT hs) assay using a well-defined population.

Methods: 1392 subjects >45 years old were randomly selected from the practice lists of seven representative local community practices. Details of the subjects were collected by questionnaire. Heart rate and blood pressure measurement, spirometry, electrocardiography (ECG) and echocardiography were performed. Subjects were venesected for fasting serum glucose and creatinine. Aliquots which were not analysed immediately were initially frozen to -20°C then transferred -70°C. Left ventricular ejection fraction (LVEF) was calculated quantitatively using Simpson's apical biplane method taking the average of three readings. LV mass was calculated using the Devereux-modified American Society of Echocardiography equation. Valvular regurgitation and stenosis were assessed qualitatively. Significant valve disease was taken as mild-to moderate or worse. Diastolic heart failure (DHF) was defined according to the European Study Group on Diastolic Heart Failure guidelines.

Total imprecision were assessed following the Clinical and Laboratory Standards Institute (CLSI) EP 15A using pools at 8 levels measured four times for five days.

Five serum samples were obtained covering the range of the assay and divided into seven aliquots. One aliquot was stored at 20° C and one at 4° C then reanalysed at 24-hour intervals for five days. The third aliquots was analysed then frozen to -20° C thawed to room temperature, reanalysed and refrozen at 24 h intervals for 5 days. 2 aliquots were frozen to -20oC and two to -70oC with one reanalysed after 15 and one after 30 days storage. All analyses were performed using the Elecsys 2010 (Roche Diagnostics) using the manufacturers recommended methods. Troponin concentrations were obtained directly from the analyser. For cTnT hs, the detection limit of the assay is 3 ng/L with an upper limit of 10000ng/L with 10% CV of 13ng/L and 99th percentile 14 ng/L.

Normal subjects were defined as all attending general population subjects with no history of vascular disease, diabetes mellitus, hypertension, or heavy alcohol intake and receiving no cardiac medication; whose blood pressure was <160/90 mmHg as the mean of two readings; whose fasting blood glucose was <6 mmol/L, whose estimated creatinine clearance (calculated by the diet modification of renal disease equation corrected to a reference creatinine method) was >60 mL/min/1.73 m2; and who had no significant valvular heart disease, left ventricular hypertrophy, diastolic heart failure, LVEF <50% or regional wall motion abnormalities on echocardiography.

Results: There were 248 normals (104 male) median age 53 years, interquartile range 49-61. Assay imprecision varied from 30.3% (6.1 ng/l) to 2.1% (1066.2 ng/l) with 10% imprecision at 18.5 ng/l. Samples were stable for up to 5 days at 20^{o}C and 4^{o}C, 30 days at -20^{o}C and -70^{o}C and through 5 freeze thaw cycles. The 99th percentile was 15.5 ng/l and was not influenced by age or gender.

Conclusion: The 99th percentile of the Roche cTnT hs assay is close to the manufacturers supplied data. Assay imprecision just fails to meet current recommendations.

C-52

High-sensitivity Troponin T will modify the clinical classification of acute coronary syndromes

J. Ordonez-Llanos[1], M. Muñoz[2], P. Calmarza[3], A. Carratala[4], C. Ortiz[5], J. Merce[1], A. Martin[2], J. Povar[3], J. V. Balaguer[4], F. Temboury[5], M. Santalo[1]. *[1]Hospital de Sant Pau, Barcelona, Spain, [2]Hospital Severo Ochoa, Leganes, Spain, [3]Hospital Miguel Servet, Zaragoza, Spain, [4]Hospital Clinico, Valencia, Spain, [5]Hospital Clinico, Malaga, Spain,*

Background: A troponin (Tn) concentration corresponding to the 99th percentile (p99th) of a reference population is recommended as the biochemical criterion for myocardial infarction (MI) diagnosis. Recently, Tn assays with improved limit of quantitation (high sensitivity assays) have been available. Preliminary data indicate that the p99th value obtained with the high sensitivity methods varies respect to the obtained with previous assays. This fact will affect MI diagnosis and be particularly relevant in patients with MI with non-sustained ST segment elevation (NSTEMI) in electrocardiogram (ECG). We analyzed the impact of measuring troponin T with a high-sensitivity assay in the clinical classification of patients admitted to Emergency Departments (ED) by non-traumatic thoracic pain and without persistent ST segment elevation in the ECG.

Methods: Five ED participated in the study and 230 patients were recruited. After obtaining informed consent, patients were sampled at admission and 2, 4-6 and 8-12h later. We measured TnT with the current available 4th generation (4th gen-TnT) assay whose p99th is 35 ng/L and with a high sensitivity assay (hs-TnT) whose reported p99th is 13 ng/L. All TnT measures were centralized in one laboratory. Final classification of the episode as no acute coronary syndromes (no ACS), unstable angina (UA) or NSTEMI was made by clinicians blinded to hs-TnT by considering the clinical records and the 4th gen-TnT values.

Results: The first sample was obtained 246 minutes (mean time) after the onset of symptoms; 40.2% of patients were sampled before 3h of evolution. Final diagnoses were no ACS in 107, UA in 80 and NSTEMI in 44 patients, respectively. Using the hs-TnT, the same number of patients were classified as no ACS, but UA patients reduced to 47 while NSETMI increased to 77 (75% increase). In NSTEMI group, the 4th-gen TnT value identified 52.3% of cases at admission, whereas hs-TnT identified 90.9%; in the subgroup of NSTEMI subjects admitted before 3h of symptoms, 4th-gen TnT exceeded the cut-off value at admission in only 35% of cases and hs-TnT in 90%. In the admission sample, the negative predictive value to rule-out NSTEMI was of 97% for hs-TnT compared to 90% for 4th-gen TnT. At any sampling time, the area under ROC curve for NSTEMI diagnosis of hs-TnT was higher than that of 4th-gen TnT.

Conclusions: In the studied population, hs-TnT values changed the clinical classifications of patients by increasing the number of those fulfilling biochemical criteria of NSTEMI. In NSTEMI patients, hs-TnT values at admission exceeded the p99th in more subjects and provided a higher negative predictive value than 4th-gen TnT values. If confirmed in other studies, the use of the hs-TnT assay in clinical practice would affect the clinical classification and hence, the management of ACS patients; therefore, before changing from current troponin assays to high-sensitivity assays a previous educational effort of the clinical and laboratory communities is required.

C-53

The clinical sensitivity of myeloperoxidase in the risk stratification of acute coronary syndromes

G. R. SYPNIEWSKA, M. Sawicki, M. Gruszka, M. Kozinski, J. Kubica.
Collegium Medicum, N.Copernicus University, BYDGOSZCZ, Poland,

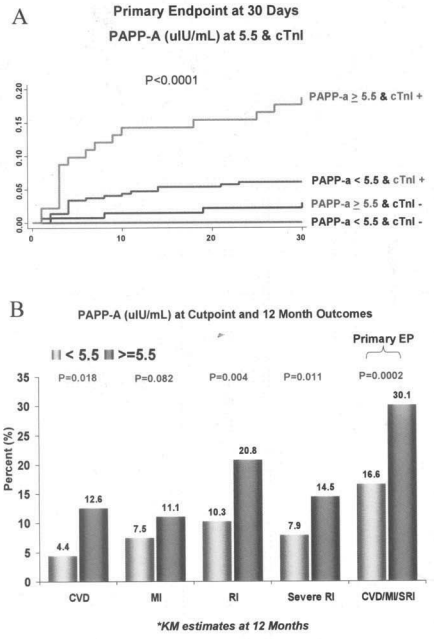
Myeloperoxidase (MPO) possessing proinflammatory and pro-oxidative properties has been proposed as a biomarker of plaque instability in acute coronary syndromes.
The aim of study was to evaluate the usefulness of plasma myeloperoxidase as a biomarker in risk stratification of acute coronary syndromes.
Study included 300 patients with clinically very well documented ACS (94 women, 159 men, aged 64 ± 12 yrs). The criteria for enrollment were as follows: typical anginal chest pain at rest, symptom onset ≤6 hrs before the hospital admission.
In patients serial ECG examinations were performed. All patients underwent coronarography and coronary angioplasty with stenting if clinically indicated. Transthoracic echocardiography was also performed. The investigated patients were discharged home with a final diagnosis of unstable angina (UA, n=100), non-ST-elevation myocardial infarction (NSTEMI, n=87) or STEMI (n=66). Control group consisted of 124 apparently healthy subjects (40 women and 84 men) aged 20 - 64 yrs.
Blood samples were collected from patients at presentation. Fasting blood samples were collected from controls. MPO was assayed in EDTA-plasma samples (446 pmol/L; 95th percentile was taken as cut-off), serum STATcTnI (cut-off value of 0,032 ng/ml), BNP, CK-MB activity, lipid profile, glucose and creatinine were assayed (ARCHITECT ci8200); hsCRP (BNII) and blood count (XE 2100) were also assayed in patient and control groups.
Characteristics of ACS patients at admission according to MPO concentration in quartiles revealed significantly higher cTnI, CK-MB activity and WBC in the third comparing to the first quartile while HDL-C was significantly lower. Calculated diagnostic efficacy (percentage of positive results over cut-off) within first 6 hours was 47% in UA patients vs 31% for cTnI, in NSTEMI patients 62,1 vs 86,2% for MPO and cTnI, respectively and in STEMI patients 86,4 vs 91,8% for MPO and cTnI, respectively. Positive MPO results were found in 30% of troponin-negative UA patients at admission as well as 7% and 8% of troponin-negative NSTEMI and STEMI patients. Using double-marker strategy (positive MPO and/or positive cTnI) for early risk stratification allowed us to recognize ACS in 61% of UA patients and in 93 or 100% of those with final diagnosis of NSTEMI and STEMI.
Conclusion : MPO as an early biomarker of risk stratification in ACS patients has shown the highest diagnostic efficacy in patients with final diagnosis of unstable angina. Within the first 6 hours from the onset of chest pain the added value of MPO was shown predominantly in troponin-negative patients with unstable angina.

C-54

Metalloproteinase PAPP-A and Outcomes in Patients with ACS: Initial Observations from the MERLIN-TIMI 36 Trial

P. Jarolim[1], M. Bonaca[1], B. M. Scirica[1], S. A. Murphy[1], J. S. Chamberlin[2], D. W. Rhodes[2], P. C. Southwick[2], E. Braunwald[1], D. A. Morrow[1]. *[1]Brigham and Women's Hospital, Boston, MA, [2]Beckman Coulter, Inc., Carlsbad, CA,*

Background: Pregnancy associated plasma protein-A **(**PAPP-A) is a high molecular weight, zinc-binding metalloproteinase. Initial studies have suggested that PAPP-A is associated with vulnerable coronary plaque and may be used as a predictor of cardiovascular disease (CVD) and mortality. We investigated whether PAPP-A would be useful for risk assessment in patients presenting with NSTE-ACS.
Methods: In this pilot study, we measured cardiac PAPP-A (Beckman Coulter DSL ELISA) at baseline in a randomly selected subset of 543 pts with NSTE-ACS randomized to ranolazine or placebo in the MERLIN-TIMI 36 trial. Patients were followed for an average of one year. Endpoints were adjudicated by a blinded Clinical Endpoints Committee. The primary endpoint for this analysis was CV death, MI, or severe recurrent ischemia (RI).
Results: PAPP-A > 5.5 uIU/mL at presentation was associated with higher rates of the primary endpoint at 30 days (RR 3.3, 95% CI 1.6 - 7.7, p=0.001). Moreover, when stratified by baseline cTnI, PAPP-A remained associated with adverse CV outcomes at 30 days (Figure A). At one year, PAPP-A was associated with higher rates of cardiovascular events (Figure B) including CVD/MI/SRI (HR 2.17; 95% CI 1.43 - 3.30), CVD or MI (HR 2.05; 95% CI 1.21 - 3.47), and severe RI (HR 2.16; 95% CI 1.17 - 3.96). After adjustment for cTnI (AccuTnI), ST deviation, age, gender, diabetes, smoking, HTN, and history of CAD, PAPP-A remained independently associated with the risk of the primary endpoint at one year (adj HR 1.98; 95% CI 1.3 - 3.0, p=0.001).
Conclusions: PAPP-A was independently associated with short and long-term risk of recurrent cardiovascular events in patients with NSTE-ACS, adding to clinical predictors and cTnI. This finding in a pilot study adds to emerging evidence supporting PAPP-A as a candidate prognostic marker in patients with ACS, and supports further investigation.



C-55

Values of BNP in outpatients with heart failure with normal ejection fraction classified by the consensus statement of the European Society of Cardiology,should be modify?

A. J. L. Jorge[1], E. T. Mesquita[1], M. L. Ribeiro[1], L. C. M. Fernandes[1], M. L. G. Rosa[1], F. V. Lício[2]. *[1]Universidade Federal Fluminense, Niteroi, Brazil, [2]Laboratory Sergio Franco, Rio de Janeiro, Brazil,*

Background: The ESC consensus established a BNP cut-off value of 200 pg/ml to confirm and 100 pg/ml to exclude heart failure with normal ejection fraction (HFNEF) in outpatients with clinical suspected of HF. These cut-off values are based on different studies, but were not tested after the publication of the guideline.
Methods: The study population consisted of 88 outpatients (mean age 69.3±12.3 years) with clinical suspected of HF and ejection fraction (LVEF) ≥ 50% which were submitted to dosage of BNP and tissue Doppler image (TDI) to assess the peak early diastolic annular velocities (E'), E/E' ratios and left atrial volume-indexed (LAV-I). Patients were classified, in accordance with the ESC guidelines, in two groups; one confirmed HFNEF and another excluded HFNEF.
Results: HFNEF was confirmed in 29 patients (33%) who presented increased LAV-I and E/E' ratio (42.8±17 ml/m² and 14.6±6.1 p < 0.0001). The average of BNP in patients with and without HFNEF was 131 pg/ml (median 95.1) and 21 pg/ml (median 16.1) (p <0,0001). BNP was correlated with worsening of diastolic function assessed by LAV-I and E/E' ratio (r = 0.60 p <0.0001 and r= 0.33 p = 0.001). The area under the receiver-operating characteristic curve for BNP to detect HFNEF was 0.95 (95% CI, 0.89 to 0.99; p < 0.0001). A BNP value of 46pg/ml had a sensitivity of 90%, a specificity of 90% and an acuracy of 90% for detecting HFNEF.
Conclusions: The determination of BNP can help to confirm or exclude HFNEF in outpatients with a cut-off (46 pg/ml) with high accuracy and less than that proposed in the ESC guidelines. Prospective studies with a larger population should be conducted to confirm this finding.

Clinical and TDI characteristics

	Total (n=88)	No HFNEF (n=59)	HFNEF (n=29)	p
Age (years)	69.3±12.3	66.1±10.9	75.7±12.7	0.527
Female gender (%)	72.1	70.2	75.9	0.578
Arterial hypertension (%)	90.7	87.7	96.6	0.182
LVEF (%)	72.6±8.3	72.8±7.5	72.1±9.9	0.659
E/E''	9.8±5.2	7.3±2.0	14.6±6.1	< 0.0001
E'' (cm/s)	8.9±3.0	9.5±2.0	7.6±3.0	0.002
LAV-Indexed (ml/m^2)	33.6±13.9	29.0±8.9	42.8±17.0	< 0.0001
Mass LV-Indexed (g/m^2)	94.8±23.4	93.0±23.2	98.6±22.9	0.290

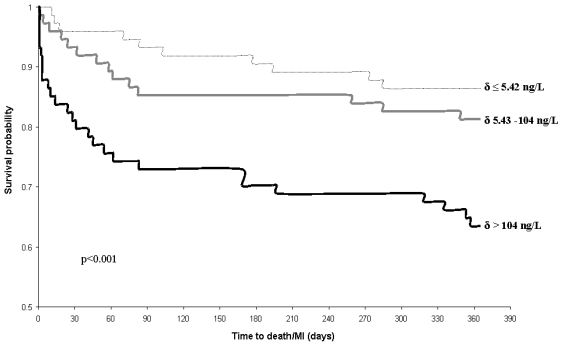
p - independent samples test and Pearson chi-square.

C-56

Absolute increase in cardiac troponin I concentration determined with a research high sensitivity assay and health outcomes in a chest pain population

P. Kavsak[1], X. Wang[2], D. Ko[2], A. R. MacRae[3], A. S. Jaffe[4]. *[1]McMaster University, Hamilton, ON, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]University of Manitoba, Winnipeg, MB, Canada, [4]Mayo Clinic, Rochester, MN,*

Background: Recent data has indicated the utility of using relative (i.e., percent) changes in cardiac troponin I (cTnI) concentrations for risk stratification in patients presenting with chest pain to the emergency department (Clin Chem 2009;55: 930-7). However, change as determined by absolute increases in cTnI concentrations, especially at the very low concentrations measurable with high-sensitivity cTnI (hs-cTnI) assays, has not been thoroughly investigated. Our objective in this study was to explore if absolute increases (δ increases) in hs-cTnI can be used to identify those patients at high risk for future adverse events.
Methods: After ethics board approval, a research hs-cTnI assay (Beckman Coulter) was used to measure stored plasma specimens from 289 subjects with 2 or more specimens collected during a chest pain presentation to the emergency department (Clin Chem 2009;55:1809-15). From this group, only those subjects where the highest (i.e., peak) hs-cTnI concentration was not at presentation were selected (n=223; median (25-75th) number of specimens/subject=5 (3-6); median time (h) from pain onset=3 (2-5)). The δ increase was calculated for each subject (i.e., peak minus presentation hs-cTnI; median time interval for δ=4h (2-10)). Kaplan Meier and Cox proportional hazard analyses based on tertiles were performed as well as ROC analysis for death/MI within 1 year.
Results: Kaplan Meier analysis indicated significant differences between the three groups (p<0.001; log rank; see Figure); however after adjusting for age and sex only the highest tertile (δ increases >104 ng/L) was at significantly higher risk for death/MI compared to the lowest tertile (δ increases ≤5.42 ng/L) (hazard ratio: 2.95 (95%CI:1.43-6.10; p=0.004)). The area under the curve for death/MI within 1 year was 0.70 (95%CI:0.61-78) with the optimal δ increase being 32.5 ng/L.
Conclusion: Larger acute absolute increases in cTnI concentrations using a high sensitivity assay are associated with more adverse events.



C-57

The Value and Clinical Signficance of Detecting Ischemia Modified Albumin in Early Diagnosis of Acute coronary syndrome

X. Cai, Y. Li, J. Gu. *Renmin Hospital of Wuhan University, Wuhan, China,*

Objective To discuss the value and clinical significance of detecting Ischemia modified albumin(IMA) in early diagnosis of acute coronary syndrome(ACS).
Methods To compare 1307 patients with acute chest pain with 761 healthy people and detect the four items of IMA,Myo,cTnI,CK-MB separately by drawing blood from vein. 368 patients were diagnose ACS finally. We detected the IMA levels of 368 patients continous . To analyze these results.
Results 368 patients were diagnose ACS in 1307 patients. And the levels of four items in ACS group was obviously higher than that in normal control group (p<0.01). The sensitivity and specificity of ACS was 82.%,92.6% , respectively. Combining the results of Myo,cTnI,CK-MB had a higher sensitivity of 91.2%.
Conclusions IMA was a useful biochemical mark for the early diagnosis of ACS. And combining the results of Myo,cTnI,CK-MB could improve early diagnosis of ACS

C-58

Prognostic Value of Combination of Cystatin C and BNP after Additional Treatment in Patients with Diastolic Heart Failure

A. Kuno, F. Kitagawa, A. Nakashima, M. Saitou, T. Hashimoto, T. Nakano, H. Naruse, S. Matsui, T. Ishikawa, I. Tanaka, Y. Ozaki, J. Ishii. *Fujita Health University, Toyoake, Japan,*

Cystatin C may improve the risk stratification of chronic heart failure (CHF) patients with reduced left ventricular (LV) systolic function compared with glomerular filtration rate estimated by the Modification of Diet in Renal Disease Study equation. We prospectively investigated whether the combination of cystatin C and BNP after additional treatment would effectively risk stratify the patients with diastolic heart failure. Serum cystatin C and plasma BNP were measured on discharge in 150 CHF patients (median age, 73yrs; male, 61%; and ischemic etiology, 31%) with preserved LV systolic function (LV ejection fraction >45%).
Results: During a mean follow-up period of 978 days after discharge, there were 61 (41%) cardiac events (cardiac death or readmission for worsening CHF). On a multivariate Cox regression analysis including 7 clinical and biochemical variables, cystatin C (relative risk 1.54 per increase of 1.0mg/L, P=0.0005) and BNP (3.76 per increase of 10-fold, P=0.0004) were independently associated with cardiac events. The tertile score was defined as the sum of an individual's cystatin C tertile (0 point, ≤1.22mg/L;1 point, 1.22 - 1.78mg/L; and 2 points, >1.78mg/L) plus BNP tertile (0 point, ≤100pg/mL; 1 point, 100 - 313pg/mL; and 2 points, >313pg/mL). Cardiac mortality and cardiac event rates according to the tertile score were shown in **Table**.
Conclusion: The combination of cystatin C and BNP after additional treatment may be useful for the risk stratification of patients with diastolic heart failure.

Cardiac mortality and cardiac event rates

Tertile score	0 n=27	1 n=47	2 n=56	3 n=36	4 n=24	P value
Cardiac mortality rate	0%	0%	13%	15%	27%	<.009
Cardiac event rate	30%	57%	52%	50%	68%	<.0001

C-59

Prognostic Value of High-Sensitive Cardiac Troponin Ton Admission in Patients hospitalized for Worsening Chronic Heart Failure

J. Ishii, F. Kitagawa, A. Kuno, A. Nakashima, M. Saitou, T. Hashimoto, T. Nakano, H. Naruse, S. Matsui, T. Ishikawa, I. Tanaka, Y. Ozaki. *Fujita Health University, Toyoake, Japan,*

Recently, it has been reported that a new high-sensitive cardiac troponin T (hsTnT) assay improves risk stratification of patients with stable chronic heart failure (CHF) compared with the traditional assay. To prospectively evaluate the prognostic value of hsTnT in acutely decompensated heart failure, we measured serum hsTnT on admission in consecutive 127 patients (median age, 73 years; male, 59%; ischemic etiology, 47%; NT-proBNP, 3611pg/mL; estimated GFR, 51mL/min/1.73m^2; and left ventricular ejection fraction, 40%) hospitalized for worsening CHF (NYHA functional class III to IV).
Results: 1) HsTnT was detectable (>0.002ng/mL) in all patients. 2) During 12-month follow-up period after admission, there were 30 (24%) cardiac events (cardiac death or rehospitalization for worsening CHF. 3) In stepwise multivariate Cox regression analyses

including 7 clinical and biochemical variables, the tertile of hsTnT was independently associated with cardiac death (relative risk 3.44, P=0.006) and cardiac event (1.78, P=0.01). 4) Cardiac mortality and cardiac event rates in patients according to tertiles of hsTnT were shown in **Table**.

Conclusion: A new hsTnT assay may be useful for the risk stratification on admission in patients hospitalized for worsening CHF.

Cardiac mortality and cardiac evnt rates

Tertile of hsTnT ng/ml	1st <0.02	2nd 0.02-0.07	3rd >0.07	P value
Cardiac mortality rate	3%	7%	19%	.006
Cardiac event rate	13%	28%	28%	.04

C-60

Admission Concentration of Cystatin C Is an Independent Predictor of Adverse Outcomes in Patients Who Underwent Emergent Coronary Angiography for Acute Coronary Syndrome

F. Kitagawa, A. Kuno, A. Nakashima, M. Saitou, T. Hashimoto, T. Nakano, H. Naruse, S. Matsui, T. Ishikawa, I. Tanaka, Y. Ozaki, J. Ishii. *Fujita Health University, Toyoake, Japan,*

We prospectively evaluated the prognostic value of serum cystatin C on admission in 431 consecutive patients who underwent emergency coronary angiography within 24 hours after admission for acute coronary syndrome (median age, 65 years; ST elevation myocardial infarction, 49%; diabetes, 23%; Killip class >2, 7%; emergent percutaneous coronary intervention, 62%; multi-vessel coronary artery disease, 38%; high-sensitive cardiac troponin T, 0.27ng/mL; NT-proBNP 279pg/mL; and high-sensitive CRP, 1.58mg/L).

Results: During a mean follow-up period of 668 days after admission, there were 65 (15%) cardiac events (cardiac death or rehospitalization for worsening heart failure or acute coronary syndrome). Stepwise multivariate Cox regression analyses, including 11 clinical and angiographic variables, revealed that tertile of cystatin C and Killip class (>2), but not estimated glomerular filtration rate, were independently associated with both cardiac deaths (relative risk 4.26, P=0.01 and 3.84, P=0.0001) and cardiac events (1.51, P=0.02 and 2.42, P=0.008). Cardiac mortality and cardiac event rates in the patients according to tertiles of cystatin C were shown in **Table**.

Conclusion: Admission concentration of cystatin C may be useful for the early risk stratification for adverse outcomes in patients who underwent emergent coronary angiography for acute coronary syndrome.

Cardiac mortality and cardiac event rates

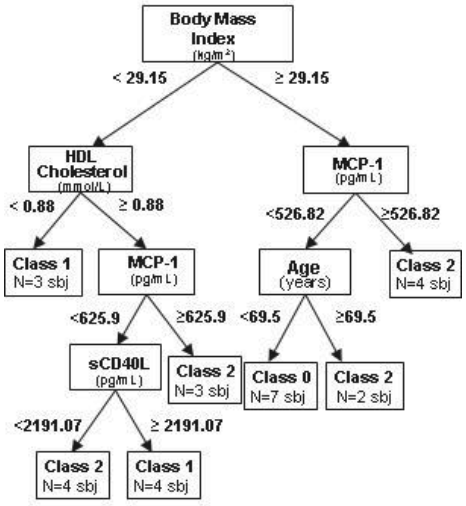
Tertile of Cystatin C mg/L	1st <0.68	2nd 0.68-0.83	3rd >0.83	P value
Cardiac mortality rate	0%	2%	12%	<.0001
Cardiac event rate	6%	16%	23%	.0004

C-61

Multiplex Beads-Based Immunoassay With Cytometric Quantitation: Validation Of A Panel Of Six Cardiovascular Biomarkers And Development Of An Algorithm For Risk Stratification.

E. Novello, M. Zaninotto, M. Sanzari, A. Padoan, G. Pantano, M. Plebani. *Department of Laboratory Medicine, University-Hospital of Padova, Padova, Italy,*

In this study we evaluate cardiovascular risk in a diabetic population using a six biomarkers assay. The FlowCytomix Human Cardiovascular 6plex (Bender MedSystems GmbH, Austria) is a bead-based immunoassay with flow-cytometric quantitation that allows the simultaneous detection of sCD40L, IL-6, IL-8, MCP-1, sP-selectin and t-PA. Samples are incubated with a mixture of two different size beads, coated with specific capture antibodies, and biotin-streptavidin-conjugated antibody. Samples fluorescences are analyzed, and cytokines concentrations are calculated by FlowCytomix Pro 2.3. After imprecision and matrix study, we measured sCD40L, IL-6, IL-8, MCP-1, sP-selectin and t-PA in 35 type 2 diabetes patients, characterized by age, BMI, lipidic and glucidic profiles, blood pressure, and diabetic complications. We developed an algorithm that on the basis of biomarkers concentrations observed in the diabetic population, was able to classify patients in three groups of cardiovascular risk (high=class2, medium=class1, low=class0), that have been compared with the Framingham risk. All data were expressed in pg/mL, except for sP-selectin expressed in ng/mL. Intra-assay and inter-assay imprecision altogether ranges respectively: 4.5-15.4%, 1.7-24.3%. The sensitivity of the method was inadequate to measure IL-6 and IL-8 (sensitivity: 27.44, 41.15 respectively) in 90% of studied samples. All biomarkers were assayed in plasma citrate samples being the only matrix adequate for sCD40L measurement. The developed algorithm is on figure below, and concordance in patients classification, Framingham and developed algorithm, was in the total patients evaluated 65%, and 73% for class 2 patients. The biochemical panel evaluated provides good analytical performance for sCD40L, MCP-1, sP-selectin and t-PA. The developed algorithm shows satisfactory concordance with Framingham classification, even if we identified some analytical critical points (the lack of sensitivity for interleukins and the need to use plasma citrate) that make incomplete the information provided by this panel to classify cardiovascular risk.



C-62

A sensitive, homogeneous assay for cardiac troponin using SPARCL™ technology

K. P. Kapsner[1], H. Akhavan-Tafti[2], K. R. Shelly[1], J. E. Gaibor[1], S. K. Lopac[1], Y. Chen[1], D. G. Binger[1], J. D. Mendoza[1], N. Shapir[1]. *[1]Beckman Coulter, Chaska, MN, [2]Beckman Coulter, Southfield, MI,*

Objective: To develop a cardiac Troponin I (cTnI) assay using SPARCL**™,** a novel homogeneous immunoassay technology.

Relevance: Rapid turnaround time is especially important for cardiac assays such as cTnI. Elimination of the time required for reagent separations and signal development can dramatically shorten this time. SPARCL**,** a novel homogenous chemiluminescent technology is a proximity-based method in which a light emitting compound (acridan) is triggered to release a photon of light after oxidation catalyzed by a closely bound horseradish peroxidase enzyme (HRP). SPARCL requires no reagent separation, and signal is instantly captured upon trigger addition.

Methodology: Sensitive, homogenous, one-step sandwich assays were developed* for the measurement of biomarkers, such as cTnI. The cTnI assay protocol pipettes 25 µL capture phase (containing both acridan and capture antibody), 35 µL buffer, 25 µL HRP-conjugated antibody, and 15 µL serum or plasma sample. The mixture is incubated for five minutes, then without wash or separation, a trigger solution is added. The resultant flash of light is measured over 250 milliseconds starting immediately upon trigger addition. Two different capture phases were studied. One capture phase is microparticle based while the other utilizes labeled soluble proteins.

Validation: The SPARCL cTnI assay dynamic range was determined to be 0 to 35 ng/mL. With the microparticle capture approach, a method comparison study to the Access AccuTnI™ assay (Beckman Coulter, Inc.) was performed and Deming regression analysis yielded the following statistics: SPARCL = 0.974AccuTnI + 0.050; r = 0.958, N = 95. Analytical sensitivity was calculated to be 0.005 ng/mL, based on measuring 20 replicates of the Access zero calibrator and calculating the dose at 2 standard deviations greater than zero. For reference interval analysis, 104 normal donor samples were assayed and the 99th percentile URL cutoff was determined to be 0.042 ng/mL. For the solution-phase approach, comparison to the Access AccuTnI assay yielded the following statistics: SPARCL = 0.974AccuTnI + .0117; r = 0.963, N = 95. Analytical sensitivity was calculated to be 0.017 ng/mL based on measuring 15 replicates of the Access zero calibrator and calculating the dose at 2 standard deviations greater than zero.

Conclusions: We have developed sensitive and rapid homogenous sandwich assays, utilizing SPARCL technology, to measure cTnI in biological samples. Two independent

designs for capturing antigen and bringing the necessary reactants into proximity were studied and both designs demonstrated a high level of agreement to the Access AccuTnI assay. The results were obtained in 5 minutes without solid-phase wash or separation thus demonstrating the potential of SPARCL technology to shorten turnaround time.
*Internal feasibility assays, not for commercial distribution of diagnostic products.

C-63

High-sensitivity cardiac troponin is the most powerful prognostic determinant and predicts progression of cardiac dysfunction in AL amyloidosis

A. Barassi[1], G. Palladini[2], G. Sarais[2], R. Capra[1], M. Milani[2], R. Albertini[3], S. Perlini[4], A. Foli[2], P. Russo[2], L. Zenone Bragotti[2], L. Obici[2], R. Moratti[3], G. Melzi d'Eril[1], G. Merlini[2]. *[1]Department of Medicine, Surgery and Dentistry, Ospedale San Paolo and University of Milan, Milan, Italy, [2]Amyloidosis Research and Treatment Center and Department of Biochemistry, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Pavia, Italy, [3]Clinical Chemistry Laboratory Fondazione IRCCS Policlinico San Matteo, Pavia, Pavia, Italy, [4]Department of Internal Medicine, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Pavia, Italy,*

Background. In AL amyloidosis the severity of heart involvement dictates prognosis. Cardiac dysfunction is assessed by measuring N-terminal natriuretic peptide type B (NT-proBNP) and troponins (cTn). Recently, more sensitive assays for cTn have been developed, aiming at identifying minimal cardiac damage. We tested a highly sensitive (hs) cTnT assay in 171 consecutive newly diagnosed patients with AL amyloidosis evaluated between January 2004 and May 2008.
Methods. The hs-cTnT was measured on a Modular E instrument with a precommercial immunoassay from Roche Diagnostics. The 99th centile of hs-cTnT concentration in sera from healthy volunteers is 14 ng/L. The detection limit is 3 ng/L. NT-proBNP and cTnI were measured with commercial assays. The upper reference limits of NT-proBNP and cTnI are 332 ng/L and 40 ng/L, respectively. Heart involvement was defined as mean left ventricular wall thickness (mLVW) >12 mm. Response to therapy was evaluated 6 months after treatment initiation.
Results. Seventy-three percent of patients had heart involvement. Of them, 100% had elevated NT-proBNP, 84% had high hs-cTnT and 65% had elevated cTnI. Sixty-two patients (36%) died, median survival was not reached and median follow-up of living patients was 48 months. A multivariate analysis including cardiac biomarkers, mLVW, ejection fraction and NYHA class at presentation showed that hs-cTnT was the only independent prognostic determinant. The hs-cTnT cutoff best predicting survival was 75 ng/L (4 year survival 74% vs. 28%, p<0.001). An increase in hs-cTnT at 6 months was associated with lack of hematologic response and shorter survival. However, at multivariate analysis, only NT-proBNP modifications after chemotherapy, and not hs-cTnT changes, independently predicted survival. Among patients who achieved a hematologic response the rate of cardiac progression (as assessed by >30% and >300 ng/L NT-proBNP and ≥2 NYHA class increase) was higher in those who presented with hs-cTnT >14 ng/L (18% vs. 0%, p=0.01). Twelve patients in whom cardiac dysfunction progressed despite hematologic response had a significantly higher pre-treatment hs-cTnT concentration (median 54 vs. 28 ng/L, p=0.05).
Conclusion. These data suggest that 2 components of cardiac damage exist in AL amyloidosis: a structural injure detected by hs-cTnT and a potentially reversible dysfunction reflected by NT-proBNP.

C-64

Levels of Neutrophil Gelatinase Associated Lipocalin (NGAL) reflect the extent of neutrophil activation in patients with acute coronary syndromes

F. Zoubouloglou[1], N. Kafkas[2], C. Demponeras[2], S. Potamitis[1], K. Triantafillou[2], D. Babalis[2], I. Drakopoulos[1], K. Makris[1]. *[1]Clinical Biochemistry Department, KAT General Hospital, Athens, Greece, [2]Cardiology Department, KAT General Hospital, Athens, Greece,*

Background: Activated neutrophils, have been reported in unstable angina (UA) and acute myocardial infarction (AMI) but not in stable coronary artery disease (SCAD). On the other hand in ACS acute worsening of cardiac function leads to kidney injury (acute cardiorenal syndrome type1). NGAL, a 25kD glycoprotein found in human neutrophil granules, is also produced by kidney tubular cells in response to ischemic or toxic insults and has been proposed as early biomarker for acute kidney injury. We hypothesized that serum-NGAL may reflect the extent of neutrophil activation in various stages of ACS and discriminate patients with SCAD from ACS and levels in urine could serve as marker for cardiorenal syndrome.
Methods:We studied 25 patients with UA, 30 with non-ST-elevation AMI (NSTEMI), 20 with ST-elevation (STEMI), 35 with SCAD and 20 controls without risk factors. Exclusion criteria included surgery, liver disease, end stage renal disease, renal cardiac or liver transplantation, neoplasia and infection. Morning collections of serum and urine samples were tested from all patients within 48hours from admission. Serum creatinine, hsCRP, and urinary-NGAL were measured on Architect-ci16200 analyzer(Abbott). Serum-NGAL was measured with an ELISA(Bioporto).
Results: Table summarizes our results. Correlation between serum-NGAL and hsCRP is linear (spearman-coefficient rho=0.685,p<0.0001), while between urine-NGAL and hsCRP is not (rho=0.568,p<0.0001). The diagnostic value for both serum and urine-NGAL in discriminating ACS from SA is high (AUC=0.963 vs AUC=0.900). Using as cut-offs 90.89ng/mL and 13.12ng/mL for serum and urine-NGAL ACS is predicted with sensitivity and specificity, 96.4% and 86.2% vs 80.4% and 82.8% respectively.
Conclusion: Levels of serum and urine-NGAL are higher in patients with ACS than in patients with SCAD and controls. Among ACS patients STEMI patients exhibit higher levels of serum-NGAL than NSTEMI and UA patients (Kruskall-Wallis test). The non-linear relationship of urine-NGAL and hsCRP show that urine-NGAL may additionally reflect renal injury secondary to ACS.

Median(range)	SA	UA	NSTEMI	STEMI	Controls
Nr	35	25	30	20	20
Sex (male/female)	(27/8)	(19/6)	(25/5)	(18/2)	(16/4)
Age	60 (49-74)	64 (42-78)	68 (49-82)	68 (49-80)	36 (25-42)
BMI	29.80 (22.65-38.10)	27.25 (22.00-44.20)	27.30 (22.90-36.70)	26.60 (22.90-29.30)	-
Diabetes	12	10	14	10	0
Hypertension	23	20	23	16	0
Dyslipidemia	24	14	16	14	0
hsCRP mg/dL	0.42 (0.13-1.31)	1.07 (0.06-4.50)	3.66 (0.12-14.93)	7.11 (0.36-15.98)	0.12 (0.02-0.25)
serumNGAL ng/mL	74.72 (40.09-133.19)	117.04 (83.74-192.21)	186.94 (108.99-325.74)	250.24 (146.00-316.78)	56.47 (44.30-70.10)
urine NGAL ng/mL	9.54 (7.02-16.46)	13.26 (9.26-27.87)	32.67 (11.20-205.06)	53.23 (15.17-206.80)	7.85 (4.80-18.50)
serum Creatinine mg/dL	0.90 (0.60-1.20)	0.90 (0.60-1.30)	0.90 (0.70-1.70)	1.00 (0.80-1.50)	0.90 (0.65-1.20)
eGFR(MDRD)	90.21 (66.09-109.20)	89.50 (51.06-127.93)	78.52 (42.44-127.16)	78.98 (48.76-109.20)	95.12 (81.15-110.34)

C-65

Clinical Evaluation of the Abbott ARCHITECT Myeloperoxidase Assay: Improving Risk Stratification in Patients Presenting With Normal Cardiac Troponin I

F. S. Apple[1], L. A. Pearce[2], S. W. Smith[1], K. Schulz[1], R. Ler[1], M. M. Murakami[1]. *[1]Hennepin County Medical Center, Minneapolis, MN, [2]Biostatistical Consulting, Minot, ND,*

Myeloperoxidase (MPO) is thought to play a role in the development of atherosclerosis and as a biomarker of plaque rupture. The purpose of this study was to assess the ability of MPO to identify patients presenting with ischemic symptoms suggestive of acute coronary syndrome (ACS) who would be at risk for major adverse cardiac events (MACE - cardiac death, MI, revascularization). Plasma (EDTA) was collected from 350 patients presenting to the emergency department with symptoms of ischemia, 11.4 % (n=40) with myocardial infarction (MI). Cardiac troponin I (cTnI) and MPO were measured on the Abbott ARCHITECT with 99th percentile reference values of <0.028 µg/L and ≤527.1 pM, respectively. Cardiac events within 30 days and 6 months were computed using the Kaplan-Meier method and compared using the log rank test . The 30-day MACE rate was 7.9 % with a normal cTnI (<99th percentile) compared to 35.8% with an increased cTnI (p<0.001). In the subjects with a normal cTnI (n=246), an increased MPO (>99th percentile) increased the MACE rate to 12.3% (p =0.03). In subjects with an increased cTnI (n=104), an increased MPO increased the MACE rate to 45.0 % (p = 0.02). The 6 month MACE rate was 10.3 % with a normal cTnI compared to 40.9 % with an increased cTnI (p<0.001). In subjects with a normal cTnI, an increased MPO increased the MACE rate to 16.2 % (p = 0.01). In subjects with an increased cTnI, an increased MPO increased the MACE rate to 50.7 % (p = 0.02). Using multivariate Cox Proportional Hazards models adjusted for age, sex, and renal function, an increased cTnI (HR 5.5 at 30 days, HR 4.9 at

6 -month, p<0.001) and increased MPO (HR 2.9 at 30 day, HR 3.0 at 6-month, p ≤0.001) were independently predictive of increased risk of MACE at follow-up. In conclusion, a combination of MPO with cTnI allowed for the identification of a greater proportion of patients at risk for MACE at both 30 days and 6 months than the use of cTnI alone. MPO, a biomarker indicative of atherosclerotic, plaque destabilization, i.e. pre-myocardial cell death, was able to identify patients presenting with ischemic symptoms suggestive of ACS, without an increased cTnI, who are at risk for a future cardiac event.

C-66

Development of an Immunoassay for BNP* on the Dimension Vista® System

L. J. Duggan, A. Amin, J. Duffy, P. Singh, R. Bauer. *Siemens Healthcare Diagnostics, Newark, DE,*

Brain natriuretic peptide (BNP) is an established marker that aids in the diagnosis of congestive heart failure. It is a 32 amino acid polypeptide, with a 17 amino acid ring structure, produced by cleavage of the parent proBNP hormone following its release from cardiac ventricles. We describe the development and initial analytical performance of a monoclonal antibody-based immunoassay for measurement of BNP in patient samples on the Dimension Vista® System.

This method is a one-step sandwich chemiluminescent immunoassay based on LOCI® technology. LOCI reagents include two synthetic bead reagents and a biotinylated monoclonal antibody fragment which recognizes an epitope located at the C-terminus of the BNP peptide. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibeads) is coated with a second antibody specific for a second independent epitope in the ring of the BNP peptide and contains chemiluminescent dye. Sample is incubated with Chemibeads and biotinylated antibody to form a particle/BNP/biotinylated antibody sandwich. Sensibeads then are added and bind to the biotin to form a bead-aggregated immunocomplex. Illumination of the complex by light at 680 nm generates singlet oxygen from Sensibeads, which diffuses to the Chemibeads and triggers a chemiluminescent reaction. The resulting chemiluminescent signal is measured at 612 nm and is directly proportional to the concentration of BNP in the sample.

The Dimension Vista BNP* method has an assay range of 5 - 5,000 pg/mL with good linearity, and has an analytical sensitivity of 1 pg/mL. Functional sensitivity (analyte concentration corresponding to 20% CV total precision) is less than 10 pg/mL. Repeatability (within-run) and within-lab (total) precision estimates were evaluated by following CLSI EP5-A2 protocol over a 20 day testing interval using patient pools and quality control materials. Repeatability and within-lab precision were less than 4.0 and 6.0% respectively across the range.

No significant interference (<10 % bias) was seen from lipemia (3000 mg/dL), hemoglobin (500 mg/dL), or icterus (20 mg/dL). Comparative results from patient samples representative of normal and abnormal levels of analyte were evaluated by Passing-Bablok regression and showed good agreement between this new Dimension Vista method and the ADVIA Centaur® BNP method. The regression statistics are as follows: Dimension Vista Method (Y-axis) versus ADVIA Centaur BNP Method (X-axis): Slope = 0.96, Y-Int = 0.03 pg/mL, r = 0.99, N = 209, range 0-4367 pg/mL.

The method provides excellent analytical performance and good agreement with the ADVIA Centaur BNP method.

* Product under development_Not available for sale

C-67

Assessment of high sensitive troponin T and I immunoassays in patients with acute chest pain without ST-segment elevation

C. Le Goff[1], C. Garweg[1], T. Laurent[1], J. Kaux[2], C. Deroyer[2], M. Merville[2], P. Mélon[1], M. Fillet[1], V. Legrand[1], J. Chapelle[1]. *[1]University Hospital of Liège, Liège, Belgium, [2]University of Liège, Liège, Belgium,*

Background: Cardiac troponin I and T are specific markers of myocardial injury that are widely used for the diagnosis of acute coronary syndrome (ACS). In acute chest pain without ST-segment elevation, they are used to differentiate unstable angina from non ST-segment elevation myocardial infarction (NSTEMI). Recently, troponin assays with higher analytical sensitivities became available to enable the detection of minor myocardial damage and identify individuals at higher risk for ACS. As a result of its high tissue-specificity, cardiac troponin T and I are cardio-specific, highly sensitive markers for myocardial damage. The aim of this study was to evaluate the new higher sensitive troponin (T and I) in patients with stable angina and acute chest pain without ST-segment elevation.

Methods: Sixty subjects (mean age : 65.5± 11 years), were included: 20 healthy controls, 20 patients with stable angina, 9 with unstable angina (troponin-) and 18 patients with NSTEMI myocardial infarction (troponin+). The protocol was approved by the ethics committee of the University of Liège (Belgium). High sensitive troponin T (hsTnT) determination was realized on heparin plasma by electrochemiluminescence immunoassay on Modular E (Roche Diagnostic). Troponin I II (TnI II) is a chemiluminescent microparticle immunoassay for the quantitative determination of cardiac troponin-I in heparine plasma on the ARCHITECT *i* System (Abbott Diagnostic). The lower detection limit of these assays was 0.005µg/L for hsTnT and 0.01µg/L for TnI II. Stastistical analysis was performed using *t* test; p-value <0.05 was regarded as statistically significant.

Results: HsTNT levels were 0.003(0.003, 0.004) [median baseline (1st, 3rd quartile)]ng/ml in controls, 0.0075 (0.00475, 0.014) ng/ml in stable angina, 0.011(0.006, 0.012) ng/ml in unstable angina and 0.3715 (0.1795, 1.00725) ng/ml in NSTEMI ACS. TnI II levels were 0 (0, 0.001) ng/ml in controls and in patients with stable angina, 0.07 (0.005, 0.014) ng/ml in unstable angina and 1.4475 (0.0407, 2.656) ng/ml in NSTEMI.

HsTNT and TnI II levels were significantly increased in NSTEMI as compared to control subjects, patients with stable and unstable angina. TnI II levels were also increased in unstable angina as compared to controls.

Conclusion: In our population, TnI II was more sensitive than hsTNT to detect minor myocardial damage in patients with unstable angina as compared to controls. Therefore, future studies will have to determine whether TnI II might contribute to better risk stratification and treatment strategy in this group of patients.

C-68

Influence of strenuous eccentric exercise on Centaur Troponin I (TnI) Ultra

G. Lippi[1], G. Salvagno[2], M. M. Mion[3], F. Impellizieri[4], F. Schena[4], M. Gelati[2], M. Zaninotto[3], M. Plebani[3], G. Guidi[2]. *[1]U.O. di Diagnostica Ematochimica, Parma, Italy, [2]Sez. Chimica Clinica, Dip. Scienze Morfologico-Biomediche, Verona, Italy, [3]Dipartimento di Medicina di Laboratorio, Università di Padova, Padova, Italy, [4]Sez. Scienze Motorie, Dip. Scienze Neurologiche e della Visione, Verona, Italy,*

Background. It has recently been reported that cardiac troponin (cTn) levels might be increased after strenuous endurance exercise, though the reasons underlying this increment are still partially unknown. To establish whether skeletal muscular injury rather than myocardial sufferance might contribute to this increase, we assessed the influence of eccentric exercise on the newly highly sensitive cTnI.

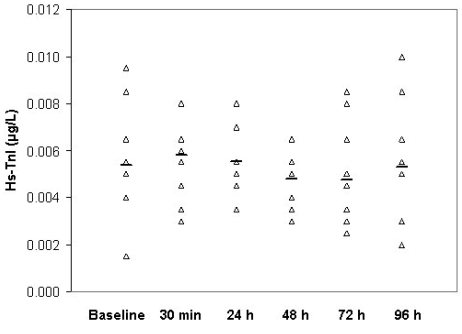
Materials and Methods. Nine healthy males performed 40-min downhill (DW) on a treadmill with a gradient of -25% and at walking velocity of 6.4 km/h. To increase the likelihood that DW would induce muscle damage, subjects were loaded with 10% of their body weight carried in a back pack, according to the standard protocol. Blood samples were drawn before exercise, and 30 min, 24, 48, 72 and 96h thereafter. Serum cTnI was measured with the new Centaur TnI Ultra (Siemens Healthcare Diagnostics). The lowest concentration measurable with the Centaur cTnI Ultra assay with a 10% CV is 0.03 µg/L, and 0.025 µg/L with a 20% CV.

Results. As shown in figure 1, no significant increase of cTnI was observed immediately after the exercise and thereafter (all p>0.05). In no case, moreover, the values of TnI exceeded the 99th percentile for apparently healthy subjects throughout the study period (0.08 µg/L).

Conclusions. Results of our investigation are consistent with the hypothesis that eccentric exercise is associated with higher levels of force that can evoke skeletal but not myocardial muscle injury and, therefore, skeletal muscular injury does not influence the sensitivity of this novel cTnI assay for diagnosing myocardial injury.

Figure 1.

Variation of highly sensitive cardiac troponin T (Hs-TnT) and myoglobin values after eccentric exercise (*n*=9). The central horizontal lines indicate the mean value.



C-69

Two center evaluation of new Beckman high sensitivity troponin indicates continuing need to validate borderline positives with recentrifugation and reanalysis

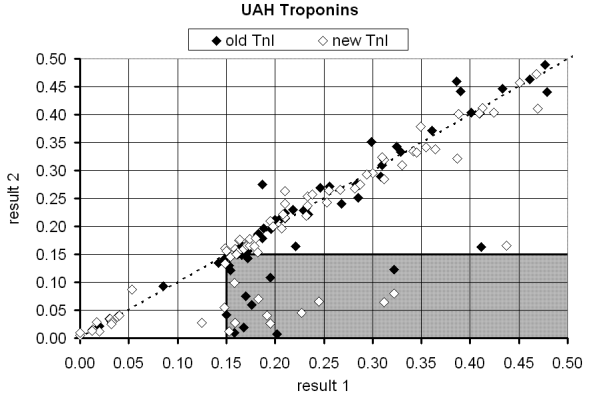
G. S. Cembrowski[1], G. B. Blakney[2], W. Skoropadyk[1], D. Gersky[2], D. Levesque[1], A. Kunst[1], D. V. Tran[1]. *[1]University of Alberta Hospital, Edmonton, AB, Canada, [2]Royal Alexandra Hospital, Edmonton, AB, Canada,*

Introduction Some laboratories have found that recentrifugation and reanalysis of positive Beckman Access troponin I plasma specimens results in clinically significant troponin reductions, even to normal troponin levels and this can increase clinicians' confidence with troponin testing. Last fall, Beckman released new Access AccuTnI (troponin I) reagents for Access Immunoassay systems. The advantages of the new troponin I included lesser heterophilic antibody interference and amelioration of false positives due to the incomplete mixing of plasma separator tubes after blood drawing. To assess the assay's improvement, we compared the proportion of plasma specimens whose troponin dropped, after recentifugation and reanalysis, from "positive and indicative of myocardial damage" (ranging from 0.15 to 0.60 ug/L) levels representing "normal to equivocal" (less than 0.15 ug/L).

Methods At two Edmonton tertiary/quaternary hospitals, during two month usages of the old and new TnI reagents, we measured the proportions of patient specimens that initially tested positive on the Beckman DxI 800 and then tested negative after recentrifugation and reanalysis.

Results The Figure shows the results for University of Alberta Hospital. Between April 23 and June 21, 2009, 13 of 85 specimens dropped from between 0.15 and 0.60 ug/L to under 0.15 ug/L. With the new reagents, tested between November 23, 2009 and January 3, 2010, 12/103 specimens normalized (not statistically different). The results were very similar for the Royal Alexandra Hospital (9/131 for old reagents vs. 18/157 for new reagents).

Conclusions Laboratories that analyze plasma with the Access AccuTnI reagent should consider recentrifugation and reanalysis of lower range positive troponins. Redefining the lower limit of normal from 0.15 to 0.10 ug/L may present difficulties as this window of values encompasses a higher density of overlap between healthy and "diseased" patients and probably a higher density for potential false positives that can be reduced by recentrifugation.



C-70

Multiple Biomarkers of Various Pathophysiologic Pathways to Determine Relationships for Diagnosis of Myocardial Infarction and Adverse Outcomes in Patients Presenting with Symptoms of Acute Coronary Syndrome

M. M. Murakami[1], L. A. Pearce[2], R. Ler[1], K. M. Schulz[1], S. W. Smith[1], F. S. Apple[1]. *[1]Hennepin County Medical Center, Minneapolis, MN, [2]Biostatistical Consulting, Minot, ND,*

We investigated multiple biomarkers (novel and contemporary) of diverse pathophysiologic pathways to determine their relationships with diagnostic accuracy for acute myocardial infarction (AMI) and adverse outcomes in patients presenting with symptoms suggestive of acute coronary syndrome (ACS). Clinical sensitivities and specificities were evaluated along with ROC curve areas (AUC) for 9 biomarkers measured in plasma specimens collected on admission in 400 patients with acute ischemic symptom onset within 12 hours of presentation: myeloperoxidase (MPO, Siemens, cut off 633 pmol/L), C-reactive protein (hsCRP, Siemens Dimension RxL, cut off 3 mg/L), cardiac troponin I (cTnI, Abbott ARCHITECT, cut off 0.028 µg/L), N-terminal pro-B-type natriuretic peptide (NT-proBNP, Siemens Dimension RxL, age and eGFR dependent cut offs), copeptin (BRAHMS Krytor, cut off 11.25 pmol/L), mid-range pro atrial NP (MR-proANP, BRAHMS Kryptor, cut off 163.9 pmol/L), BNP (Abbott ARCHITECT, cut off 100 ng/L), ST2 (Critical Diagnostics, cut offs 30.6 U/mL men, 20.9 U/mL women), eGFR (cut off <60 mL/min). Forward and backward stepwise logistic regression modeling techniques were used to identify biomarkers independently predictive of AMI. Cox proportional hazards models were used to estimate relative risk of death during 6 mo follow-up and to identify biomarkers independently predictive of death. Fifty patients (12.5 %) received an AMI diagnosis. ROC AUCs were: cTnI 0.80 (sens 83%, spec 77%), MPO 0.59 (84%, 33%), copeptin 0.59 (65%, 52%), NT-proBNP 0.57, hsCRP 0.56, BNP .55, eGFR 0.55, ST2 0.54, MR-proANP 0.51. Increased cTnI, particularly in patients with normal eGFR and normal BNP, was powerfully predictive of AMI, with MPO or copeptin adding marginally to the model. In patients presenting with normal cTnI, 5 of 6 (83%) of patients diagnosed with AMI had increased copeptin vs. 43% of the 23 who were not diagnosed with AMI. Twenty deaths occurred within 6 months. cTnI (HR 2.0), MPO (HR 8.3), NT-proBNP (HR 6.2), MR-proANP (HR 5.7), copeptin BNP (HR 2.1), ST2 (HR 4.4) and eGFR (HR 2.9) were all univariately associated with increased risk of death, but not hsCRP. Copeptin (HR 15.5, $p < 0.001$) and MPO (HR 5.9, $p = 0.05$) were independently predictive of death, with 17 of the deaths occurring in patients with both markers elevated. In conclusion, cTnI was the most accurate biomarker for the diagnosis of MI. Whether MPO and/or copeptin add earlier diagnostic value requires further investigation in a larger group of patients presenting within the early 6 hour window after onset of symptoms. For 6 mo mortality risk, our findings suggest that only copeptin and MPO, biomarkers indicative of different underlying pathophysiologic mechanisms, are independently predictive of increased risk in patients presenting suggestive of ACS. The cost effectiveness of adding additional biomarkers to cTn still needs to be determined.

C-71

Does echocardiographic stress test induced release of hsTnT and TnI II?

C. Le Goff[1], T. Laurent[1], C. Garweg[1], J. Kaux[2], C. Deroyer[2], M. Fillet[2], P. Lancellotti[1], L. Pierard[2], J. Chapelle[2]. *[1]University Hospital of Liège, Liège, Belgium, [2]University of Liège, Liège, Belgium,*

Background: Cardiac troponins (cTn) are considered as the best biomarkers for detection of myocardial cell injury. In this study, cTnT and cTnI were measured by new commercially available high-sensitive methods in patients undergoing brief exercise- or pharmacologic- induced stress. Our aim was to compare cTnT and cTnI levels before and after the stress tests, in the patients with or without reversible ischemia.

Materials and Methods: Fifty patients (28 men and 22 women) underwent an echographic stress test (ST) for suspected ischemic heart disease. Of these 50 patients, 28 received pharmacological ST (dobutamine injection) and 22 dynamic ST (bicycle exercise). The patients were subdivided into two groups according to the presence or absence of documented transient reversible ischemia: 14 with reversible ischemia (mean age: 67.71±9.66 y) and 36 without ischemia (mean age: 63.17±11.72 y).

In all patients, cTnT and cTnI concentrations were measured by high sensitive methods (hsTnT, Roche Diagnostics and TnI II, Abbott Diagnostics) on heparin plasma immediately before (T0) and after ST (T1).The lower detection limit of these assays was 0.005µg/L for hsTnT and 0.01µg/L for TnI II.

The protocol was approved by the ethics committee of the University of Liège (Belgium). All patients gave informed consent. All statistical analyses were performed using Medcalc version 8.1 for Windows. P value <0.05 was regarded as statistically significant.

Results: There was no significant difference between hsTnT concentrations at T0 and T1, neither in the whole patient group, nor in the subgroups of subjects who received pharmacological ST or dynamic ST. The same was true for TnI II.

Although there was no change in hsTnT levels during test in ischemic and in non ischemic patients, the latter tend to demonstrate higher median T0 levels (25^{th}, 75^{th} percentiles) than the others [0.011 (0.007, 0.029) vs 0.007 (0.0047, 0.1125) ng/ml, p=0.09]. They also showed higher median T1 levels [0.014 (0.065, 0.03) vs 0.007 (0.003, 0.0102) ng/ml, p=0.08]. Higher TnI II levels were also recorded in ischemic patients as compared to non ischemic patients at T0[0.014 (0.0072; 0.0265) vs 0.005 (0.003; 0.01) ng/ml, p=0.08] and T1[0.013 (0.0085-0.03) vs 0.006 (0.0035-0.008) ng/ml, p=0.08]. Also, TnI II levels did not change during test in both subgroups.

Conclusions: Measurement of cardiac troponins by high sensitive methods did not allow to detect significant release of biomarkers from the heart during exercise-or pharmacologic-induced ST, even in patients who demonstrated reversible myocardial ischemia. The type of test - pharmacological or dynamic - was without effect. The patients with induced transient ischemia had however higher troponin T and I levels at baseline, this difference remaining during test.

C-72

Clinical Evaluation of the Beckman Coulter Access and Siemens Stratus CS Cardiac Troponin I Assays in Patients Suspected of Acute Coronary Syndrome: Impact of MI Classification Using Strict Guideline Adherence

F. S. Apple[1], L. A. Pearce[2], K. M. Schulz[1], S. W. Smith[1], M. M. Murakami[1]. *[1]Hennepin County Medical Center, Minneapolis, MN, [2]Biostatistical Consulting, Minot, ND,*

Cardiac troponin (cTn) testing, which can be very assay dependent, plays an important role in assessing patients presenting to an emergency department with ischemic symptoms. In 2007 the guidelines for diagnoses of MI were established to include classification of 5 types of myocardial infarction (MI). Type I MI is defined as spontaneous MI related to a primary coronary event such as plaque rupture, while a Type II MI is defined as MI secondary to ischemia due to imbalance between oxygen demand & supply. The goal of this study was twofold. First, we evaluated both the diagnostic accuracy and the prognostic value for risk assessment of the Beckman Coulter Access and Siemens Stratus CS cardiac cTnI assays in a heterogeneous population of patients presenting with ischemic symptoms suggestive of acute coronary syndromes (ACS). Second, we compared classification of MI using the local ICD-9 coded MI rate with an adjudicated MI diagnosis rate following strict guideline criteria. cTn was measured on admission plasma specimens in 662 patients. The local MI diagnosis (from ICD-9 codes) was established in 39 patients (5.9%). Adjudication by three independent clinicians following strict Global Task Force guidelines established the diagnosis of MI in 139 patients (21.0%); a 3.5-fold increase. 92% of the additional MIs were Type II classifications. Based on the 99th percentile cutoffs (Beckman 0.04 µg/L and Siemens CS <0.1 µg/L), baseline specimen clinical sensitivity and specificity were: Beckman 66 % and 76%, and Siemens CS 56 % and 86%, respectively (local ICD-9 diagnoses). Diagnostic accuracy based on ROC curve areas were identical for the Beckman Coulter Access assay (0.79) and the Siemens Stratus CS assay (0.79). Excluding the 39 MI subjects, the 99th percentile concentrations for all remaining non-ACS patients were: Beckman 1.19 µg/L and Siemens CS 1.99 µg/L. Sixty-five patients had an MI or died within 60 days of presentation. The 60-day combined MI and all cause death event rates (estimated by the Kaplan-Meier method and compared with the log rank statistic) based on the LoD (Beckman 0.01 µg/L; Siemens CS 0.04 µg/L) and the 99th percentile values (<LoD, LoD to 99th percentile, >99th percentile) were as follows: Beckman ($p < 0.001$) 5.3% (7 events) vs 9.9% (19) vs. 24.9% (38); Siemens CS ($p < 0.001$) 7.1% (24 events) vs, 28.0% (15) vs. 26.9% (26). Our findings demonstrate that both the Beckman Access and the Siemens Stratus CS cTnI assays are sensitive diagnostic biomarkers for the early detection of MI as well as predictors of increased risk for adverse events at any measurable cTnI value in patients presenting with symptoms suggestive of ACS. When the criteria established for the Universal MI diagnosis is strictly followed, additional, under-diagnosed non-ST elevation MIs (NSTEMI), predominantly Type II, were identified. Finally we show that 14 to 24% of non-ACS patients have increased presentation cTnI concentrations that overlap with concentrations found in patients presenting with an MI.

C-73

Diagnostic Accuracy and Risk Outcomes Assessment of Roche's High Sensitivity cTnT and cTnI Assays Compared to the Beckman AccuTnI and Siemens Ultra cTnI Assays in Patients With Symptoms Suggestive of Acute Coronary Syndrome

F. S. Apple[1], L. A. Pearce[2], R. Ler[1], K. M. Schulz[1], S. W. Smith[1], M. M. Murakami[1]. *[1]Hennepin County Medical Center, Minneapolis, MN, [2]Biostatistical Consulting, Minot, ND,*

The purpose of this study was to clinically evaluate Roche's new high sensitivity (hs)-cTnT and cTnI assays compared to two contemporary cTnI assays both for diagnostic accuracy for ruling out acute myocardial infarction (AMI) and for risk outcomes assessment in a non-selected, heterogeneous population of patients presenting with ischemic symptoms suggestive of acute coronary syndromes (ACS). Plasma was collected from patients (n=427) presenting with symptoms suggestive of ACS at presentation and approximately 6 to 24 hours post admission. cTnT was measured by Roche's hs-cTnT and 4th generation cTnT assays, with 99th percentile concentrations of 0.014 µg/L and <0.01 µg/L, respectively. cTnI was measured using the Roche Elecsys, Siemens Stratus CS, and Beckman Access assays; with 99th percentile concentrations of 0.16 µg/L, <0.1 µg/L, and 0.04 µg/L, respectively. Combined endpoint of first of MI or cardiac death (MACE) within 30 days and 6 mo from time of presentation were used for risk outcomes assessment with rates estimated by the Kaplan-Meier method and compared using the log rank statistic. 43 (10.0 %) of patients had the diagnosis of AMI. Clinical sensitivities and specificities based on the presentation specimen in descending order of sensitivity were Roche hs-cTnT 81%/47%, Beckman cTnI 67%/74%, Siemens CS cTnI 60%/79%, Roche cTnT 58%/70%, Roche cTnI 33%/89%. Using maximum cTn concentrations all assays showed sensitivities between 88 to 98% with specificities similar to the presentation sample. The ROC curve areas ranged from 0.65 to 0.78 for presentation sample and from 0.88 to 0.94 for maximal concentration samples. Each of the Roche hs-cTnT, Siemens Stratus CS cTnI, and Beckman Access cTnI assays had higher accuracy for the presentation sample than the Roche 4th generation cTnT assay (all $p < 0.01$); the Beckman Access cTnI and Siemens cTnI had higher accuracy than the Roche 4th generation cTnT assay for maximum cTn concentration (both $p \leq 0.01$), and the Siemens cTnI was more accurate than the Roche hs-cTnT assay ($p = 0.02$). The 30 day MACE rate was 11.1% (46 events). MACE rates for the presentation sample by 99th percentile cutpoint were hs-cTnT 16.2 % v. 4.2 % ($p = 0002$); cTnT 20.0 % v. 6.3% (< 0.001); Beckman cTnI 24.8% v. 4.7 % (< 0.0001); Siemens CS cTnI 25.9 % v. 5.8% (< 0.0001); Roche cTnI 25.5% v. 8.6% ($p = 0.0002$). Similar results were found utilizing 6-month follow up data. Our findings demonstrate subtle differences in diagnostic accuracy (sensitivity and specificity) between the multiple cTnT and cTnI assays evaluated, implying that assays should not be interchanged within a medical center. Risk outcome assessments appear similar between assays, although patient numbers within each risk group varied

C-74

Development of a Next Generation Cardiac Troponin I Method for the Dimension Vista® System.

G. Hickey, B. Bedzyk, V. Desai, M. Drinan, K. Gibney, T. Johnson, E. Magee, M. Sharma, P. Singh, Z. Teng, R. Bauer. *Siemens Healthcare Diagnostics Inc, Newark, DE,*

Methods for quantitation of cardiac troponin have evolved considerably over time in terms of performance, with recent generations having achieved significantly improved analytical sensitivity and lower limits of detection. Over the past several years, analytical and clinical evaluations of "next generation, high sensitivity" troponin assays have appeared in the literature. These assays have these performance characteristics in common: 1) ability to detect troponin at pg/mL levels 2) ability to detect troponin in normal individuals and develop a reference interval and 3) ability to demonstrate acceptable functional sensitivity (10% total CV) at the upper end of the reference interval. The purpose of this study was to determine the performance characteristics of a new, high sensitivity Troponin I method* for the Dimension Vista® System from Siemens Heathcare Diagnostics Inc. As with the current Troponin I method, the new high sensitivity method is a fully automated homogeneous sandwich immunoassay based on LOCI® technology, with a time to first result of 10 minutes. The LOCI reagents contain high affinity antibodies for both capture and detection of cTnI. The analytical sensitivity of the method was determined to be less than 1.5 pg/mL, based on 20 replicates of analyte free sample, and the upper end of the assay range is 20,000 pg/mL. Good linearity was observed throughout the assay range. The functional sensitivity, defined as the lowest analyte level with a 10% total CV was determined to be less than 4 pg/mL based on a two replicate per day, 20 day ANOVA study using patient pools. Method reproducibility was determined using patient pools, QC material and spiked serum samples with a two replicate per day, 20 day ANOVA study. Repeatability (Within-Run) %CV was 1.1%, 1.0% and 1.0% at 175.7, 246.1, and 16,650.1 pg/mL, respectively. Within-lab (Total) %CV at those levels was 2.7%, 1.4% and 2.4%. Method comparison to the current commercial Dimension Vista Troponin I method using serum and heparin plasma samples (n = 47 serum and 47 plasma) over the range of 0.00 to 14,600 pg/mL yielded the following Passing-Bablok regression statistics: slope = 1.00, y-intercept = -6.5, r = 0.95. The 99th percentile of a reference interval study comprised of 219 blood bank donors ranging in age from 20 to 60 years was determined to be 12.5 pg/mL. We conclude that this new method has demonstrated sufficient sensitivity to measure detectable levels of cTnI in most normal individuals and meets the joint recommendation of the American and European cardiology societies that the precision (total CV) of the method is less than 10% at the 99th percentile of the reference interval.

*Product under development - not available for sale.

C-75

Highly sensitive cardiac troponin assays in hypertrophic cardiomyopathy: new tool for a better patient management?

M. Zaninotto[1], M. M. Mion[1], A. Karydi[1], P. Melacini[2], C. Calore[2], S. Iliceto[2], F. Corbetti[3], M. Plebani[1]. *[1]Department of Laboratory Medicine, University-Hospital, Padova, Italy, [2]Department of Cardiac Thoracic and Vascular Sciences, University, Padova, Italy, [3]Department of Radiology, University, Padova, Italy,*

International guidelines recommend cardiac troponin (cTnI and cTnT) as the biomarker of choice to detect myocardial necrosis. Recently, the improved analytical performance of the cTn methods has allowed the commercial availability of a new generation of cTn assays namely highly sensitive cTn assays (hs-cTn) characterized

by an increased analytical sensitivity and precision, particularly at the low-end of the measuring range (cTn concentration corresponding to 10% Coefficient of Variation : cTn concentration corresponding to 99th percentile Upper-Reference-Limit = ≤ 1). Hypertrophic cardiomyopathy (HCM) is a primary myocardium disease characterized by an heterogeneous clinical presentation and natural history. Myocardial ischemia is responsible for myocyte death and replacement fibrosis that play a crucial role in disease progression. Aim of the study was to assess the role of hs-cTn assays in HCM patients (pts) management and outcome prediction. cTn plasma concentrations (lithium-heparin) were determined using 2 hs-cTn assays: CTNI LOCI, Siemens (hs-cTnI, 99th percentile=0.045 µg/L) and High Sensitivity Troponin T, Roche Diagnostics (hs-cTnT, 99th percentile=14 ng/L. The biomarkers concentration was studied in relation to clinical and instrumental parameters. Age, gender, cardiac symptoms, electrocardiographic abnormalities, arrhythmias at 24 hours-Holter monitoring, echocardiographic and cardiac magnetic resonance data were collected in 67 consecutive HCM pts enrolled in the study. Pts with evidence of epicardial coronary artery disease were excluded. Studied population, main characteristics: males, 45 (67%); age, mean±SD=43±16 years; NYHA (New York Heart Association) class I+II, 58 (86%); LVEF (Left Ventricular Ejection Fraction), mean±SD=59±9%. Pts with positive hs-cTn values (hs-cTnI: n=14, 21%; hs-cTnT: n=24, 36%) showed: lower LVEF (hs-cTnI: 54±10% vs 60±8%, p=0.035; hs-cTnT: 55±11% vs 61±7, p=0.05); most severe diastolic dysfunction (i.e. prevalence of restrictive pattern; hs-cTnI: 21 vs 5%, p=0.02; hs-cTnT: 22 vs 0%, p=0.002); ischemic ECG abnormalities (i.e. ST-segment downslopping; hs-cTnI: 57 vs 11%, p=0.0002; hs-cTnT: 42 vs 9%, p=0.002). At multivariate analysis, using models considering also age and gender, inverse-linear relationships were found among ln hs-cTnT, LVEF and diastolic dysfunction (R^2 0.26, adjusted R^2 0.23, p=0.0002) as well as among ln hs-cTnI, ST-segment downsloping and the presence of ventricular tachiarrhythmias (R^2 0.33, adjusted R^2 0.30, p < 0.0001). Pts with at least 1 hs-cTn positive value (higher than 99th percentile) during the follow-up (mean±SD=26±18 months) had worse outcome (composite end-point: progressive severe heart failure, heart-failure related death, cardiac transplantation; Kaplan-Meier curves, p=0.04). In HCM pts with impaired systolic function and/or severe diastolic dysfunction, cardiac troponins measured by highly sensitive assays resulted valuable biomarkers of HCM progression to advanced heart failure and useful predictors of prognosis.

C-76

Utility of Plasma Concentration of Myeloperoxidase as Screening for Cardiovascular Disease in Outpatients with Type 2 Diabetes

F. Kitagawa, A. Suzuki, S. Asano, S. Sekiguchi, A. Kuno, A. Nakashima, K. Nakamura, T. Fujita, T. Ishikawa, I. Tanaka, M. Itou, J. Ishii. *Fujita Health University, Toyoake, Japan,*

Myeloperoxidase (MPO) has emerged as a critical mediator in the physiopathology of atherosclerosis from plaque formation and growth until destabilization and rupture leading to acute coronary syndrome. We evaluated the utility of plasma concentration of MPO as screening for cardiovascular disease in 349 outpatients with type 2 diabetes.

Results: 1) A history of cardiovascular disease was present in 77 (21%) patients. 2) Patients with increased MPO (> 75th percentile of 382pmol/L) had higher levels of white cell counts, high-sensitive CRP, hemoglobin A1c, NT-proBNP and cystatin C, had a lower level of hemoglobin, and had a higher prevalence of cardiovascular disease compared with those without increased MPO (**Table**). 3) On a stepwise logistic analysis including 13 clinical and biochemical variables, high-sensitive CRP (odds ratio 2.98 per increase of 10-fold, P<0.0001), hemoglobin A1c (1.51 per increase of 1.0%, P=0.009) and cystatin C (2.64 per 1.0mg/L, P=0.002) were independently associated with increased MPO.

Conclusion: Plasma MPO concentration may be useful for screening cardiovascular disease in outpatients with type 2 diabetes.

Data are expressed as median or %

Increased MPO(>382pmol/L)	+ (n=86)	- (n=263)	P value
MPO (pmol/L)	468	262	<.0001
Age (yrs)	68	60	.08
Male (%)	50	61	.08
White blood cell counts (/µL)	6400	5600	.0007
High sensitive CRP (mg/L)	1.08	0.39	<.0001
Hemoglobin A1c (%)	7.2	6.6	.003
Hemoglobin (g/dL)	12.9	13.6	.004
NT-proBNP (pg/mL)	22	9	.0007
Cystatin C (mg/L)	0.83	0.72	.0007
Cardiovascular disease (%)	31	19	.02

C-77

"New" highly sensitive and "Old" troponin assays in a real-world setting: which advantages for a proper management of acute coronary syndrome patients?

M. Zaninotto[1], M. M. Mion[1], A. Karydi[1], G. Biasillo[2], L. M. Biasucci[2], M. Plebani[1]. *[1]Department of Laboratory Medicine, University-Hospital, Padova, Italy, [2]Institute of Cardiology, Catholic University, Roma, Italy,*

The early diagnosis of acute coronary syndrome (ACS) and the proper management of chest pain patients (pts) still represents a serious clinical challenge in the emergency department (ED). International guidelines recommend cardiac troponins (cTnI, cTnT), as the biochemical gold standard for the detection/exclusion of myocardial infarction (MI). The recent commercially available highly sensitive cTn assays (hs-cTn) shortening the time to test's positivity promise a higher sensitivity for the diagnosis of ACS among pts admitted early to ED. We compared highly sensitive and conventional cTn assays for the early diagnosis of ACS. n=356 consecutive pts (216 males, 140 females; age, 50th percentile, range: 63, 15-94 years) admitted to ED within 12 hours from the onset of chest pain (<3 hours=32%, 3-6 hours=17%, >6 hours=51%) were enrolled. n=114 out of n=356 pts (32%) were admitted to hospital whereas n=242 out of n=356 (68%) were discharged from ED. EKG and previous history of ACS were obtained in all pts. cTn serum levels were determined in the ED admission samples using 3 conventional and 3 highly sensitive assays: Troponin T (cTnT-R, Roche Diagnostics), ST AIA-PACK cTnI-3rd generation (cTnI-T, Tosoh), Access AccuTnI (cTnI-B, Beckman Coulter) and High Sensitivity Troponin T (hs-cTnT-R, Roche Diagnostics), CTNI LOCI (hs-cTnI-S, Siemens), TnI-Ultra (hs-cTnI-A, Siemens). The diagnostic accuracy for the ACS diagnosis of the different cTn assays was evaluated calculating the Area Under the Curve (AUC) by the Receiver-Operating Characteristic (ROC) curve analysis. n=37 out of n=356 pts (10%) were discharged with a diagnosis of ACS according to current guidelines. Diagnostic accuracy was similar among the different cTn tests being AUC (95% CI): cTnT-R=0.81 (0.73-0.90), cTnI-T=0.82 (0.74-0.90), cTnI-B=0.84 (0.75-0.93), hs-cTnT=0.90 (0.84-0.96), hs-cTnI-S=0.82 (0.73-0.90), hs-cTnI-A=0.88 (0.80-0.95). A statistically significant difference was obtained comparing the AUC of the hs-cTnT-R assay vs all the 3 traditional cTn assays and vs 1 out of the other 2 hs-cTn assays (p): hs-cTnT-R vs cTnT-R (0.0073), hs-cTnT-R vs cTnI-T (0.0045), hs-cTnT-R vs cTnI-B (0.0224), hs-cTnT-R vs hs-cTnI-S (0.0036). The results obtained in this study show that in a real-world setting with low prevalence of ACS like chest pain patients in ED, hs-cTn assays could improve significantly the diagnosis of ACS in comparison to traditional troponin assays. Moreover, the observed diagnostic accuracy of the studied hs-cTn assays might be underestimated due to the large proportion of pts (>50%) presented to ED with a time from symptoms onset to ED admission longer than 6 hours: the long time elapsing between pain onset and ED sampling may reduce the potential advantage of the highly sensitive troponin assays utilization.

C-78

Utility of Very Low Concentrations of Cardiac Troponin T as Screening for Cardiovascular Risk in Outpatients with Type 2 Diabetes

A. Kuno, F. Kitagawa, A. Suzuki, S. Asano, S. Sekiguchi, A. Nakashima, T. Sakuishi, M. Saitou, Y. Akiyama, T. Ishikawa, I. Tanaka, M. Itou, J. Ishii. *Fujita Health University, Toyoake, Japan,*

A new high-sensitive assay for cardiac troponin T (hsTnT) makes it possible to measure concentrations >5-fold lower than the limit of the traditional assay. We evaluated the utility of serum hsTnT as a risk marker of cardiovascular disease in 409 outpatients with type 2 diabetes.

Results: 1) HsTnT was detectable (>0.002ng/mL) in 80% of the patients. 2) A history of cardiovascular disease was present in 89 (22%) patients. 2) Patients with increased hsTnT (>0.014ng/mL) are older, had higher values of NT-proBNP, high-sensitive CRP, cystatin C and urine albumin/creatinine ratio, had lower values of hemoglobin and estimated GFR, and had a higher prevalence of cardiovascular disease compared with those without increased hsTnT (**Table**). 3) On a stepwise logistic analysis including 13 clinical and biochemical variables, NT-proBNP (odds ratio 7.40 per increase of 10-fold, P<0.0001) and cystatin C (18.0 per 1.0mg/L, P<0.0001) were independently associated with increased hsTnT.

Conclusion: A new hsTnT assay may be useful for screening cardiovascular risk in outpatients with type 2 diabetes.

Data are expressed as median or %

Increased hsTnT (>0.014ng/ml)	+ n=79(19%)	- n=330	P value
HsTnT (ng/ml)	0.023	0.005	<.0001
Age (yrs)	73	66	<.0001
Male (%)	50	61	NS
White blood cell counts (/µL)	6000	5800	NS
Hemoglobin (g/dL)	12.2	13.6	.004
Hemoglobin A1c (%)	6.8	6.7	NS
NT-proBNP (pg/mL)	303	54	.0007
High-sensitive CRP (mg/L)	0.72	0.45	<.0001
Estimated GFR (ml/min/1.73m2)	46	78	<.0001
Cystatin C (mg/L)	1.17	0.72	<.0001
urine albumin/creatinine ratio (mg/g•Cr)	263	28	<.0001
Cardiovascular disease (%)	37	18	.0007

C-79

BNP Measurements by Two Assays Utilizing Antibodies with Different Epitope Specificity

N. N. Tamm[1], A. G. Semenov[1], K. R. Seferian[1], M. M. Murakami[2], F. S. Apple[2], E. V. Koshkina[3], M. I. Krasnoselsky[4], R. V. Malanichev[5], A. G. Arutyunov[6], A. G. Katrukha[1]. *[1]HyTest Ltd., Turku, Finland, [2]Hennepin County Medical Center, University of Minnesota, Minneapolis, MN, MN, [3]67 City Hospital, Moscow, Russian Federation, [4]Moscow State Medico-Stomatological University, Moscow, Russian Federation, [5]4 City Clinical Hospital, Moscow, Russian Federation, [6]Pirogov Russian State Medical University, Moscow, Russian Federation,*

Background: B-type natriuretic peptide (BNP) is a reliable biomarker of prognosis and to aid in the diagnosis of chronic heart failure (HF). Commercial assays have been developed for quantitative immunodetection of BNP. Most are designed as a sandwich-type immunoassay utilizing two monoclonal antibodies (MAbs) specific to different epitopes. At least one of these two antibodies is specific to the ring structure of the BNP molecule, while the other can be specific to the N- or C-terminus of peptide. Plasma from HF patients contains multiple forms of BNP truncated from both the N- and C-terminus. Only a small portion of BNP circulates as the full-length form (BNP-32). Thus measurement of BNP by current commercial assays is susceptible to proteolytic degradation. Recently we reported a "Single Epitope Sandwich Immunoassay" (SES assay), in which one MAb 24C5 is specific to the ring fragment of the BNP molecule and the second MAb - Ab-BNP2 only recognizes the immune complex of MAb 24C5 with BNP or proBNP. This assay demonstrated greater stability of BNP and proBNP compared to commercial assays. In the current study we have compared BNP concentrations measured by the SES and Siemens ADVIA Centaur BNP immunoassays to determine differences in the content of BNP and/or proBNP in blood of HF patients.
Materials and Methods: Plasma samples (n=103) from HF patients were analyzed by the SES and Siemens assays. Both assays were calibrated using recombinant proBNP (expressed in *E. coli*). The Siemens assay utilizes one MAb specific to the C-terminus (epitope 27-32) and another MAb specific to the ring structure of BNP molecule (epitope 14-21). MAb 24C5 (epitope 11-17), MAb Ab-BNP2 and recombinant proBNP (expressed in *E. coli*) were provided by HyTest.
Results: 100% of the plasma samples measured by the SES assay contained significantly more BNP; from 1.2- to 7.2-fold; 2.1±0.89 (mean±SD) compared to the Siemens BNP immunoassay. Six of the samples (5.8%) measured by SES assay were 3 to 7.2-fold greater than the Siemens assay. BNP concentration in the plasma samples was ranged from 0.003 to 1.8 nmol/L and from 0.005 to 2.65 nmol/L as determined by the Siemens BNP immunoassay and by the SES assay, respectively.
Conclusion: BNP measured by the commercial Siemens assay underestimates circulating BNP concentrations in HF patients. The HyTest prototype SES assay appears to better determine the absolute concentrations of circulating BNP and proBNP. Clinical studies will be necessary to determine the clinical implications of this observation for the diagnosis, outcomes assessment and management of patients presenting with symptoms of HF.

C-80

Which marker of kidney dysfunction for prognosis in acute cardiovascular patients?

P. Cappelletti[1], D. Rubin[1], P. Bulian[2], M. Cassin[1], G. Nicolosi[1]. *[1]AOSMA, Pordenone, Italy, [2]CRO, Aviano, Pordenone, Italy,*

Objective. We evaluated the performance of two markers of kidney dysfunction, eGFR (estimated Glomerular Filtration Ratio) and uAER (urinary albumin excretion ratio) as predictors of the risk of adverse events at long term (6 months) in population of ACS (acute coronary syndromes) patients admitted in CCU.
Relevance. In ACS identification of high-risk patients might be helpful for selection of more aggressive pharmacological or interventional treatment. Level of kidney function is a known independent factor for short and long term prognosis in acute cardiovascular patients.
Materials and Methods. In 862 patients (72% males; age range 26-99, median 68 years), consecutively admitted in CCU with NSTEMI (n. 435) and STEMI (n. 211) diagnosis, eGFR by MDRD formula and AER (µg per mg of creatinine) by spot urine, together usual biochemical and hematologic tests [Clin Chem 2009;55(6Suppl):A70], were determined. The variables were analyzed for prognostic power vs composite end-point (n.112: death 35%, heart failure 44%, and myocardial re-infarction 21%), by calculating Spearman's rho and Fisher test: those with a p<0.20 were entered in a logistic regression model.
Results. AER values distribution is log normal (median 28 µg/mg; range 1-10.380; 1° quartile 12, 3° quartile 147 µg/mg, respectively). With a AER cut-off 30 µg/mg (AER>30), 47% of all patients, 48% of STEMI patients and 43% of STEMI patients are over the decision level (NS difference). eGFR values distribution is gaussian (median 83 ml/min; range 7-320; 1° quartile 62, 3° quartile 104 ml/min, respectively). With a eGFR cut-off 60 ml/min (GFR<60), 25% of all patients, 24% of STEMI patients and 13% of STEMI patients are over the decision level (significant difference). There is an inverse correlation between AER and eGFR (Spearman's rho -0.29) and the concordance between AER>30 and GFR<60 is 48%. In univariate analysis (Fisher test) the predictive power is similar for both markers in all patients: AER>30 odd ratio (OR) 3.28 and GFR<60 OR 3.29. Nevertheless there are significant differences between subgroups: in NSTEMI AER>30 OR 3.35 and GFR<60 4.57; in STEMI AER>30 OR 3.61 and GFR<60 3.29. In multivariate analysis (logistic regression) AER>30 and GFR<60 resulted independently associated with end-points events, but, adjusting for co-morbid conditions, AER>30 remains the only independent predictor.
Conclusions. In our CCU NSTEMI and STEMI populations, the predictive power of uAER and eGFR as marker of renal dysfunction is confirmed. Nevertheless in multivariate analysis only AER>30 µg/mg is an independent predictor of total adverse events (death, re-MI, and CHF) at long term (6 months) in both ACS populations, in presence of co-morbid conditions including diabetes.

C-81

The Role of Furin and Corin in Processing of Human B-type Natriuretic Peptide Precursor

A. G. Semenov[1], N. N. Tamm[1], K. R. Seferian[1], A. B. Postnikov[1], N. S. Karpova[2], D. V. Serebryanaya[2], A. G. Katrukha[1]. *[1]HyTest Ltd., Turku, Finland, [2]Moscow State University, Moscow, Russian Federation,*

Background: B-type natriuretic peptide (BNP) is a peptide hormone mainly expressed by ventricular myocardium in response to volume overload and increased filling pressure. An active BNP hormone, along with physiologically inactive N-terminal fragment (NT-proBNP), is formed from the precursor molecule, proBNP, by the specific enzyme cleavage. The clinical significance of proBNP-derived peptides as biomarkers of heart failure (HF) has been explored thoroughly, whereas little is known about the mechanisms of proBNP processing itself. However, the comprehensive assessment of proBNP processing mechanisms could be of value for better understanding HF development and reliable interpretation of the results of BNP, NT-proBNP and proBNP measurements.
It has not yet been elucidated exactly what enzyme converts proBNP to mature BNP. Two proprotein convertases, furin and corin, are discussed as the most likely proBNP processing enzymes. In the present study, the role of furin and corin in human proBNP processing and the specificity of the proBNP cleavage by the designated convertases were analyzed.
Methods: Coexpression of human proBNP with either furin or corin was performed in HEK 293 cells and the rate of proBNP processing was analyzed using proBNP/NT-proBNP-specific immunoassays. Recombinant proBNPs (non-glycosylated and glycosylated peptides) and synthetic BNP were incubated with corin-expressing HEK 293 cells to test the ability of recombinant corin to convert proBNP. Affinity chromatography was used to purify two forms of endogenous proBNP, glycosylated and non-glycosylated in the region located close to the cleavage site. The ability of these fractions to be cleaved by furin was tested. Gel-filtration and mass spectrometry (MS) were applied to analyze the products of furin- and corin-mediated proBNP cleavage.
Results: Coexpression of proBNP in HEK 293 cells with furin or corin increased the rate of proBNP processing (3.1- and 2.2-fold respectively). Corin was able to cleave recombinant non-glycosylated proBNP, as well as the proBNP non-glycosylated in the cleavage site region, but failed to process the proBNP modified by O-glycans in the cleavage site region. MS analysis revealed that proBNP cleavage by furin results in formation of BNP 1-32, whereas corin-mediated cleavage leads to the production of the truncated BNP form - BNP 4-32. It was found that some portion of proBNP (~30% of total proBNP) in the plasma of HF patients is not glycosylated in the cleavage site region. This proBNP form is

susceptible to furin-mediated cleavage resulting in formation of mature BNP 1-32.
Conclusions: Our data indicate that both furin and corin could be involved in the proBNP processing pathway, giving rise to distinct BNP forms: BNP 1-32 (furin) and BNP 4-32 (corin). The formation of BNP 4-32, due to the specific processing activity of corin, could explain the diversity of N-terminal truncated BNP forms found in the plasma of HF patients. The phenomenon of the presence in circulation of unprocessed proBNP that could be processed by the endogenous convertase(s) should be further investigated for better understanding the BNP physiology.

C-82

Analytical and Clinical Validation of a Next-Generation "High-Sensitivity" Cardiac Troponin I Assay on the Dimension Vista® System
R. H. Christenson[1], M. L. Gantzer[2], S. H. Duh[1], D. R. Cervelli[2], C. R. deFilippi[1]. *[1]Univ of Maryland Sch of Med, Baltimore, MD, [2]Siemens Healthcare Diagnostics, Newark, DE,*

Objective: To characterize performance of a prototype* high-sensitivity cardiac troponin I (hs-cTnI) assay on the Dimension Vista® System.
Relevance: Diagnostic criteria for myocardial infarction (MI) includes elevated cTn with a rising and/or falling pattern This hs-cTnI assay may offer advantages for diagnosing MI in patients with suspected acute coronary syndromes (ACS).
Methods: Hs-cTnI was measured on the Vista system in 304 healthy subjects, 129 men (age 20-59y) and 175 women (age 18-56y). Functional Sensitivity, defined as the lowest cTn concentration with 10% total CV, was determined over 10 days using six serum pools ranging from 2 to12 ng/L (40 replicates each). Imprecision was characterized with a 20-day ANOVA according to CLSI EP15-A2. Linearity was determined with nine mixtures of a high patient and a normal individual. Clinically, hs-cTnI was evaluated for diagnosing MI in 653 (48 MI, 605 non-MI) patients from PROBE-ACS, a patient cohort presenting with suspected ACS, adjudicated using the ADVIA Centaur Ultra-TnI method; MI diagnostic criteria included Ultra-TnI cutoff of >0.04ng/mL and a 20% rise and/or fall.
Validation: Functional sensitivity (10% CV) of the hs-TnI method was 3.0 ng/L. The 99th percentile of the healthy population was 9.0 ng/L (see Figure). Total and (within-run) imprecision were 8.5% (7.1%) at 4.4 ng/L and 4.6% (4.1%) at 11.8 ng/L. Linearity was 1.3 to15,314.0 ng/L. The ROC curve-area for MI diagnosis using the hs-TnI method was 0.976 (95% Confidence Interval (CI): 0.961-0.986). At a cutoff of 23ng/L, diagnostic sensitivity was 93.7% (95% CI: 82.8-98.6%) and specificity was 91.1% (95% CI: 88.5-93.2).
Conclusion: The Vista hs-cTnI assay has functional sensitivity of 3.0 ng/L, good imprecision and measured cTnI in almost all healthy subjects. This hs-cTnI assay may be a powerful aid for the diagnosis of MI in suspected ACS patients.
*Product under development; not available for sale.
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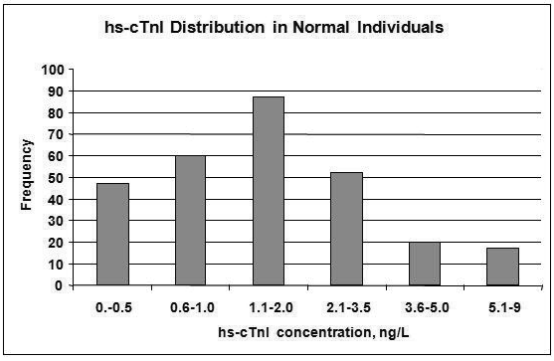


C-83

Evaluation of the Beckman Enhanced AccuTnI precision profile and 99th centile reference limit in a fully characterised reference population.
D. C. Gaze[1], P. O. Collinson[1], R. Senior[2]. *[1]St George's Healthcare NHS Trust, London, United Kingdom, [2]Northwick Park Hospital, London, United Kingdom,*

Objective: To determine the assay precision performance and 99th centile of the Beckman Coulter enhanced AccuTnI cardiac troponin I (cTnI) assay.
Methods: 1392 general population subjects >45 years old were randomly selected from seven representative local community practices. Details were collected by questionnaires and subjects' heart rate, blood pressure measurement (the average of two readings), spirometry, electrocardiography (ECG), echocardiography and venesected for fasting serum glucose and creatinine. Samples were then frozen to -20°C then transferred for long-term storage to -70°C and subsequently analysed for AccuTnI. Total imprecision was using pools at 8 levels measured four times for five days according to protocol EP 15A. All analyses were performed using the Access 2 (Beckman Coulter) using the manufacturers recommended methods and manufacturer provided quality control material. The detection limit of the assay is 10 ng/L with an upper limit of 100,000 ng/L. The claimed 10% CV is 60ng/L with an upper 99th centile of 40 ng/L. Normal subjects were defined as all attending general population subjects with no history of vascular disease, hypertension, or heavy alcohol intake, receiving no cardiac medication; whose blood pressure was <160/90mmHg as the mean of two readings; whose fasting blood glucose was <6mmol/L, whose estimated creatinine clearance was >60mL/min/1.73 m2; and normal cardiac function. Statistical analyses were performed using the Analyse it add-in Excel (Analyse-it for Microsoft Excel (version 2.12).
Results: 699/1392 (50.2%) of those invited attended for screening, (336 male) median age 58.0 years, interquartile range 53-71years. There were 248 normals (104 male) median age 53 years, interquartile range 49-61 years. Total assay precision varied from 2.33% (530 ng/L) to 13% (14 ng/L) with 10% imprecision at 16 ng/L. The median cTnI was 7.35±6.9 ng/L and the upper 99th centile was 34 ng/L. The 99th centile was not influenced by age or gender (p=1.000).
Conclusion: The 99th centile of the Beckman enhanced AccuTnI cTnI assay in a screened population is lower than the manufacturers supplied data. Assay imprecision of the enhanced AccuTnI assay is superior compared to the manufactures supplied data and meets the current clinical recommendations.

C-84

Assessment of the Siemens TnI-Ultra Cardiac Troponin I Assay and the Impact of its Implementation on laboratory workload
R. W. Peake, J. Reeve, H. Turner, I. Philip, J. Allison, B. L. Croal. *NHS Grampian, Aberdeen, United Kingdom,*

Background. Troponin assays play a valuable role in risk stratification in acute coronary syndromes. Consensus recommendations have adopted a concentration of troponin above the 99th percentile of the reference group at assay imprecision of 10% CV. We have determined the functional sensitivity of the Siemens Ultra troponin I assay (TnI-Ultra) and assessed the impact of the new detection level on troponin I (cTnI) requesting within a large central laboratory.
Methods. Imprecision studies were performed on the Siemens ADVIA Centaur XP/CP using the TnI-Ultra assay. Functional sensitivity at 10% CV was evaluated by analysing 10 serum pools of cTnI concentrations ranging from 0.002 to 0.11ng/mL. The detection limit (LOD) was calculated as the lowest cTnI concentration corresponding to a signal 3 SD above the mean of 20 replicates of a cTnI-free serum-based matrix. cTnI requesting was audited during September 2009 using the previous detection level (≥0.1ng/mL) and compared to February 2010 following introduction of the new detection (≥0.04ng/mL).
Results. Imprecision data is summarised in Table 1. The LOD was calculated at a mean cTnI concentration of 0.006ng/mL. From plots of CV vs log-transformed values of cTnI concentration in the range 0.00-0.10ng/mL, the cTnI concentration that corresponded to 10% total CV was 0.032ng/mL. This compares favourably with the manufacturer's reported level (0.03ng/mL). The total number of cTnI requests from all sources increased by 7% following the implementation of the new detection level (1st to 21st September 2009; 1471 requests vs 1st to 21st February 2010; 1577 requests). The increase in positivity rate (and therefore referral rate) was 8% following adoption of the lower detection limit.
Conclusions. The study demonstrates that our TnI-Ultra method has performed with necessary precision at the lower detection level. Implementation of this level may translate to an increase in workload for both the Laboratory and Cardiology Department.

Serum Pool	n	Mean (ng/mL)	SD	Intra-assay CV (%)	Inter-assay CV (%)
1	10	0.002	0.0040	80.81	111.71
2	10	0.011	0.0020	28.50	23.65
3	10	0.015	0.0036	26.40	16.33
4	10	0.021	0.0018	9.80	19.75
5	10	0.030	0.0018	8.30	16.61
6	10	0.036	0.0018	5.00	9.30
7	10	0.041	0.0017	3.80	2.67
8	10	0.044	0.0013	3.20	6.58
9	10	0.068	0.0047	10.40	4.74
10	10	0.073	0.0021	3.10	6.28
11	10	0.106	0.0032	4.46	5.21

C-85

Implication of serum CA125 and plasma BNP level Changes in patients with heart failure

D. Zhang. *Chinese PLA General hospital, Beijing, China,*

Background: Heart failure is a serious clinical syndrome that threats to the human health. It has high prevalence and poor prognosis which is the leading cause of death to Cardiovascular disease. It is particularly important to enhance the early prediction, detection and intervention to heart failure. It has great significance to look for the predictive value of the indicator. At present, BNP's role in the diagnosis and treatment of heart failure has been fully affirmed. As an important ovarian cancer-associated antigen, CA125 has been widely recognized as the tumor markers, but little report about the relationship with heart failure. The purpose to study the relationship between serum CA125 levels and cardiac function in patients with heart failure through measuring CA125, BNP levels and simultaneous evaluation of cardiac function. Additionally, it also explores whether the level of CA125 expression has an impact on combined pleural effusion, pericardial effusion, or atrial fibrillation.

Methods: 118 patients with heart failure were divided into three groups based on the standard of NYHA classification. 50 patients with cardiovascular disease but without heart failure and 50 healthy people were served as control. Level of CA125 and BNP was measured by electrochemiluminescence immunoassay. LVEF was detected by echocardiography.

Results: Serum CA125 and plasma BNP level in heart failure patients is higher than non-heart failure group and the healthy group, and its level is closely related to cardiac function. Increasing at higher levels of heart function.Serum CA125 and plasma BNP level is negatively correlated with LVEF, irrelevant to the original underlying diseases resulting in the heart failure.The increase of serum CA125 level was positively correlated with the severity of heart failure and left ventricular enlargment.When Heart failure with hydrothorax or atrial fibrillation occurs, CA125 level is significantly higher than those without hydrothorax or atrial fibrillation; while those hydrothorax patients with pericardial effusion, the CA125 level is not more significantly increased than that of those who don't have pericardial effusion.

Conclusion: Serum CA125 and plasma BNP levels have clinical significance on the heart failure diagnosis and the assessment of severity among patients with heart failure. For patients with suspected heart failure, it can be diagnosed by detecting the CA125 level. For moderate and worst degree of heart failure patients, CA125 can be used as a simple laboratory indicator, together with symptoms, signs and other non-invasive indicators to effectively monitor the severity and the progress of heart failure.

C-86

Anti-inflammatory and anti-remodeling effects of Cardiac Resynchronization Therapy

A. E. Stanciu[1], M. Stanciu[2], R. Vatasescu[3], C. Iorgulescu[3], M. Dorobantu[3]. *[1]"Prof.Dr.Al.Trestioreanu" Institute of Oncology, Bucharest, Romania, [2]University "Politehnica" of Bucharest, Bucharest, Romania, [3]Clinic Emergency Hospital Bucharest, Bucharest, Romania,*

Background: Remodeling reflects the structural and functional deterioration that occurs in heart failure. Indices of remodeling constitute an important marker of the severity of heart failure, and reverse remodeling is an accepted goal in the treatment of heart failure. In chronic heart failure (CHF) patients, cardiac resynchronization therapy (CRT) leads to reverse left ventricular (LV) remodeling. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are matrix-degrading enzymes that have been demonstrated to influence LV properties and serve as targets of potential anti-remodeling agents. Our objective was to investigate the effects of CRT on serum levels of N-terminal pro-brain natriuretic peptide (NT-proBNP), pro-inflammatory cytokines (interleukins IL-1β, IL-6, IL-8, tumor necrosis factor TNF-α) and MMP-2, MMP-9, TIMP-1 and TIMP-2 in patients with CHF.

Methods and patients: 29 patients (21M/8F, aged 64 ± 11 years) with CHF (III-IV NYHA functional class) were investigated for immune activation before and 1 hour, 1 week, 3, 6, 12, 18 months after CRT treatment. In all patients, blood specimens were drawn from a peripheral vein. The serum levels of MMP-2, MMP-9, TIMP-1, TIMP-2, interleukins IL-1β, IL-6, IL-8, TNF-α and NT-ProBNP were measured at the same time by an ELISA method. Routine chemical methods were used to measure serum concentrations of lipids and acid uric. Cardiac function was assessed echocardiographically.

Results: After 18 months from CRT, all patients had improved in NYHA-class with 1 or 2 classes and there was a significant reduction in serum levels of the Nt-proBNP, IL-6 and IL-8. It is important to indicate a correlation between changes in Nt-proBNP and IL-6 ($r = 0.76 \pm 0.23$, $p < 0.05$), Nt-proBNP and IL-8 ($r = 0.72 \pm 0.23$, $p < 0.05$), NT-proBNP and IL-1β ($r = 0.54 \pm 0.47$, $p < 0.05$), NT-proBNP and TNF-α ($r = 0.39 \pm 0.48$, $p < 0.05$). CRT positively influences extracellular matrix remodeling by decreasing serum levels of MMP-2 and increasing TIMP-2. The MMP-2/TIMP-2 ratio had decreased from 8.3 before CRT to 0.6 at 18 months after CRT treatment. Serum levels of Nt-proBNP had a significant positive correlation with circulating levels of MMP-2 ($r = 0.68 \pm 0.25$, $p < 0.05$) and MMP-2/TIMP-2 ratio ($r = 0.58 \pm 0.34$, $p < 0.05$).

Conclusions. At 18 months follow-up, CRT was associated with a discordant change in serum MMP-2 and TIMP-2 levels. Changes in pro-inflammatory cytokines activity were related to changes in serum MMP-2 levels. This suggests that CRT reduces peripheral markers of immune activation in patients responding to CRT. These changes in MMP-2/TIMP-2 ratio lead to reverse LV remodeling in patients with CHF.

C-87

Time course proteomic profiling of human myocardial infarction plasma samples as approach to biomarkers discovers

V. N. Silbiger[1], A. D. Luchessi[1], R. D. C. Hirata[1], L. G. Lima-Neto[1], C. P. Pastorelli[1], E. S. dos Santos[2], M. P. Pereira[2], R. Ramos[2], M. F. Sampaio[2], D. Armaganijan[2], S. H. Paik[3], Y. Murata[3], G. O. Oi[3], M. H. Hirata[1]. *[1]Faculdade de Ciências Farmacêutica - Universidade de São Paulo, São Paulo, Brazil, [2]Instituto Dante Pazzanese de Cardiologia, São Paulo, Brazil, [3]Sun BioMedical Technologies, Ridgecrest, CA,*

Background: Cardiovascular diseases (CVD) continue to be a very important cause of morbidity, mortality rising health care costs far into this new century. However, acute myocardial infarction (AMI) symptoms are frequently atypical or absent and alterations in the ECG can be unspecific or absent. Furthermore, serum markers, such as CK-MB and cTnI, can have low sensitivity (35%) and specificity (86%) for diagnosis of the coronary heart arrest. To investigate plasma proteomic profile of patients with AMI who underwent angioplasty using the SELDI-TOF-MS technology and to analyze the relationship of this profile with clinical and biochemical markers within 48 h after cardiac ischemia.

Methods: Ten individuals with ST-segment elevation AMI (STEMI) were selected at the Instituto Dante Pazzanese de Cardiologia, São Paulo, Brazil, all patients with acute ischemic-type chest pain (< 8 h duration). Patients were classified according to the first (1STEMI; n=5) or second (2STEMI; n=5) cardiac event, which took place within one year after the first episode. The blood was drawn from an antecubital vein six times (stages) after the STEMI diagnose. The first stage (T_0) was in Emergency Unity before receiving any medication, the second (T_2) was in a Hemodynamic Unity just after the primary angioplasty within 2 h after T_0 and next four stages took place at the Coronary Unit by each 12 h, the third (T_{12}) was at 12 h, the fourth (T_{24}) at 24 h, the fifth (T_{36}). Individuals without AMI with similar risk factors for CVD and normal ergometric test were selected as control group (CG) and the blood was collected just one time. Biochemical analyses were carried out by routine laboratory methods. Plasma Proteomic profiling analysis was performed using the top-down (*i.e.* intact proteins) SELDI-TOF-MS, after process in Multiple Affinity Removal Spin Cartridge System (Agilent, USA). Background-adjusted and normalized data was analyzed using the R-statistics based BRB Array Tools v.3.7.

Results: Compared with CG individuals, 1STEMI group presented 91, 329, 84, 88 and 123 protein peaks differentially expressed at T_2, T_{12}, T_{24}, T_{36} and T_{48} ($p<0.05$) respectively. 2STEMI group presented 27, 23, 4, 13 and 19 protein peaks differentially expressed at T_2, T_{12}, T_{24}, T_{36} and T_{48} ($p<0.05$) respectively, in comparison with CG individuals. In 1STEMI, nine proteins (MW 9217Da, 17788Da, 17792.5Da, 17797Da, 17803Da, 17804.5Da, 17806Da, 17807.5Da and 17825.5Da) were down-regulated at 48 h past-AMI ($p<0.05$). In 2STEMI, five of these proteins were down-regulated (17797Da, 17803Da, 1784.5Da, 1780 Da and 17807.5Da).

Conclusion: Proteomic analysis by SELDI-TOF-MS ProteinChip technology combined with bioinformatics tools demonstrates differential protein profile in AMI patients with one or two coronary ischemic events. Differential expression during 48 h course time suggests a potential role of some these proteins as biomarkers for AMI follow up in the most critical period of cardiac ischemic recovery.

C-88

Performance of a new rapid automated high-sensitive TroponinT (hsTnT) immunoassay

T. C. Aw, S. K. Pua, L. Yew, W. R. Lim, S. P. Tan. *Changi General Hospital, Singapore, Singapore,*

Background Troponin is the preferred cardiac biomarker. For the management of acute coronary syndromes (ACS) a Troponin result that is both rapid and highly sensitive is vital. A new rapid (9 minute) hsTnT chemi-luminescent assay on the Cobas 6000 autoanalyzer (Roche) was recently launched. We compared it to the current Cobas TnT assay (18 minutes) to determine the reference range for healthy subjects.

Methods Three hundred and eighty apparently healthy ambulant subjects (180 males aged 31-60 years, and 200 females aged 41-60 years) were recruited for the study. The functional sensitivity of the hsTnT was determined by measuring replicates (n=20) of

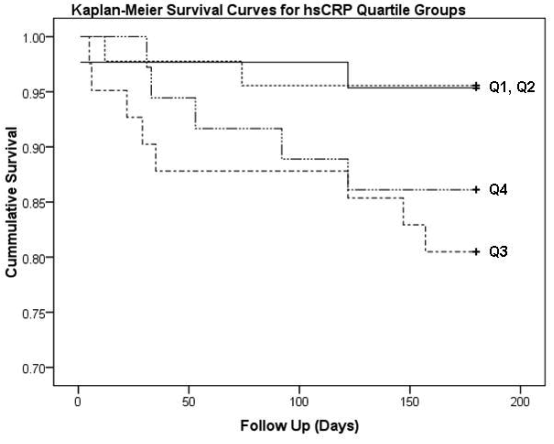
several pooled sera varying in concentration from 6 to 26 pg/mL.
Results The functional sensitivity of the hs-TnT assay at different inter-assay coefficients of variation (cv's) was as follows: 7.5 pg/mL @ 20%cv, 9 pg/mL @ 15%cv, 11.5 pg/mL @ 10%cv, and 17 pg/mL @ 5%cv. The inter-assay precision for the manufacturer supplied assay controls was 2.8% at 23 pg/mL and 1.9% at 1.9 ng/mL. There was good agreement between the TnT and hsTnT assays: r = 0.996845; y (hsTnT) = 0.9787x (TnT) + 0.0051. In the 380 subjects the 99th centile cut-off for hsTnT was 15 pg/mL versus 30 pg/mL for the TnT assay. Assay turnaround time (TAT) was 9 minutes.
Conclusion We have established the 99th centile cut-off for normal healthy subjects - 15 pg/mL for the new hsTnT compared to 30 pg/mL for the current TnT assays. The improved assay turnaround time should enable more labs to meet the desired door-balloon time of under 45 minutes for angioplasty.

C-89

The clinical utility of admission hsCRP levels in acute coronary syndrome patients

R. W. A. Peake, J. Reeve, H. Turner, W. Mutch, E. Grant, V. Mills, R. Soiza, G. Hillis, J. Allison, B. L. Croal. *NHS Grampian, Aberdeen, United Kingdom,*

Background. Patients presenting with acute coronary syndrome (ACS) are a heterogeneous group with a highly variable prognosis. Atherosclerosis is a pro-inflammatory condition and as such C-reactive protein (CRP), an acute phase protein, may play a direct role in promoting ACS. Measurement of hsCRP in ACS patients, using a high sensitivity assay, may provide useful prognostic information.
Methods. Serum hsCRP (Siemens Prospec) and cardiac troponin I (cTnI; Siemens ADVIA Centaur) were measured in 238 ACS patients on admission to Aberdeen Royal Infirmary. Patients were followed up at 6 months for all-cause mortality with hospital length of stay (HLOS) recorded.
Results. By 6 months 18 patients had died. Admission hsCRP levels were 6.90 mg/L in patients who had died compared to 4.67 mg/L in those who were still alive (p = 0.071). Kaplan Meier survival analysis demonstrated increased mortality as hsCRP levels increased (log rank 4.085; p = 0.043). Peak cTnI levels (ng/ml) showed an increasing trend through quartile groups of admission hsCRP (Q1 = 0.20, Q2= 0.21, Q3 = 1.26 and Q4 = 2.00). In addition, HLOS (days) increased with increasing quartiles of hsCRP (Q1 = 4, Q2 = 4, Q3 = 6 and Q4 = 6; p<0.001). Admission hsCRP levels were predictive of longer HLOS (OR 1.008; p = 0.035) in a univariate logistic regression model.
Conclusion. Admission hsCRP was associated with subsequent mortality in this cohort. The relationship between hsCRP and subsequent peak cTnI suggests that hsCRP may be more reflective of emerging infarct size rather than the underlying inflammatory status of the coronary vasculature. Such patients with higher hsCRP levels also had longer HLOS which may reflect the additional care and recovery period associated with larger infarcts. hsCRP may therefore be a useful predictor of both outcome with implications for HLOS and subsequent burden of care.



C-90

Prognosis in ACS patients: BNP, left ventricular hypertrophy, and renal dysfunction

P. Cappelletti[1], P. Bulian[2], D. Rubin[1], M. Cassin[1], G. Nicolosi[1]. *[1]AOSMA, Pordenone, Italy, [2]CRO, Aviano, Pordenone, Italy,*

Objective. We evaluated the relative effects of the kidney dysfunction and left ventricular dysfunction on plasmatic concentrations of natriuretic peptides in a population of ACS (acute coronary syndromes) patients admitted in CCU.
Relevance. Luchner et al demonstrated (Hypertension 2005;46:118-23) that left ventricular hypertrophy and renal damage work together to magnify the plasmatic concentrations of natriuretic peptides in the cardiovascular patients and, consequently, their cut-off concentrations should be stratified according to renal function.
Materials and Methods. In 862 patients (72% males; age range 26-99, median 68 years), consecutively admitted in CCU with NSTEMI (n. 435) and STEMI (n. 211) diagnosis, renal dysfunction was evaluated by eGFR (MDRD formula) and AER (µg per mg of creatinina) and left ventricular dysfunction was determined by echocardiography (systolic ejection fraction, LVEF). BNP was measured by immunoassay on Advia Centaur. The cut-off (224 mg/L) was determined by ROC curve for prognostic power vs composite end-point (n.112:death 35%, heart failure 44%, and myocardial re-infarction 21%). The χ^2 and Spearman's rho tests were applied.
Results. In patients with eGFR<60 ml/min BNP is over cut-off in 58%, while in patients with eGFR>60 ml/min in 26% (ratio 2.2; χ^2 <0.0001). In patients with AER>30 µg/mg BNP is over cut-off in 46%, while in patients with AER<30 µg/mg in 26% (ratio 2.0; χ^2 <0.0001). In patients with LVEF<40% BNP is over cut-off in 81%, in patients with LVEF>40% in 28% (ratio 2.9; χ^2 <0.0001). Spearman's rho is -0.44, -0.29, -0.46, respectively.
In patients with a eGFR<60 ml/min and LVEF<40%, BNP is over cut-off in 77% and in patients with eGFR>60 ml/min and LVEF>40% BNP is over cut-off in 31% (ratio 2.5; χ^2 <0.0001). In patients with AER>30µg/mg and LVEF<40% BNP is over cut-off in 73%, in patients with AER<30µg/mg and LVEF>40% BNP is over cut-off in 32% (ratio 2.1; χ^2 <0.0001).
Conclusions. In our ACS population renal dysfunction, evaluated by both AER and eGFR, determined a ~2-fold increase of patients with a risk of worst prognosis as predicted by BNP. Nevertheless, left ventricular dysfunction, as decreased systolic ejection fraction (LVEF), was the main factor for a bad prognosis, leading to a ~3-fold increase of patients with an increased BNP. The risk increased 2.5-fold in patients combining both renal and ventricular dysfunction. However, 30% of patients with ACS presented an increased risk of adverse events in absence of both renal and hemodynamic dysfunction.

C-91

Evaluation Of Sentinel Ck-Mb Liquid Assay On Synchron®Lx20 And Unicel®Dxc Clinical Chemistry Systems

F. Rota, E. Marelli, A. Cugini, F. Vespasiani, L. De Angelis. *Sentinel CH. SpA, milano, Italy,*

OBJECTIVE: analytical performance of Sentinel CK-MB Liquid assay was evaluated on the Beckman Coulter Synchron®LX20 UniCel®DxC Clinical Chemistry Systems. Goals of the study were to verify the analytical performance and establish this assay's agreement with the desirable analytical specification based on biological variability as given by Ricos.
MATERIALS/INSTRUMENTS: CK-MB Liquid reagent, manufactured by SENTINEL CH., is a ready to use format, based on the catalytic reaction between creatine phosphate and ADP with formation of creatine and ATP. The ATP formed, in presence of glucose and hexokinase (HK), yields ADP and glucose-6-phosphate. The glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase (G6P-DH), with reduction of β-NADP+ to β-NADPH. Reagent 1 contains mouse antibodies, which inhibit the CK-MM isoform, allowing the determination of CK-B isoenzymes (CK-MB and CK-BB). The CK-MB activity is obtained by multiplying the CK-B activity by two. The increase of absorbance at 340 nm is directly proportional to CK-MB activity in the sample. The determination of isoenzymes, in particular the quantification of CK-MB, is of considerable clinical importance because in presence of elevated value of CK Total, it is possible to discriminate the pathology of the cardiac muscle.
The SynchronLX20 is a fully automated, high-throughput clinical chemistry system, able to run colorimetric, enzymatic, as well as turbidimetric assays, which does allow consolidation of Lab workflow on a unique platform.
STUDY DESIGN: study protocols were based on CLSI guidelines. Acceptance criteria were defined to meet desirable analytical specifications as per biological variation (TE< 24%) at clinical decision level (24 U/L). Total Imprecision (EP05A), acceptable CV% </= 10%. Linearity (EP06A), acceptable % bias +/-5%. Limit of Quantitation (LOQ_EP17) was defined as the analyte concentration at which the Total Error is less than 10%. Method

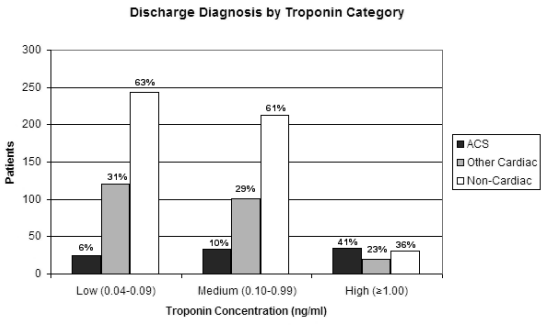
comparison was evaluated by comparing the results of paired patient serum samples on SynchronLX20, on UnicelDxC and on Abbott Aeroset analyzers, according EP09A. Endogenous substances interferences were investigated following EP07A, allowed bias was 6 U/L. In Use Stability was tested in terms of On Board reagents only, since calibration is not required.
RESULTS: Total Imprecision as CV% were on SynchronLX20 6.6% at 18.9 U/L and on UniCelDxC 9.8% at 16.8 U/L. Assay was found to meet Linearity specifications from 6 up to 600 U/L. LOQ was 6 U/L. Comparison A) SynchronLX20 vs Aeroset® analyzer: n 96, slope 1.07, intercept 0.60, r 0.99; B) SynchronLX20 vs. UniCelDxC: n 55, slope 1.04, intercept -1.8, r 0.99. Assay was free of interferences up to Hemoglobin (100 mg/dL), Lipids (600 mg/dL), conjugated and unconjugated Bilirubin (60 mg/dL), Ascorbate (60 mg/dL) and Pyruvate (3 mg/dL).
In use stability study proved 28 days On Board Reagent stability.
CONCLUSION: performance of Sentinel CH. CK-MB Liquid assay on Beckman Coulter SynchronLX20 and UniCelDxC Clinical Chemistry Systems did meet the Acceptance criteria based on Ricos Biological Variation database specifications. The study supported the use of the assay on clinical laboratory routine, thus allowing consolidation of the lab workflow on a unique instrument platform and offering an alternative solution to the Immunochemistry methods.

C-92

Diagnostic performance of a highly sensitive cardiac troponin I assay in an emergency department setting

S. E. Melanson, J. M. Kosowsky, M. Yiadom, M. J. Conrad, P. Jarolim.
Brigham and Womens Hospital, Boston, MA,

Introduction: Improving performance of cardiac troponin (Tn) assays increases sensitivity for detection of myocardial injury but decreases specificity for acute coronary syndrome (ACS). This is particularly apparent in Emergency Departments (ED) where the assay is often used as a screening test rather than a confirmatory assay for ACS. In this retrospective review, we evaluated a highly sensitive TnI-Ultra assay (Siemens Healthcare Diagnostics) in a busy tertiary care ED.
Methods: We reviewed ED visits between 3/1/2007 and 7/31/2007, during which 23,731 patients were seen. Patients with positive TnI results were divided into three categories according to the first positive result - low positive TnI, 0.05-0.10 ng/ml; medium positive TnI, 0.11-0.99 ng/ml; and high positive TnI≥1.00 ng/ml. Discharge diagnoses were reviewed for all patients with positive TnI results and categorized as primary ACS (i.e, Type 1 Myocardial Infarction according to the ESC/ACCF/AHA/WHF Task Force definition), other cardiac diagnoses, and non-cardiac diagnoses.
Results: Of the 4,928 (21%) patients who had TnI-Ultra ordered, 821 (17%) had positive results ranging from 0.05 to 52.6 ng/ml. 388 (47%) results were in the low positive range, 347 (42%) were in the medium range and 86 (11%) were in the high range. Primary ACS accounted for 11% (93/821) of discharge diagnoses, other cardiac, primarily CHF, atrial fibrillation and other arrhythmias, accounted for 30% (241/821) and non-cardiac, primarily fall, sepsis, and pneumonia, accounted for the remaining 59% (487/821).(Figure)
Conclusions: Our review of the diagnostic performance of a highly sensitive TnI assay in an ED setting demonstrates that the specificity for primary ACS of this and most likely other Tn assays is considerably lower than current estimates. Additional diagnostic tools, such as relative TnI change, velocity of TnI change, or other biomarkers should be used to confirm or exclude the diagnosis of primary ACS in the ED.

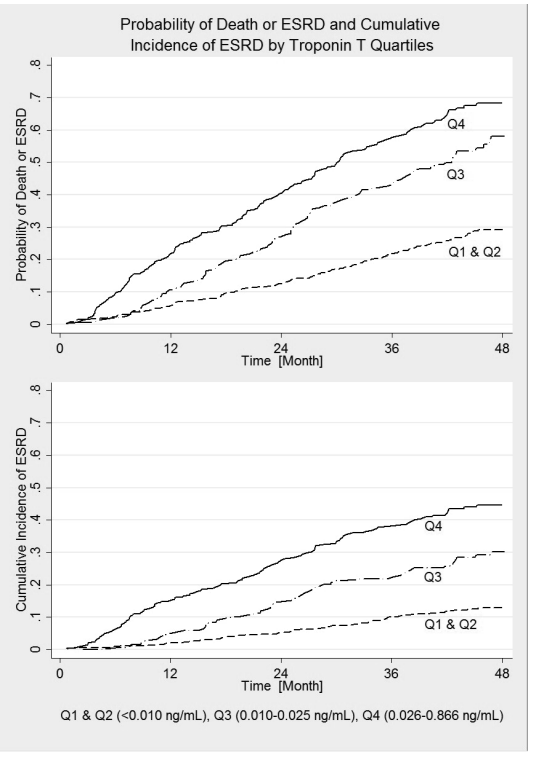


C-93

Serum Levels of Both Cardiac Troponins T and I Are Associated with Renal Disease Progression in Patients with Diabetes Mellitus, Anemia, and Chronic Kidney Disease

P. Jarolim[1], A. Desai[1], H. Uno[1], K. Eckardt[2], P. Finn[1], A. S. Levey[3], E. F. Lewis[1], J. Lin[1], J. J. V. McMurray[4], H. Parving[5], P. Parfrey[6], S. Keithi-Reddy[7], S. Solomon[1], R. Toto[8], O. Vasylyeva[1], M. A. Pfeffer[1]. *[1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, [2]University of Erlangen-Nuremberg, Erlangen, Germany, [3]Tufts Medical Center, Boston, MA, [4]British Heart Foundation, Glasgow, United Kingdom, [5]Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [6]Health Sciences Centre, St. John's, NL, Canada, [7]Amgen, Thousand Oaks, CA, [8]University of Texas Southwestern Medical Center, Dallas, TX,*

Background: Elevations in cardiac troponins are associated with increased risk for cardiovascular events in patients with chronic kidney disease (CKD). Many cardiovascular risk factors are also associated with renal disease progression. We hypothesized that levels of cardiac troponins might also predict renal outcomes in patients with CKD.
Methods: Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) randomized 4038 subjects with type 2 diabetes, CKD not on dialysis (eGFR 20-60 ml/min/1.73 m^2), and anemia (hemoglobin≤11 g/dl) to treatment with darbepoetin alfa or placebo. We measured serum cardiac troponin T (TnT, 4th generation, Roche) and troponin I (TnI-Ultra, Siemens) in baseline serum samples from the first 1000 patients enrolled. The relationship between biomarker levels and both the composite of death or progression to end-stage renal disease (ESRD) and ESRD alone was analyzed in multivariable Cox models.
Results: Levels of TnI and TnT were modestly correlated in the sample (Spearman's r=0.45, p<0.0001). TnT concentrations ≥0.010 ng/ml, exceeding the 99% upper reference limit (URL), were noted in 45% of patients. TnI levels exceeded the URL (0.04 ng/ml) in 11% of patients. A strong and graded relationship with the composite of death or ESRD was noted for both TnT (p<0.001, figure) and TnI (p<0.001). The cumulative incidence of ESRD alone also increased across quartiles of both TnT (figure, HR [for highest vs. lowest 2 quartiles] 4.5, 95% CI 3.3-6.2, p<0.001) and TnI (not shown). Relationships with both the composite of death or ESRD and ESRD alone remained robust even after adjustment for age, gender, blood pressure, hemoglobin A1c, eGFR, urine protein/creatinine ratio, history of cardiovascular disease, and smoking status.
Conclusions: In ambulatory patients with diabetes, anemia, and pre-dialysis CKD, levels of both cardiac TnT and TnI are strongly associated with renal disease progression, independent of established risk factors including eGFR and proteinuria.



C-94

Reevaluation of a Cardiac Profile Testing Protocol

S. E. Dawson, T. Ocasio. *Swedish Covenant Hospital, Chicago, IL,*

Background: Our Emergency Department (ED) has utilized a Rule out Myocardial Infarct profile (ROMI) for screening all possible chest pain patients for the past 14 years, that includes 4 specimens covering from 0-8 hours post presentation in ED. The first two specimens, acquired on arrival and at 2hr, include a troponin and myoglobin, and the 4hr and 8hr specimens are for troponin only. In recent years, the sensitivity of troponin assays has greatly improved.

Objective : In light of the increased sensitivity of troponin assays, the objective of this study was to determine if the acquisition of specimens in the ROMI profile should be adjusted, either in timing or in number.

Method: A retrospective study of all patients admitted through ED during November and December 2009, and diagnosed with MI during the first 12 hours of hospital stay was performed. The results of the ROMI profile were analyzed to determine when (in terms of specimen acquisition time) the troponin level increased at least 0.10 ng/ml in 2hr or exceeded 0.40ng/ml, two defining diagnostic criteria for MI at our institution.

Results: There were 92 patients included in the study who met the troponin MI criteria, 50 males and 42 females. 54% of the patients met the criteria (troponin>0.40ng/ml) on arrival. 26% met the criteria by the 2hr specimen (0.10 ng/ml rise or >0.40 ng/ml troponin). 17% met the criteria by the third specimen which was 4 hours post arrival. 2% met the criteria with the 8hr specimen.

The patients of concern are the two who didn't meet the criteria until the 8hr specimen. One did not have a 4hr troponin , which probably would have met criteria based on the result of the 8hr specimen. The second patient was admitted with ACS following the third troponin which showed a serial increase of 0.08ng/ml over 2 hours. In the past, this increase may have been too small to measure using the less sensitive assays.

No patients in the study had non-detectable troponin levels at 0, 2 and 4hr and then detectable levels at the 8 hour specimen.

Conclusion: The inclusion criteria for MI patients, based on a troponin serial rise and/or troponin levels exceeding 0.40ng/ml, are substantiated by the study data. With 26% of the patients meeting the troponin MI criteria at 2hr, choosing to eliminate the 2hr specimen may delay or confound treatment for patients with normal or inconclusive ECGs. With 98% of patients diagnosed by the 4hr specimen, reducing or changing any of the first three draw times does not seem prudent. The use of the 8 hour draw may be reduced based on this study, especially for patients with elevated troponins at presentation. The use of the 8 hr specimen for patients with no measurable troponin using a sensitive assay for the 0, 2 and 4 hr specimens may be questionable.

C-95

Stability of galectin-3 in serum and plasma at various temperatures as measured with the BGM Galectin-3™ assay.

C. Adiletto[1], S. Wang[1], N. Gordon[1], R. H. Christenson[2]. *[1]BG Medicine, Inc., Waltham, MA, [2]University of Maryland, Baltimore, MD,*

Objective: To establish the stability of galectin-3 in serum and EDTA plasma at multiple storage temperatures, over multiple time points and several freeze-thaw cycles.

Relevance: Galectin-3 is a cardiac biomarker that is implicated in adverse ventricular remodeling and the progression of heart failure. The BGM Galectin-3™ assay is a microtiter plate based ELISA that quantitatively measures galectin-3 in serum and plasma and is intended to be used in conjunction with clinical evaluation as an aid in the stratification of patients diagnosed with heart failure.

We report the results of an evaluation of the stability of galectin-3 in two sample matrices, at four storage temperatures with multiple freeze-thaw cycles, and over multiple time points.

Methodology: Fresh serum and EDTA plasma samples were collected from study subjects at a heart failure clinic. Eighteen individual serum and EDTA plasma pools were prepared and tested within four hours of collection to establish the baseline galectin-3 levels. Subsequently, the pools were divided into aliquots for storage at -70ºC, -20ºC, 2-8ºC and 22-28ºC. In addition, the frozen samples were thawed and re-frozen multiple times to assess the stability of galectin-3 when exposed to freeze-thaw cycles. The Bland-Altman method was used to calculate the % difference of the galectin-3 at the testing time point compared to the baseline value. The final stability determination was based on the time point that achieved % difference ±25% of baseline.

Results: The results are summarized in the table below.

Storage temperature	Stability		Freeze-thaw stability (# freeze-thaw cycles)	
	EDTA plasma	Serum	EDTA plasma	Serum
2-8ºC	22 days	1 month	n/a	n/a
22-28ºC	22 days	22 days	n/a	n/a
-20ºC	6 months	6 months	9	9
-70ºC	6 months	6 months	9	9

Conclusions: These data support the stability of galectin-3 in serum and EDTA plasma for several days or months. Labs may batch specimens and store up to 22 days at 2-8ºC and 22-28ºC. Long term storage of clinical specimens may be achieved by storing under frozen conditions, either at -20ºC or -70ºC. This study also demonstrates that galectin-3 is stable upon multiple freeze-thaw cycles.

C-96

Correlation of the severity of Coronary Artery Disease with serum Neutrophil Gelatinase-Associated Lipocalin (NGAL).

A. Haliassos[1], T. Zografos[2], S. Korovesis[2], E. Giazitzoglou[2], D. Rizos[3], E. Voridis[2], D. G. Katritsis[2]. *[1]Diamedica SA, Athens, Greece, [2]Athens Euroclinic, Athens, Greece, [3]Hormone Laboratory "Aretaieio" University Hospital, Athens, Greece,*

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa secretory glycoprotein, originally identified in human neutrophil granules, which has been recently associated with insulin resistance and atherogenesis. The demonstration of increased NGAL expression in atherosclerotic plaques in conjunction with evidence that NGAL correlates with risk factors for atherosclerosis, supports a direct role of NGAL in the development and progression of coronary artery disease (CAD). In the present study, we investigated the association of serum NGAL levels with validated angiographic indices of the severity of CAD (i.e., number of diseased vessels and modified Gensini score).

We enrolled in our study 73 consecutive patients who underwent first-time coronary angiography for suspected CAD. Blood specimens were collected before angiography and determination of serum NGAL concentration was performed using an enzyme immunoassay concentration was performed using an enzyme immunoassay (ELISA Bioporto, Denmark).

Median serum NGAL levels in patients with angiographically confirmed CAD were significantly higher than those in patients with normal coronary arteries (29.0 ng/mL [interquartile range 25.2 to 36.8] vs 22.4 ng/mL [interquartile range 17.3 to 32.0], p= 0.004). Statistically significant correlations were observed between serum NGAL level and the number of diseased vessels (r_s=0.390, p=0.01) and modified Gensini score (r_s=0.356, p=0.002). Using multivariate analysis, serum NGAL level was independently associated with the presence and severity of CAD.

We conclude that serum NGAL levels are significantly higher in patients with CAD and are correlated with the severity of the disease as assessed by coronary angiography. Further clinical studies are needed to confirm the potential use of serum NGAL as a biomarker for the detection and the staging of the CAD extent.

Wednesday AM, July 28

Poster Session: 10:00 am – 12:30 pm
Immunology

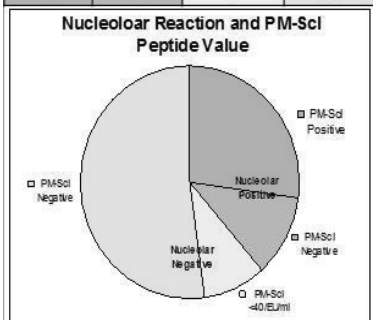
C-97

Do We Need Immunofluorescence Tests For PM/SCL Antibody Detection?

V. Ramsperger, M. Lindemann, V. Kumar. *IMMCO Diagnostics. Inc., Buffalo, NY,*

Introduction: PM/Scl overlap syndrome is an autoimmune disease. Patients with antibodies to PM/Scl have symptoms of both systemic scleroderma and either polymyositis or dermatomyositis. A characteristic serological feature of PM/Scl and sclerodema is the presence of antinuclear antibodies (ANA) of a nucleolar reaction pattern; however, the limitation of this method is that it is subjective and requires significant skills for the identification of a specific nucleolar reaction pattern associated with PM/Scl antibodies. The antigens to which PM/Scl antibodies react have been identified, resulting in the availability of specific immunoassays that are easy to perform. The objective of our study was to determine if ELISA is sufficient for detecting PM/Scl antibodies or if testing on HEp-2 may be desired for confirmation, especially when the ELISA results are weak positive.
Method: Serum samples were examined on HEp-2 for ANA and for PM/Scl antibodies by ELISA using Euroimmun, Dr. Fooke and in-house kits. The test results were read blinded and an association of the ANA reaction pattern to the ELISA was determined.
Results: Of the 111 samples tested for PM/Scl antibodies, seventeen were positive for ANA with nucleolar pattern. Twelve of these seventeen were positive and five negative for PM/Scl antibodies. Of the nucleolar negative samples only four were positive for PM/Scl antibodies. All these four samples had low positive results on the ELISA.
Comments: The use of ELISA in combination with nucleolar antibody detection on HEp-2, especially when the ELISA results are low positive provide a reliable combination for detecting PM/Scl antibodies.

Nucleolar Positive		Nucleolar Negative	
PM-Scl Positive	PM-Scl Negative	PM-Scl <40/EU/ml	PM-Scl Negative
12	5	4	23



C-99

Recirculating Phagocytes are a novel source of Biomarkers for Multiple Sclerosis.

R. C. Nayak[1], T. Vollmer[2], R. Bomprezzi[3], D. Campagnolo[3], R. F. Jacolik[1]. *[1]MSDx, LLC, Tucson, AZ, [2]University of Colorado, Denver, CO, [3]Barrow Neurology Institute, Phoenix, AZ,*

Progress in the development and use of biomarkers for Multiple Sclerosis (MS) has been limited by inherent barriers associated with studying the brain. We have postulated that phagocytic leukocytes recruited into the brain to clear away damaged brain tissue re-enter the blood from the brain while containing brain molecules as cargo as a result of their damage clearance function. Interrogation of the cargo in these blood borne cells may represent a novel biomarker strategy that will report pathophysiological activity in the brain. Consequently, we have studied peripheral blood mononuclear cells (PBMCs) from relapsing -remitting MS patients (RRMS, n=18) and healthy controls (n=12) in order to detect neural antigens carried as cargo. Hypo-osmotic lysates of PBMCs were coated onto ELISA wells at 5ug/ml and probed for the presence of the neuronal microtubule associated protein Tau and the neuronal calcium sequestering protein Hippocalcin 1 like-1 using rabbit polyclonal antibodies and standard assay procedures. Tau was present in 7 of 18 RRMS lysates and 1 of 12 healthy control lysates. Hippocalcin1 like-1 was present in 5 of 18 RRMS lysates and 1 of 12 healthy control lysates. Neither the Tau nor Hippocalcin1 like-1 results were statistically significant by the two tailed Fisher's exact test. However, positivity for either Tau OR Hippocalcin1 like-1 resulted in 9 of 18 RRMS patients being positive and 1 of 12 healthy controls being positive. Fisher's exact test: two-tailed p value equals 0.0235. The association between rows (groups) and columns (outcomes) is statistically significant. The results support the concept of interrogating circulating mononuclear cells for neural antigens as a "window into the brain" that may form the basis of a novel biomarker strategy to develop diagnostic and prognostic aids for MS.

C-100

Tacrolimus (FK506) may cause liver transplantation patient produce anti-nuclear antibody against nucleolar

Y. Wu, L. Wang, B. Cai, Y. Bai. *West China Hospital of SiChuan University, Chengdu, China,*

Background: Liver transplantation was one of the most important ways to treat liver cirrhosis and hepatocellular carcinoma. Tacrolimus (FK506) as immunosuppressive drug was used to reduce the graft rejection after operation and the immune balance of recipient was bound to be broken because of tacrolimus usage and exogenous liver transplantation. Anti-nuclear antibody (ANA) as the indicator was detected for liver transplantation patients used tacrolimus and controls with matched liver diseases to evaluate the immunity status of subjects.
Methods: All subjects including 94 liver transplantation patients and 94 controls were obtained from inpatients and outpatients of West China Hospital of Sichuan University from 2007 to 2009. ANA was detected by indirect immunofluorescence with HEp-2 cell as substrate at an initial serum titer of 1:100, exactly titer was got by further diluted as 1:320, 1:1000, 1:3200, 1:10000 if positive. ANA pattern included speckled, homogeneous, nucleolar, centromere and so on (Kit from EUROIMMUN Germany). Data was analyzed by SPSS 16.0 software. $P<0.05$ was considered statistically significant.
Results: The demographics had no significant differences between the liver transplantation patients and controls for mean age, sex and disease distribution ($P>0.05$). ANA prevalence of patients with liver transplantation was 20.21%, mean titer was 264.21 ± 278.69, matched control group was 12.76% and 191.67 ± 113.28 respectively, no statistical difference was found between two groups ($P> 0.05$). But the prevalence of nucleolar pattern of ANA in liver transplantation patients was up to 63.16%, significantly greater than matched control (63.16% vs 16.67% $P = 0.011$). The mean titer of nucleolar pattern of ANA in two groups was 191.67 ± 113.28 and 210.00 ± 155.56 respectively, no statistical difference was found between two groups ($P = 0.896$).
Conclusion: Nucleolar ANA could increase the possibility of suffering from scleroderma. But in our study, the liver transplantation patients with nucleolar ANA had no obvious symptoms of scleroderma related. Therefore, we speculate that tacrolimus may cause liver transplantation patient produce nucleolar ANA. Whether scleroderma will be induced in liver transplantation patients used tacrolimus needs further investigation.

C-101

Prokaryotic expression and purification of procalcition

C. Wang, X. Zhou. *Wuhan University Zhongnan Hospital, Wuhan, China,*

Objective: Procalcitonin (PCT) is the precursor of calcitonin (CT), which has no calcitonin hormone activity. It consists of 116 amino acid residues, and shows a molecular weight of 13kD. The half-life of PCT is 20-24h, after that PCT was digested into two fragments: PCT-I and PCT-II, which combine to form CT. The low serum concentration of PCT makes it difficult for detection by ordinary methods. Under pathological conditions, such as medullary thyroid carcinomas, or neuroendocrine tumors, severe bacteria infections, sepsis, multiple organ dysfunction syndrome (MODS), organs like thyroid gland, adrenal gland, brain and pancreas, in response to external stimulus, can produce large amount of PCT, significantly increasing the serum concentration of PCT, sometimes to greater than 10ng/ml. Monitoring the serum concentration of procalcitonin (PCT) is an effective means for detecting infection, but most of the reagents for PCT tests are imported, which adds cost to clinical application. We perform prokaryotic expression and purification of procalcitonin to provide experimental basis for preparing the pracalcitonin antibody for clinical application.
Methods: Full length mRNA of PCT was obtained from cultured medullary thyroid carcinoma cells (TT cells) through RT-PCR. BamHI, HindIII restriction site were added to the 5' end of forward and reverse RT-PCR primers, respectively, for convenient insertion into PET-32a expression vector. The recombinant PET-32a-PCT plasmid was transferred into DH5alpha E coli cells for cloning. Positive clones were sent for sequencing to confirm the sequence fidelity of cloned PCT mRNA sequence. The plasmids extracted from clones

with the correct sequence were then transferred into DE3 cells for recombinant protein production by IPTG induction. The recombinant protein with His tag was purified by Ni-NTA affinity chromatography column, and verified by 10% sodium dodecy1 sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), with further characterization by Western blot.

Results: The PCT mRNA was extracted from cultured TT cells, and 1.5% agarose electrophoresis analysis of the PCT RT-PCR amplification product showed a band of 348bp. Sequencing of RT-PCR product showed that the cloned sequence corresponds with known PCT mRNA sequence. The RT-PCR product was inserted into PET-32a vector. Recombinant plasmids were transferred into E. coli DE3 cells. Sequencing of positive clones confirmed the identity of the recombinant vector. The recombinant proteins were extracted after 3h, 4h, 5h, 6h IPTG induction, respectively, and 12% SDS-PAGE electrophoresis showed that the 31kD target band appeared at all sampling time points and peak protein expression appeared at 5h.The recombinant protein with His tag purified by Ni+ affinity chromatography column was analyzed by 10% SDS-PAGE electrophoresis and Western blot, and results showed acceptable purity.

Conclusions: We successfully cloned the full length PCT mRNA, and created recombinant PET-32a-PCT plasmid for expression in DE3 E. coli cells. Relatively high level of PCT expression was obtained, and Ni+ affinity chromatography column purification produced recombinant proteins with acceptable purity. The recombinant protein we produced lays the foundation for further study on the function and pathophysiological role of procalcitonin, and provides experimental basis for preparing the antibody for pracalcitonin for further clinical applications.

C-102

Effect of JAK/STAT signaling pathway on extracellular release of HMGB1 in lipopolysaccharide-induced hepatocytes

G. Chen, J. Zhang. *Wuxi People's Hospital, Wuxi, China,*

Objective: To study the effect of JAK/STAT signaling pathway on extracellular release of high mobility group box 1 (HMGB1) in endotoxin-induced hepatocytes.

Methods: HMGB1 concentration was determined in the culture medium of 100 μg/L lipopolysaccharide-induced BRL-3A hepatocytes by enzyme-linked immunosorbent assay (ELISA), and the change of HMGB1 mRNA expression was observed by RT-PCR method.The effects of JAK/STAT pathway inhibitors (tyrphostin AG 490 and fludarabine) with various concentrations were investigated on HMGB1 mRNA expression and extracellular release.

Results: The level of HMGB1 mRNA expression and HMGB1 concentration in the culture medium significantly increased after BRL-3A hepatocytes were induced by lipopolysaccharide for 24 hours(P<0.01). 25 μmol/L tyrphostin AG 490 and 50 μmol/L fludarabine partly inhibited HMGB1 mRNA expression and extracellular release in lipopolysaccharide-induced BRL-3A hepatocytes(P<0.05).

Conclusion: Lipopolysaccharide induces hepatocytes to release HMGB1, which mechanism is involved with intracellular JAK/STAT signaling pathway.

Tab Levels of HMGB1 in the culture medium of lipopolysaccharide-induced BRL-3A hepatocytes (μg/L, n=5,±s)

Groups	Cell culture time		
	12 h	24 h	36 h
Control	3.1±1.1	3.7±1.0	5.1±1.6
100 μg/L LPS-induced	12.6±2.7*	24.3±7.2*	27.4±6.9*
Compared with control,*P<0.01			

C-103

Development and validation of a multicolor flow cytometry assay for clinical laboratory assessment of CD4 recent thymic emigrants (RTEs)

X. Dong, M. V. Hoeltzle, R. S. Abraham. *Mayo Clinic, Rochester, MN,*

Background: Quantitation of thymic output is helpful in the analysis of the naïve T cell repertoire and diagnostic evaluation of cellular primary immunodeficiencies, HIV disease, immune reconstitution after hematopoietic stem cell transplant and the use of immunomodulatory therapy. Quantitation of T-cell receptor excision circles (TREC) is the most-commonly used direct measurement for thymic output. However, the longevity of naïve T cells and dilution by cell proliferation affects TREC levels. The presence of CD31 on CD45RA+ CD4 T cells coincides with high TREC levels (non-divided naïve CD4 T cell) and therefore is a useful marker for CD4 recent thymic emigrants in blood.

Objective: To develop and validate a multi-color flow cytometry assay for quantitation of CD4 RTE.

Methods: Quantitative immunophenotyping of CD4 RTE was performed on a BD FACS Canto II ™flow cytometer. CD4 RTE ($CD4^+CD45RA^+CD45RO^-CD31^+$) analysis was performed on EDTA-anticoagulated whole blood with relevant antibodies. Appropriate isotype controls were used for gating flow data and subsequent analysis was performed with FACS Diva 6.0 software (BD BioSciences). The CD4 RTE data was obtained both as % (of total CD4 T cell) and absolute count number (cell number/μL of whole blood). The CD4 absolute counts were obtained using BD TruCount™ tubes. TREC levels in blood were measured by quantitative real-time PCR using the Taqman® chemistry (ABI7500 Fast Real Time PCR).

Validation Results: Analytical validation was performed using blood from healthy donors. Ten samples were used to determine stability (time and temperature). The CD4 RTE was found to be stably expressed at ambient temperature up to 72 hours post-blood collection. The inter- and intra-assay precision was determined using 10 replicates of 2 independent samples in 2 different experiments. The actual inter and intra-assay precision for CD4 RTE was determined to be 5.9 and 7.5% respectively. CD4 RTE and TREC were evaluated in 44 samples from healthy individuals aged 19 to 74 years old. There was an overall correlation between TREC levels (copies per million CD3 T cells) and CD4 RTE (cells/uL) for the same sample. Age-related decrease in CD4 RTE correlated with similar age-related decrease in TREC levels. CD4RTE reference values were developed for pediatric and adult individuals using 90 and 168 healthy donors respectively. Clinical validation for CD4 RTE was performed on 112 patient samples across a spectrum of relevant clinical diagnoses. Though the test is to be used in context with other laboratory and immunological evaluation along with clinical presentation since it is not directly diagnostic for a specific disease, the CD4 RTE counts correlated well with the appropriate clinical diagnosis, providing information on the clinical accuracy of the assay. An assay to measure CD8 RTE (CD8+CD62L+CD45RO-CD103+) was also developed and validated. It was determined that in older children and adults the contribution of CD8 RTE to peripheral CD8 T cell homeostasis is minimal.

Conclusion: Multicolor flow cytometric quantitation of CD4 RTE is helpful in the evaluation of thymic output in several appropriate clinical contexts.

C-104

Development of an Immunoassay for NGAL for the ARCHITECT® $i2000_{SR}$ and $i1000_{SR}$ Analyzers

R. F. Workman, C. Lipowsky, A. Hoffman, L. Templin, J. Brady, D. Wiesner. *Abbott Laboratories, Abbott Park, IL,*

Background NGAL (Neutrophil Gelatinase-Associated Lipocalin) is a 25 kDa globular protein in the lipocalin family that was initially identified in the co-purification of a gelatinase protease. Lipocalins contain a hydrophobic pocket or calyx which is designed to bind small hydrophobic molecules in the blood for transportation into cells. Under inflammatory stress, the kidney produces NGAL and releases it into the urine. There are many published studies that have examined the clinical utility of NGAL levels in conjunction with kidney damage.

NGAL ARCHITECT® Assay

The ARCHITECT NGAL assay is a chemiluminescent automated assay (CMIA). Specific anti-NGAL antibodies are coated on paramagnetic particles and capture the urinary NGAL. A second NGAL-antibody tagged with acridinium is used to complete the NGAL sandwich and the signal generated is proportional to the NGAL present in the sample. The ARCHITECT NGAL assay is standardized using recombinant human NGAL and to ensure similarity to native NGAL, it is expressed in a mammalian cell line.

Previously, in the research phase we showed that the ARCHITECT Urine NGAL assay had good precision (< 10% total CV) and sensitivity (< 3 ng/mL) and that it was robust to several common interferences found in urine. We have expanded the list of interfering substances tested and included commonly used antibiotics and a wider list of cross-reactants and endogenous substances (including bicarbonate). We completed an expected values study on 200 patients that exhibited normal levels of blood creatinine and urinary protein. We performed an expanded 20-day precision study including panels and controls ranging from ~5 ng/mL (under our claimed LOQ of 10 ng/mL) to ~1350 ng/mL (within 10% of our highest calibrator). We did a robust statistical evaluation of linearity including samples ranging from below the LOQ to the highest calibrator. We also re-examined several other standard assay measurements including: the limit of quantitation (LOQ), manual vs auto-dilution performance, spike recovery, sample storage stability, and within-assay sample carry-over.

Results Precision CVs ranged from 1.8 -7.0 %. Antibiotic interference ranged from -2.9-2.3 %. Cross-reactant interference ranged from -2.5-0.7 %. Endogenous substance interference ranged from -6.1-2.8 %. Expected values study ranged from 0-128.4 ng/mL (p=0.05, one-sided). Linearity acceptable range was 2.3 to 1509.6 ng/mL. LOQ ranged from 1.5-3 ng/mL. Manual vs Auto-dilution % difference ranged from -0.5-4.8 %. Spike recovery ranged from 89.5-103.5 %. Sample storage for 4 °C, 22 °C, and 37 °C was 0.3% over 7 days, 3.2% over 3 days, and 2.9% over 3 days, respectively. Within-assay sample carry-over ranged from -0.6-0.0 %.

Conclusion The results demonstrate that the ARCHITECT NGAL assay is a convenient, well-standardized, precise method for measuring NGAL in urine.

C-105

Clinical Sensitivity and Specificity of Anti-Cyclic Citrullinated Peptide IgG, Rheumatoid Factor, and C-Reactive Protein Assays Singly or in Combination to Aid in the Diagnosis of Rheumatoid Arthritis

A. Wang[1], R. Barten[2], Y. Attia[1], S. Thompson[1], D. Sustarsic[1]. *[1]Siemens Healthcare Diagnostics Inc., Los Angeles, CA, [2]Siemens Healthcare Diagnostics Products, GmbH, Marburg, Germany,*

Background: In 2009, the new rheumatoid arthritis (RA) criteria released by the American College of Rheumatology (ACR) and the European League Against Rheumatism were revised to include the measurement of anti-cyclic citrullinated peptide (CCP) antibodies to aid in the classification of RA. As is true for the other serological markers mentioned in the criteria_rheumatoid factor (RF) and C-reactive protein (CRP)_elevated anti-CCP IgG is predictive of RA. RF has good sensitivity (50%-90%) but low specificity for RA, whereas anti-CCP IgG is highly specific (82%-98%) with moderate diagnostic sensitivity (60%-85%).
Objective: The objective of this study was to determine if the combination of the IMMULITE® 2000 Anti-CCP IgG*, Dimension Vista® RF, and Dimension Vista hsCRP or CRP assays improve the diagnostic sensitivity and specificity for RA.
Methods: The fully automated IMMULITE 2000 Anti-CCP IgG assay is a two-cycle chemiluminescent immunoassay for the detection of specific IgG autoantibodies to CCPII. Samples were measured for RF, hsCRP, and CRP on the Dimension Vista according to the package insert. If the hsCRP result exceeded the assay range, the value was then obtained with the CRP assay.
Results: The clinical sensitivity, specificity, and overall agreement for 339 samples (138 confirmed rheumatoid arthritis and 201 apparently healthy patients) tested with the IMMULITE 2000 Anti-CCP IgG assay were 79%, 100%, and 91%, respectively. When tested with the Dimension Vista RF, the sample results provided clinical sensitivity, clinical specificity, and overall agreement of 83%, 98%, and 92%, respectively. Receiver operator curve analysis for anti-CCP IgG, RF, and hsCRP provided an area under the curve (AUC) equal to 0.93, 0.93, and 0.79, respectively. The clinical sensitivity, specificity, and overall agreement for the combination of anti-CCP IgG and RF assays were 93%, 98%, and 96%, respectively.
Conclusion: Overall, the IMMULITE 2000 Anti-CCP IgG assay performed comparably to the Dimension Vista RF assay for the diagnosis of rheumatoid arthritis, and in combination, the clinical sensitivity was improved from 79% (anti-CCP) or 83% (RF) to 93%. The overall average for CRP was significantly higher (15.91 mg/L) in the rheumatoid arthritis samples than in normal samples (2.49 mg/L).
* Under development. Not available for sale.

C-106

Dehydroepiandrosterone inhibits the proliferation and inflammatory response in endothelial cells

X. Zunen, L. Yan, W. Ming, D. Wen. *Renmin Hospital of Wuhan University, Wuhan City of Hubei Province, China,*

Objective Several lines of evidence suggest that Dehydroepiandrosterone (DHEA) seem to exert protective effects against atherosclerosis, diabetes, and osteoporosis. Based on the few study of DHEA action on endothelial cells proliferation and apoptosis and regulated factors, the major purpose of our present work was to investigate the effect of DHEA on endothelial cells proliferation and apoptosis and inflammatory response, primarily to discuss the possible mechanism of effects of DHEA on endothelial cells and provide a part of the cellular and molecular theory basis of protective roles of DHEA.
Methods Human umbilical vein endothelial cells(HUVECs) were stimulated for 24 hours by different level of DHEA. MTT technique was used to determine HUVECs proliferation. HUVECs were incubated with 10^{-9}~10^{-6} mol/L DHEA for 1h and stimulated with 10 ng/ml TNF-α for 24 hours, apoptosis of HUVECs was measured by Annexin V-FITC and PI. HUVECs were incubated with DHEA for 1h and stimulated with 1 μg/ml CD40L for 24 hours. The mRNA expression of ET-1, eNOS, E-selectin and ICAM-1 were measured by RT-PCR. The protein expression of E-selectin and ICAM-1 were detected by western blot.
Results 10^{-9}mol/L DHEA had no effect on HUVECs proliferation. 10^{-8}~10^{-6} mol/L DHEA inhibited HUVECs proliferation. DHEA inhibited endothelial cells apoptosis induced by TNF-α in a concentration dependent manner. The expression of ET-1, E-selectin and ICAM-1 were increased by CD40L, DHEA inhibited the mRNA and protein expression of these inflammatory cytokine induced by CD40L.
Conclusion DHEA inhibited HUVECs proliferation with a dose-dependent manner and time-dependent manner. DHEA attenuated TNF-α induced endothelial cells apoptosis. DHEA inhibited the mRNA and protein expression of inflammatory cytokine, provided a protect effect on endothelial cells.

C-107

Evaluation of Thyroid Function and Autoimmunity in Women Older than 35 Years Old Infected by HIV

L. Carvalho[1], P. F. S. Teixeira[1], A. L. Pânico[1], M. Cohen[1], M. F. M. C. Pinheiro[2], P. F. Barroso[3], M. Vaisman[1]. *[1]Division of Endocrinology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2]Sérgio Franco Medicina Diagnóstica, Rio de Janeiro, Brazil, [3]Division of Infectious Diseases, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,*

Background: Thyroid autoimmune diseases (TAID) are the most important cause of thyroid dysfunction and the more prevalent autoimmune disease in the general population, affecting 13.1% of women older than 35 years old in Rio de Janeiro city. The association between these diseases and the effects of HAART in the induction of immune restoration of patients infected with HIV has been studied. The objective of this study was to evaluate the prevalence of thyroid dysfunction and thyroid autoimmune diseases in women older than 35 years old infected by HIV, and the association between these diseases and HAART (highly active anti-retroviral therapy) and immune restoration parameters.
Methods: HIV infected women were selected from the infectious diseases outpatient clinic of University Hospital. Patients with recent hospitalization or in use of drugs that might alter thyroid function were excluded. Laboratory evaluation included: CD4 lymphocytes levels (flow cytometer, FACS Calibur, Multitest, Beckton Dickinson, USA), viral copies (b-DNA, Siemens, USA) and also serum TSH, FT4 and TPO-Ab levels (ECLIA, Modular, Roche, German). In the patients with low levels of TSH we also performed anti-TSH receptor antibodies (TRAB) measurements (RIE, RSR, UK).
Results: One hundred fifty three HIV infected women older than 35 years old were studied, their mean age was 46.5 ± 9.1 years. The prevalence of thyroid disorders (abnormal serum TSH levels and / or positive serum TPO-ab) in the whole group was 7.8 % (12 patients). All of them were on HAART at the time of diagnosis of thyroid disorder. One hundred thirty patients were on HAART at the time of the study. In this subgroup, the prevalence of thyroid disorder and TAID were 9.2 and 4.6%, respectively. Hypo and hyperthyroidism were detected in 4.6 and 3.1% of these. We detected differences in viral copies amounts between patients with or without hyperthyroidism (0 vs 2801 copies; p=0.002) and between patients with or without thyroid immune disease. In patients with thyroid immune disease we also detected higher levels of CD4 cells (825 ± 357 vs 581 ± 299; p=0.054). As expected, the absolute numbers of CD4 correlated negatively with viral copies (rs= -0.422; p<0.001).
Conclusions: We did not demonstrated higher prevalence of thyroid dysfunction and autoimmunity in women infected with HIV, despite the use or not of HAART. A possible association between the use of HAART and the immune restoration, with the development of thyroid disease was suggested by the presence of higher levels of CD4 and lower levels of viral copies in the patients with the diagnosis of thyroid immune disease.

C-108

Th17/Treg imbalance is associated with rheumatoid arthritis

Q. Niu, Z. C. Huang, B. Cai, L. L. Wang, W. H. Feng. *Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China,*

Background: RA is an inflammatory autoimmune disease regulated by T lymphocyte subsets. Recently, Th17 cells and $Foxp3^+CD4^+CD25^+$ regulatory T (Treg) cells have been identified as two distinct subsets from Th1 and Th2 cells. Due to their opposite effects, the balance between Th17 and Treg cells is important in controlling autoimmunity and inflammation, and Th17/Treg imbalance may play a critical role in pathogenesis/development of RA.
Objective: To assess whether the Th17/Treg balance was broken in patients with RA, and to explore the relationship between Th17/Treg imbalance and RA progression.
Methods: The frequencies and related cytokines secretion of circulating Th17 cells and $Foxp3^+CD4^+CD25^+$Treg cells in 36 active RA, 32 stable RA and 20 healthy controls were determined respectively by flow cytometry (FCM) and ELISA.
Results:The frequencies of Th17 cells were markedly higher in patients with active RA (2.17±1.20%) and stable RA (1.45±0.73%) than controls (0.53±0.11%) (P<0.05), and Th17 frequency in active RA group was significantly higher than that of stable RA group (P<0.05). The frequencies of $Foxp3^+CD4^+CD25^+$Treg cells decreased markedly in active (0.74±0.43%) and stable RA (1.07±0.52%) groups when compared with control group (2.92±1.24%) (P<0.05), while there was no obvious difference between two RA groups (P>0.05). In addition, plasma concentrations of Th17 related cytokines IL-17, IL-23, IL-6 and TNF-α in active [IL-17:2.85(1.84-4.91) pg/ml; IL-23:54.33(49.49-65.37) pg/ml; IL-6:32.98(8.90-176.88) pg/ml; TNF-α: 1158.10 (338.63-2303.12) pg/ml] and stable RA [IL-17:2.69(1.91-5.11) pg/ml; IL-23: 65.84 (51.88-76.69) pg/ml; IL-6:14.59(3.83-62.44) pg/ml; TNF-α:1417.81(707.15-2289.46) pg/ml] groups were significantly higher as compared with controls [IL-17:1.43(0.96-2.57) pg/ml; IL-23:36.07(32.99-48.82) pg/

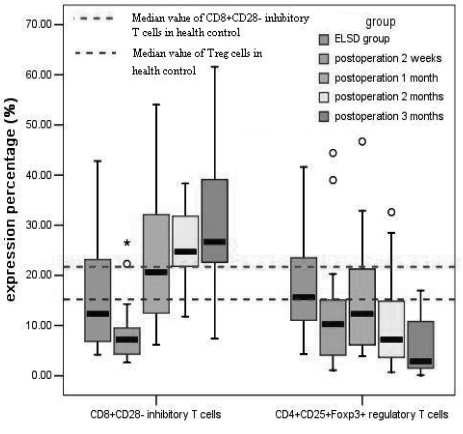
ml; IL-6:1.55(1.27-1.78) pg/ml; TNF-α:13.50(9.73-19.08) pg/ml] (P<0.01), and the differences of all these cytokine concentrations were no significance between two RA groups (P>0.05), respectively. Concentration of Treg related cytokine TGF-β1 was obviously lower in active [4.36 (2.70-7.09) ng/ml] and stable RA [6.90 (4.78-8.70) ng/ml] groups than that of control group [24.19 (16.58-27.07) ng/ml] (P<0.01), and TGF-β1 concentration was significantly lower in active RA than that of stable RA group (P<0.01).
Conclusions: The Th17/Treg imbalance exists in patients with RA, suggesting a potential role for Th17/Treg imbalance in development of RA.

C-109

Immunoregulatory and immunotolerant effect of tacrolimus on peripheral $CD4^+CD25^+$ regulatory T cells and $CD8^+CD28^-$ inhibitory T cells in allo-liver recipients

L. L. Wang[1], B. Cai[1], Y. J. Bai[1], Y. G. Zou[1], W. H. Feng[1], H. Wu[2], L. N. Yan[2]. *[1]Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China, [2]The Center of Liver Transplantation, West China Hospital of Sichuan University, Chengdu, China,*

Background: Regulatory T (Treg) cells and inhibitory T cells both play the critical role in transplantation tolerance. Researches about regulatory effects of tacrolimus on these T cells were rare.
Objectives: To define tacrolimus regulatory role on Treg cells and $CD8^+CD28^-$ inhibitory T cells in allo-liver recipients we explored peripheral $CD4^+CD25^+Foxp3^+$ Treg cells, $CD8^+CD28^-$ inhibitory T cells and plasma IL-10 and TGF-beta1 in allo-liver recipients treated with tacrolimus.
Methods:111 allo-liver recipients treated with Tacrolimus, 27 patients with end-stage liver diseases (ESLD) and 24 Healthy volunteers were included. Flow cytometry was applied to determine peripheral $CD4^+CD25^+Foxp3^+$ Treg and $CD8^+CD28^-$ inhibitory T cells. Plasma IL-10 and TGF-beta1 were determined by ELISA. Treatment drug monitoring was made by enzyme-multiplied immunoassay.
Results: 1.Blood drug levels in four different post-transplantation period groups were similar. 2. $CD4^+CD25^+Foxp3^+$ Treg cells expression in recipients was the lowest among three groups, the expression of $CD8^+CD28^-$ inhibitory T cells in recipients was more than those in ESLD group and similar to health control. 3.During post-transplantation 3 months $CD8^+CD28^-$ inhibitory T cells gradually increased and $CD4^+CD25^+Foxp3^+$ Treg gradually decreased. (Figure) 4. Plasma TGF-beta1 concentration gradually increased from disease control, health control to recipients. IL-10 gradually increased among three groups, characterized by the lowest in health control and the top in recipients group. 5. The positive relationship between $CD8^+CD28^-$ inhibitory T cells expression and plasma TGF-beta1 levels was shown, r_s=0.372 (P< 0.05). The relationship between serum IL-10 level and Treg cells or $CD8^+CD28^-$ inhibitory T cells was not shown.



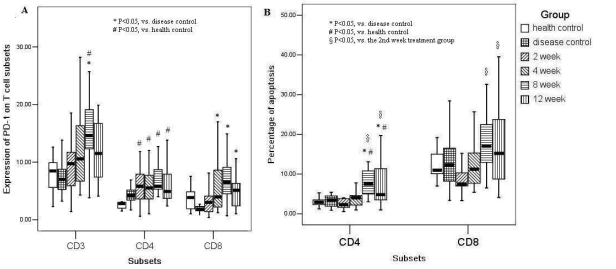
Conclusions: 1. Abnormal immunoregulation of allo-liver recipients treated with tacrolimus was characterized by decreased Treg cells and increased $CD8^+CD28^-$ inhibitory T cells. 2. Tacrolimus exerts the immune regulation effects through increasing $CD8^+CD28^-$ inhibitory T cells, but not $CD4^+CD25^+Foxp3^+$ regulatory T cells in allo-liver recipients. 3. TGF-beta1 plays immune regulatory role by synergizing with $CD8^+CD28^-$ inhibitory T cells.

C-110

Tacrolimus immunoregulatory effects on PD-1 and apoptosis pathway in allo-liver recipients

B. Cai[1], Y. J. Bai[1], L. L. Wang[1], W. H. Feng[1], Y. G. Zou[1], H. Wu[2], L. N. Yan[2]. *[1]Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China, [2]The Center of Liver Transplantation, West China Hospital of Sichuan University, Chengdu, China,*

Background and Objectives: Negative costimulator and apoptosis pathways both participate in transplantation immunoregulation. Here we explored important molecules in both pathways to investigate tacrolimus regulatory effects on Programmed death-1(PD-1) and activated Caspase-8 on/in peripheral T cells subsets, and T lymphocytes apoptosis in allo-liver recipients.
Methods: 55 allo-liver recipients treated with tacrolimus, 20 patients with end-stage liver diseases (ESLD) and 20 health volunteers were included. Flow cytometry was applied to determine PD-1 and activated Caspase-8 expression on/in T cells, apoptosis of T cell subsets. Treatment drug monitoring was made by enzyme-multiplied immunoassay.
Results: 1.Blood drug levels among each treatment group were similar. 2. PD-1 expression on T cell subsets was gradually increased with transplantation time going. PD-1 on $CD3^+$T cells in 8th week group reached the top and was significantly higher than that in health control and disease control; PD-1 on $CD4^+$T cells in 2nd week group was higher than that in health control (P<0.05) and those on $CD8^+$T cells in 8th week increased to the top. (Figure A) 3. Intracellular activated Caspase-8 expression in T cell subsets in post-transplantation 2nd week group was the lowest and increased with treatment progression. Caspase-8 expression in CD4+T cells and $CD8^+$T cells reached the top in 8th week group. 4. Apoptosis percentage of $CD4^+$T cells and $CD8^+$T cells gradually increased after transplantation and both of them in 8th week group increased to the top. The ratio of apoptosis percentage of CD4+ T cells to CD8+T cells increased with time going. (Figure B)



Conclusions: 1. Upregulatory role of tacrolimus on PD-1 on $CD4^+$T cells might be one of reasons to cause $CD4^+$T cells decrease. 2.Tacrolimus induced more CD4+T cells apoptosis than CD8+T cells by activating Caspase-8 pathways. 3. The 8th week after treatment was the optimal effective fastigium of tacrolimus for the immune tolerant regulation.

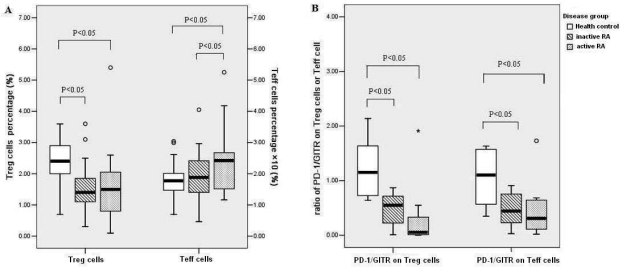
C-111

Costimulators disturbance on peripheral regulatory T cells and effector T cells in patients with rheumatoid arthritis

Z. C. Huang[1], B. Cai[1], L. L. Wang[1], W. H. Feng[1], G. Liu[2]. *[1]Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China, [2]Department of Rheumatology, West China Hospital of Sichuan University, Chengdu, China,*

Background and Objective:Costimulatory pathways and regulatory T (Treg) cells-effector T (Teff) cells system play most important role in immune regulation. To define the regulatory effects of glucocorticoid-induced TNF receptor (GITR) and Programmed death-1(PD-1) on Treg cells we explored GITR, PD-1, Treg cells, Teff cells and serum cytokines in patients with Rheumatoid Arthritis (RA).
Methods:47 patients with RA (23 active RA patients and 24 inactive RA patients) and 28 healthy volunteers were included. Flow cytometry was applied to detect GITR, PD-1 and Treg cells or Teff cells. Serum TGF-beta1, IL-6 and TNF-alpha were detected by ELISA.
Results:1.T cells subpopulations disturbance in RA was characterized by significantly CD3+CD4+ T cells increasing and CD3+CD8+ T cells decreasing. 2. In RA the disturbance between Treg cells and Teff cells was characterized by peripheral Treg cells decreasing and Teff cells increasing. With RA activity developing, the imbalance was exacerbated. (Figure A) 3. In RA GITR on Treg cells or Teff cells increased and PD-1 decreased. In health control PD-1 expression on Treg cells was significantly higher than that on Teff cells, in RA PD-1 on Treg cells was lower than that on Teff cells. (Figure B) 4. Compared with health control, serum TGF-beta1 decreased in RA, and decreased to the bottom in active RA (P<0.05). There was no correlation between TGF-beta1 levels

and Treg cells expression. 5. Serum TNF-alpha and IL-6 increased in RA, compared with health control, and they were both higher in active RA than those in inactive RA ($P<0.05$). There was no correlation between them and Teff cells.



Conclusions:In RA abnormal PD-1 and GITR signals may participate in inducing the disturbance of Treg cells and Teff cells. TGF-beta1 played immunoregulatory role in RA independent on Treg cells. High Teff cells may participate in immune response mainly by GITR pathway not inflammatory cytokines.

C-112

Clinical significance and changes of serum cytokines concentrations in patients with systemic lupus erythematosus

P. Zhang, X. Zhou, Z. Xia, J. Fu. *Renmin Hospital of Wuhan University, Wuhan, China,*

objective Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by several immunological abnormalities. T cell abnormalities, B cell hyperactivity and abnormal cytokine production have been implicated to be of pathogenic importance in SLE. The aim of this study was to investigate the changes of serum levels of tumor necrosis factor-α(TNF-α), interleukin-6 (IL-6) and soluble interleukin-2 receptor (sIL-2R) of SLE patients before and after hormone treatment.

Methods Slice immune biological fluorescence of ANA and anti-dsDNA antibodies ,the application of Western blot sticks of ENA, A total of 40 SLE patients and 30normal control people were subjected to detect the serum TNF-α, IL-6 and sIL-2R with a solid phase enzyme-labeled chemiluminesent immunometric assay and immunoglobulin G(IgG), complement 3(C3) with immune rate nephelometry.

Results The levels of serum IgG, C3, TNF-α, IL-6 and sIL-2R in SLE patients were (20.96±5.08)g/L, (0.57±0.29)g/L, (52.34 ±8.61)ng/L, (19.33 ±6.89)ng/L and (688.40 ±191.50)U/ml, respectively. The levels of serum IgG, C3, TNF-α, IL-6 and sIL-2R in control group were(11.35±2.56)g/L, (1.09±0.24)g/L, (7.29±2.44)ng/L, (5.25±1.33)ng/L and (416.62 ± 117.03)U/ml. Compared with those of the control, the levels of IgG, TNF-α, IL-6 and sIL-2R in SLE patients were higher, however,C3 level was lower($P<0.01$).The levels of IgG, TNF-α, IL-6 and sIL-2R in SLE patients treated with hormone were (13.55 ±4.11)g/L, (0.87 ±0.33)g/ L, (16.15±4.57)ng/L, (13.58±4.88)ng/L and (436.60 ±118.3) U/ml, respectively. Compared with those of the activity SLE, the levels of IgG, TNF-α, IL-6 and sIL-2R decreased significantly, however, C3 level increased($P<0.01$).But serum IgG, C3, TNF-α, and IL-6 levels in between stable SLE patients and control have still significant difference($P<0.01$).

Conolusion The results showed that patients with SLE expressed much more TNF-α,IL-6 and sIL-2R.It strongly suggested that immune dysfunction and cytokines imbalance would contribute to the pathogenesis of SLE.

C-113

Utility of free light chains ratio in the monitoring of the treatment of a patient with light chain lambda multiple myeloma.

J. García de Veas Silva, C. Bermudo Guitarte, T. Arrobas Velilla, V. Sánchez Margalet, J. Martín Ruiz, M. Merchan Iglesias, R. Goberna Ortiz. *HUVM, SEVILLE, Spain,*

Introduction: An excess of free light chains (FLC) are homogeneous populations of kappa (k) or lambda (λ) immunoglobulin light chain molecules produced by malignant B-cell clones. Approximately 15% of Multiple Myeloma patients have only monoclonal free light chain and no monoclonal intact immunoglobulin secreted by the malignant clone. These patients are difficult to diagnose and monitoring as they frequently show no abnormality when tested by Serum Protein Electrophoresis (SPE). The free light chains are very important markers for monitoring patients with multiple myeloma (MM) and other monoclonal gammopathies. An abnormal k/λ FLC ratio indicates an excess of one light chain type versus the other. The k/λ FLC ratio allows us to detect a relapse of the patient before any symptoms are present or could be detected later by another diagnostic test. In this case, the k/λ FLC ratio shows that chemotherapy applied wasn´t completely effective in the patient and the clinician had to change the treatment.

Case report: A 66 years old man was diagnosed in May 2008 of Light Chain Lambda Multiple Myeloma (LCMM) with orbital plasmacytoma. In June 2008 he began treatment with six cycles of VAD (Doxorubicin, Vincristine and Dexamethasone) and one month later the k/λ FLC ratio was normal while serum protein electrophoresis (SPE) and serum immunofixation (IFE) were negative. In late October 2008, after the first chemotherapy cycles, the k/λ FLC ratio was abnormal (0.002) while SPE and IFE were negative. This value of the ratio predicted a relapse in the patient's condition. Due to the partial response of the disease, the patient began a treatment of five cycles of Bortezomib and Dexamethasone from January 2009 to May 2009 and received twenty sessions of radiotherapy in May 2009. These treatments improved ocular motility and the associated symptoms but the k/λ FLC ratio remained altered (0,01) until June 2009. Then the patient started a new cycle of treatment with Lenalidomide and Dexamethasone improving the patient's condition. The k/λ FLC ratio was normal during this new treatment and this ratio didn´t predict a relapse of the patient. Currently (January 2010), the SPE and IFE are negative and the k/λ FLC ratio is normal (0,73). The patient doesn´t have any symptoms associated with multiple myeloma and he presents a good evolution with improvement of osteolytic lesions.

Conclusion: This case is a good example of the utility k/λ FLC ratio in the monitoring of multiple myeloma and it can predict future relapses in the patient. The value of the k/λ FLC ratio helps us to determine if the patient responds to chemotherapy or the patient has a relapse and we need to change treatment. This is due to the high specificity of free light chains and the high sensitivity of this assay that enables an early identification of residual activity that otherwise couldn´t have been detected.

C-114

Mast Cell Tryptase Controls and Standards for Acute Anaphylaxis Monitoring

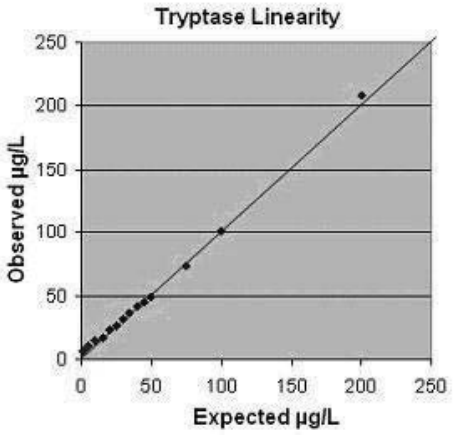
S. Packer[1], P. Jewess[1], D. Patel[2], C. Barber[2], W. Egner[2]. *[1]Scipac Ltd, Sittingbourne, United Kingdom, [2]Northern General Hospital, Sheffield, United Kingdom,*

Background: Mast cells are the key effector cells in allergic responses. When stimulated by an allergen they release several mediators of inflammation, amongst which is the serine protease tryptase. Tryptase is released into the circulation after a patient suffers an anaphylactic reaction caused by drugs, insect venom or food. Measurements of tryptase help identify and assess the extent of the reaction. Basal levels of tryptase are 0.2 to 14.0 µg/L, with peak levels > 40 µg/L following allergic stimulation. Tryptase comprises a multigene family, although the catalytically active β-tryptase is the form released during systemic anaphylaxis. Our current work utilises the Phadia ImmunoCAP 1000 *in vitro* test, which measures both α- and β-tryptase.

Objectives: To evaluate the suitability of purified tryptase diluted in patient normal human serum for EQA distribution.

Methods: We have purified tryptase from human lung tissue and spiked it into normal human serum to generate material for assessing linearity, recovery and stability. Samples were assessed for linearity between 0.2 and 200 µg/L tryptase, recovery from spiked samples and stability at -20ºC, 4ºC and room temperature, using both our internal and Phadia controls and Phadia calibrators.

Results: Linearity: results using the spiked sample showed a high degree of linearity between 0.2 and 200 µg/L tryptase ($r^2 = 0.999$). Precision: the precision of results when a known patient sample was run alongside the purified mast cell tryptase samples on the Phadia ImmunoCAP 1000 was very good, with CVs of less than 3 and SDs of 0.6 and 0.8 respectively. Stability: without heparin samples showed moderate stability at -20ºC and 4ºC: during two weeks tryptase declined to 62±5% (both temperatures) when purified tryptase was spiked into normal human serum. There was no significant difference between stability at 4ºC and -20ºC.



C-115

Analytical and Clinical Comparison of an Automated CCP Assay for the Diagnosis of Rheumatoid Arthritis

D. R. Block, D. A. Dalenberg, J. G. Balsanek, M. R. Snyder, A. K. Saenger. *Mayo Clinic, Rochester, MN,*

Background: Rheumatoid arthritis (RA) is characterized by chronic joint inflammation and certain extra-articular manifestations. The diagnosis of RA is made by assessment of clinical symptoms and autoantibody serology. Rheumatoid factor (RF) is the classic marker for RA. Although moderately sensitive, it lacks clinical specificity. Autoantibodies that recognize citrullinated peptides, referred to as anti-cyclic citrullinated peptide (anti-CCP) antibodies, are a newer diagnostic marker for RA. The primary advantage of anti-CCP antibodies is improved specificity when compared with RF testing.

Objective: Determine the analytical performance and clinical utility of a new automated assay for anti-CCP in the diagnostic assessment of RA.

Methods: A plate-based anti-CCP enzyme immunoassay (EIA) (QUANTA Lite CCP 3.1, INOVA Diagnostics, Inc.) was compared to an electrochemiluminescent immunoassay (ECLIA) on the Roche Modular E170 (Roche Diagnostics, Indianpolis, IN). Serum samples from normal donors (n=91), patients with RA (n=99), and patients with other connective tissue diseases (CTDs) (n=97) were analyzed to determine clinical sensitivity, specificity, PPV, and NPV. Analytical concordance was assessed using samples submitted for routine clinical testing (n=100).

Results: Imprecision studies using serum samples on the automated ECLIA demonstrated intra-assay CV's of 2.14% and 0.54% and inter-assay CV's of 6.5% and 3.3% at concentrations of 45.71 U/mL and 227.3 U/mL, respectively. The EIA had intra-assay CV's of 9.5% and 14.3% at concentrations of 29.1 U/mL and 137.4 U/mL, respectively and inter-assay CV's of 4.6% and 11.1% at concentrations of 52.0 U/mL and 143 U/mL. The LOD of the ECLIA was 8.47 U/mL (CV = 1.65 %), while the LOD of the ELISA was 9.4 U/mL (CV = 4.3%). Both assays showed similar linearity within the manufacturer's defined reportable ranges with an average r^2 of 0.99 and slope of 1.01 (8.00 U/mL to 500.0 U/mL) for the ECLIA and an r^2 of 1.00 and slope of 0.99 (15.6 U/mL to 250.0 U/mL) for the EIA. Overall, analytical concordance between the two assays was found to be 62%. Although the concordance between positive results was acceptable at 95.2%, the negative concordance was poor at 53.2%. This was primarily because a significant number of samples were identified as negative on the ECLIA but positive on the EIA. The ECLIA was 81.8% sensitive for the diagnosis of RA, which was similar to the 77.8% sensitivity of the EIA. In the healthy control population, the ECLIA demonstrated a clinical specificity of 98.9% with the EIA showing a specificity of 100%. However, in the CTD population, both the ECLIA and EIA had decreased specificities of 92.8% and 89.7%, respectively. Overall, this leads to a PPV of 92.1% and 88.5% and a NPV of 83.3% and 79.8% for the ECLIA and EIA, respectively.

Conclusion: The ECLIA demonstrated similar analytical performance, although with improved precision, in comparison to the EIA. The two methods also demonstrated similar clinical sensitivity and specificity, despite poor analytical negative concordance. The Roche automated immunoassay is a viable alternative to the classic plate-based EIAs for the detection of anti-CCP antibodies, with the advantage of being performed on an automated platform.

C-116

Variation of biochemical markers in patients with multiple sclerosis versus patients with non-demyelinating diseases.

T. Arrobas Velilla, M. Garcia Sánchez, M. Gamero García, A. Martin Bastida, V. Sánchez Margalet, F. Fabiani Romero, G. Izquierdo Ayuso. *Hospital Universitario Virgen Macarena, Sevilla, Spain,*

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system which affects the brain and spinal cord by presence of autoreactive T lymphocytes, creating a latent autoimmune state. In acute lesions, helper T cells (CD4+) are present and, moreover, B cell activation is detected.

Aims: To quantify lymphocitary subpopulations and intrathecal immunoglobulin synthesis in MS patients and controls in order to identify variables that will allow us to differentiate the immunological state of patients in each group.

Material and Methods: this study has been carried out by the Neurology Unit (MSU) and the Clinical Biochemistry and Molecular Biology Unit of the Virgen Macarena University Hospital (Seville, Spain). Finally, 123 patients were included between 2008 and 2009. Lumbar puncture was performed and flow cytometry was carried out on CSF and blood from patients without previous treatment, after obtaining informed consent. Two groups were defined: *Group 1* control patients (n=70), non-demyelinating neurological disorders; *Group 2* MS patients (n=53), relapsing-remitting (RRMS). Subsequently, IgG, IgA and IgM were quantified by nephelometry and BOCG were determined by isoelectric focusing. Lymphocyte subpopulations were classified by flow cytometry analysis using BD FACSCantoTM (Becton Dickinson) using the following markers: CD3, CD19, CD4, CD8, CD4/CD8, NKt, CD16CD56NKCD8+, NKCD8- and IgG, IgA, IgM index.

Results: Sample distribution was not normal for any variable. The Mann-Whitney test was used. A CD19 (B cell) increase was observed in CSF but not in blood of MS patients, suggesting a higher inflammatory activity in the CNS. A decrease of NKCD8- in blood serum can be seen also. These cells mediate tissue damage and regulate autoimmune response by T cells through cytokine secretion and cytotoxicity in the inflamed CNS. A decrease of this subpopulation may prove the efectiveness of initial therapy with interferon. Moreover, a decrease in CD4 T-cells (p=0,008) and a increase in CD8 T-cells in CSF of RRMS patients, which involves a lower CD4/CD8 ratio (p=0,001) is observed. No previous research work was made to corroborate this results, the studies mainly describe MS patients with different pharmacological treatments in blood samples. These publications suggest that B-cell and CD4+ T-cell populations may change, modifying the CD4+/CD8* T-cell ratio.

Respect to Ig indexes, higher IgG values in RRMS patients in comparison to control patients were expected as intrathecal IgG synthesis is a characteristic observed in 95% of MS patients. There is also an increase in IgA index in RRMS patients, suggesting that IgA may contribute to axonal damage in the CNS.

Conclusions: B-cell subpopulation in CSF of RRMS patients increase in a statistically significant manner (p=0,005) in comparison to control patients. This accumulation of B cells is correlated with inflammatory CSF parameters as the presence of oligoclonal bands (p=0,000) and IgG (p=0,000) intrathecal synthesis. The results support the idea of B and T cells are one of the key in the pathogenesis of MS.

C-117

Vitamin D Insufficiency and High Parathyroid Hormone Levels in Brazilian Patients with Systemic Lupus Erythematosus

M. I. D. Souto[1], A. Coelho[1], C. Guo[1], L. M. C. Mendonça[2], M. F. M. C. Pinheiro[3], S. V. L. Argolo[3], J. A. S. Papi[1], M. L. F. Farias[4]. *[1]Division of Internal Medicine, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2]Division of Reumathology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [3]Sérgio Franco Medicina Diagnostica, Rio de Janeiro, Brazil, [4]Division of Endocrinology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,*

Background: Vitamin D is a hormone with immunomodulatory properties and its influence in systemic lupus erythematosus (SLE) severity has been studied. Prevalence, associated risk factors and consequences of its insufficiency are of great interest in the course of SLE. The objectives of this study were to determine the vitamin D status and the parathyroid hormone (PTH) levels in patients with SLE and their relationship with bone mineral density (BMD), demographic and clinical variables.

Methods: 141 brazilian patients who met at least three American College of Rheumatology (ACR) criteria for SLE and had clinical definition of SLE were studied. Serum levels of intact PTH and 25-hydroxyvitamin D (25OHD) were measured by ICMA (Immulite 2000, Siemens, USA) and HPLC (Chromsystem, German), respectively. Values of 25OHD <30ng/ml were defined as vitamin D insufficiency. Bone mineral density (BMD) was evaluated by dual-energy X-ray absorptiometry (Prodigy GE) at the hip, lumbar spine and radius 33%. BMD was considered lower than expected when Z-score was ≤

-2 standard deviations. The lowest Z-score was considered for analysis. Data collected included: sex, age, ethnicity, postmenopausal status, disease duration, cumulative daily dose of oral glucocorticoids (GC) in the last 6 months, highest oral dose of GC ever used, duration of GC use, calcium and vitamin D supplementation, photosensitivity, use of sunblocks, sun exposition, diabetes mellitus, creatinine clearance, sedentarism, smoking and knowledge about familiar history of osteoporosis. Bivariate statistical analysis used Mann-Whitney test, Spearman correlation coefficient, Chi-square test and Fisher's Exact Test. Multivariate analysis used multiple linear regression.

Results: Most patients (93.8%) met 4 ACR SLE criteria. The mean age was 42.5 ± 11.5 yrs, 94.3% were female (48.1% postmenopausal) and 68.1% were caucasian. Mean SLE duration was 12.2 ± 7.5 yrs, 25.4% were smokers, 81.2% were sedentary, 4.3% had type 2 diabetes mellitus, 24% reported familiar history of osteoporosis, 43.5% used calcium and vit D supplementation, 65.2% had photosensitivity, 21.7% had regular sun exposition and 65.9% used sunblocks. The median duration of GC use was 6 yrs (25^{th} = 3; 75^{th} = 11). Insufficient levels of 25OHD were found in 39% patients. The prevalence of low bone mass at any region was 33%. Levels of 25OHD had significant and inverse correlation with PTH levels (r = -0,284; p = 0,001), but not with the lowest Z-score (r = 0,047 ; p = 0,60). Z-score had significant and inverse correlation with PTH (r = -0,283 ; p = 0,001). Vitamin D insufficiency had significant association with diabetes (p = 0,034). There was no association between 25OHD levels and the other variables studied.

Conclusions: Even in a tropical country there is a high prevalence of vitamin D insufficiency in SLE, which was associated with high levels of PTH and with type 2 diabetes. There was also a high prevalence of low bone mass in SLE, although the demographic and clinical risk factors are still not clear. More studies are needed to define the many mechanisms for vitamin D and calcium deficiencies that contribute to PTH elevation and low BMD.

C-118

Evaluation of IgG subclass assays on the Roche cobas c6000 analyser

F. Mitchell, M. D. Coley, P. J. Showell. *The Binding Site Group Ltd, Birmingham, United Kingdom,*

Measurement of IgG subclasses (IgGSc) is useful in the diagnosis of IgG deficiencies where a deficiency in one IgGSc is masked by normal total IgG levels. Assays for measurement of IgGSc in serum are routinely used in many immunology laboratories. Here we evaluate the performance of The Binding Site IgGSc assays for use on the Roche cobas® c6000. The main assay characteristics are summarised in the table below:

Assay	IgG1	IgG2	IgG3 Latex	IgG4 Latex
Range (mg/L)	983 - 19665	570 - 9450	68 - 1286	69 - 837
Sample dilution	1/10	1/5	1/15	1/15
Sensitivity (mg/L)	327	57	13.6	9.2
Assay time	10 mins	10 mins	10 mins	10 mins
Intra-assay precision (n=20) %CV (Mean)	2.5 (1627mg/L)	2.6 (970mg/L)	4.1 (130mg/L)	1.9 (117mg/L)
	2.8 (4070mg/L)	2.5 (2796mg/L)	5.8 (579mg/L)	2.1 (378mg/L)
	2.4 (16111mg/L)	1.7 (8205mg/L)	4.1 (1145mg/L)	1.6 (690mg/L)
Inter-assay precision (n=10) %CV (Mean)	6.6 (1658mg/L)	4.3 (953mg/L)	3.2 (137mg/L)	1.5 (126mg/L)
	4.9 (3993mg/L)	3.4 (2699mg/L)	6.3 (584mg/L)	3.4 (362mg/L)
	4.4 (16519mg/L)	2.6 (8292mg/L)	3.5 (1165mg/L)	1.2 (749mg/L)

Interference was within ±8% when bilirubin (200mg/L), hemoglobin (5g/L) or chyle (1,540 formazine turbidity units) were added to serum samples with known IgGSc concentrations. Linearity was assessed by assay of serially diluted serum samples and comparison of expected with measured results by regression analysis. All assays showed acceptable linearity over the range tested:- IgG1: y=1.004x - 33mg/L, r=1.00; IgG2: y=0.995x + 32.9mg/L, r=1.00; IgG3: y=0.1.02x - 16.0mg/L, r=1.00; IgG4: y=0.989x - 2mg/L, r=1.00. Comparison was made with the IgGSc assays for the Roche Modular P. Serum samples from normal and clinical subjects were assayed for IgGSc on both systems. Regression analysis of the results shows good agreement:- IgG1: y=0.92+ 272mg/L, r=0.98; IgG2: y=0.96x + 124mg/L, r=0.99; IgG3: y=0.96x + 12mg/L, r=0.98; IgG4: y=0.99x + 17mg/L, r=0.99. We conclude that the Binding Site IgGSc assays on the Roche cobas c6000 provide a rapid, precise method of measuring IgGSc in serum and show good agreement with existing assays.

C-119

Comparison of five automated immunoassays for measuring thyroglobulin antibodies

J. Harrington, R. Kreller, J. Waletzky, M. Gupta, T. Daly. *Cleveland Clinic, Cleveland, OH,*

Thyroglobulin antibody (TgAb) measurement is challenging because of the heterogenous nature of the autoantibodies produced, and the lack of standardization between TgAb assays. As a result, method changes between analyzers can be difficult. We evaluated the intra-assay agreement of five different automated TgAb assays, and correlated TgAb results from these assays against other markers of autoimmune thyroid disease and against inhibition of thyroglobulin measurement. 101 clinical samples that had been sent for TgAb analysis were tested using the Tosoh AIA-pack, Abbott Architect, Siemens Centaur, Beckman DxI, and DPC Immulite TgAb assays. Samples were chosen for the study without knowledge of clinical histories, but were selected to provide a range of TgAb values based on the predicate assay in our laboratory (Tosoh AIA pack).

Quantitative results for TgAb varied considerably between assays. However, when samples were classified as "positive" or "negative" for TgAb using the manufacturer-recommended cutoffs for each assay, overall agreement between assays was relatively good. 90/101 samples showed agreement in at least 4 of the 5 assays. Overall agreement was highest for Tosoh vs Architect (93%) and DxI vs Immulite (93%) comparisons, but the majority of pairwise comparisons between assays showed >85% concordance in this sample set. The positive call rate varied between assays, with the Centaur assay giving the most positive results (41%), the Tosoh and Architect assays giving an intermediate number (30%) and the DxI and Immulite assays having the fewest positive calls (20%). The majority of disagreement was observed for "weak-positive" samples where TgAb levels were <4x the assay-specific upper limit of normal.

Although thyroid peroxidase antibody (TPOAb) was present in 40% of the samples, there was no correlation between TPOAb and TgAb results for any of the five TgAb assays tested. However, TgAb-positive samples did show an increased rate of interference with thyroglobulin (Tg) measurements. Both the frequency and magnitude of Tg inhibition were increased in samples that were called TgAb-positive by multiple assays, with 95% of samples that were TgAb positive in >= 4 assays showing diminished recovery of spiked Tg relative to control samples.

In conclusion, we have characterized the relative performance of five automated TgAb assays. Although there was generally good agreement in classifying samples as positive vs negative between these assays, variation does occur, particularly in samples with lower levels of TgAb. Laboratories should be aware of the specific characteristics of their assay in order to appropriately advise clinicians on the significance of such results.

C-120

Nephelometric immunoassay measurements of IgMκ and IgMλ for the assessment of patients with IgM monoclonal gammopathies

N. J. B. Fourrier[1], K. Sharp[1], P. Walsh[1], G. L. F. Wallis[1], M. T. Drayson[2], A. R. Bradwell[2], S. Harding[1]. *[1]The Binding Site Group Ltd., Birmingham, United Kingdom, [2]Division of Immunity and Infection, The Medical School, University of Birmingham, Birmingham, United Kingdom,*

Detection and quantification of IgM monoclonal proteins by serum protein electrophoresis (SPE) can be hampered by its failure to migrate from the origin and its heavily polymerised nature. Immunofixation (IFE) can improve the sensitivity of detection but is non quantitative. Here we describe automated, nephelometric immunoassays on the Siemens Dade Behring BN™II analyser for the quantification of IgMκ and IgMλ in serum. Determination of the IgMκ/IgMλ ratio may provide a sensitive method for detecting and monitoring monoclonal disease. The main assay characteristics are summarised below:

Assay	IgMκ	IgMλ
Range (mg/L)	200 - 6400	175 - 5600
Sample dilution	1/100	1/100
Min. sample dilution	1/5	1/5
Sensitivity (mg/L)	10	8.8
Assay time (mins)	12	12

Purified polyclonal IgMκ and IgMλ reference proteins were produced; the two preparations were greater than 98% pure by silver-stained SDS-PAGE, and light chain specific as indicated by ELISA, dot blot and Western blotting. Quantification was by established nephelometric methods referenced to DA470k. IgMκ and IgMλ concentrations were measured in 120 normal (blood donor) sera; median IgMκ 526mg/L (range 136mg/L - 1417mg/L), median IgMλ 395mg/L (range 111mg/L - 1076mg/L), median IgMκ/IgMλ ratio 1.398 (range 0.790 - 2.013). IgMκ + IgMλ summated well to total IgM (Passing Bablok, Y= -0.004+0.92X). IgMκ + IgMλ concentrations were measured in 19 IgM (14

IgMκ / 5 IgMλ), archived patient sera collected from the Department of Immunity and Infection, University of Birmingham, UK. In all cases the IgMκ/IgMλ ratio correctly identified the monoclonal IgM type. We conclude that highly purified polyclonal reference material allowed for the correct assignment of IgMκ and IgMλ values in the calibrator fluid. Measurement of IgMκ and IgMλ in serum and the presence of an abnormal ratio may be useful in identifying and monitoring patients with IgM monoclonal gammopathies. `

C-121

MELISA® In-Vitro Methodology for the Detection of Metal Allergy in an American Population

P. D. Khare[1], M. P. Kellermann[2], G. H. Kellermann[1]. *[1]NeuroScience Inc., OSCEOLA, WI, [2]Pharmasan Labs Inc., OSCEOLA, WI,*

Background: Although heavy metals such as copper, iron and zinc are essential to maintain normal physiologic functions, many metals, including aluminum, mercury, lead, nickel, and titanium, among others, may be a cause of metal allergy, which can be a root cause of chronic inflammation and disease. Lymphocyte testing is more sensitive than dermal patch testing for metal allergies because the cellular response to dermal contact is often different than that in an in-vivo environment. Dermal patch testing may also induce allergies, and there are no standardized tests for dermal patch testing for all relevant metals. There is an urgent need for a functional in vitro test that can determine heavy metal allergy in US population.

Objective: The aim of this study is to evaluate the reproducibility, sensitivity, specificity and reliability of an in vitro MELISA® (Memory Lymphocyte Immuno-Stimulation Assay) test for detecting type IV allergy to metals in a US population. Multiplex cytokine analysis was also performed in patients identified with metal allergy to assess inflammatory status.

Design: 525 MELISA® tests for metal allergy against aluminum, mercury, lead, methyl mercury, nickel, thimerosal, and titanium were performed on enriched lymphocytes obtained from the peripheral blood of 75 healthy US subjects. The frequency and distribution of reactivity, and the sensitivity and specificity of the assay were analyzed. In addition, 308 metal tests were performed to determine inter-assay variability and 116 metal tests were performed to evaluate intra-assay variability. Proficiency testing was performed in laboratories in the US and Geneva, Switzerland. Microscopic examination on 126 metal tests was performed for accuracy studies with lymphocyte reactivity. The effect of varied heavy metals on cytokine production was also examined using Multiplex technology.

Results: MELISA® testing identified 36% of asymptomatic subjects tested positive for metal allergy. Of these, 66.6% were identified with allergy to 1 metal, 25.9% to two metals, and 7.4% to three metals. The most frequent allergy was nickel (24%), followed by mercury (13.3%), lead (6.6%), titanium (2.6%), thimerosal (2.6%) and aluminum (1.3%). The accuracy of the MELISA® test was 99% using the cut-off of Stimulation index ≥3. Intra-assay variability was maximum with lead (18.9%), followed by titanium (16.1%), aluminum (14.1%), mercury (11.6%), thimerosal (11.2%), nickel (10.1%) and methyl mercury (5.3%). Proficiency testing for nickel, thimerosal and methyl mercury was 14.6%, 11.7% and 14.8%, respectively when data were compared between labs. Cytokine assessment on subjects with positive MELISA® test results indicated an activated immune system, producing high amounts of pro-inflammatory cytokines and chemokines (IL-1β, IL-2, IL-6, IL-8, IL-17 and MIP-1β).

Conclusion: The MELISA® assay is a highly reproducible, sensitive, specific, and reliable in detecting metal allergy in an American population. In vitro MELISA® and Multiplex cytokine testing is clinically useful in patients with suspected metal allergy and chronic diseases. To our knowledge, this is the first comprehensive study that identifies metal allergy in an American population by MELISA®, and the effect of heavy metal allergies on cytokine production.

C-122

Induction of Unfolded Protein Response in HEp-2 cells exposed to Glucocorticoids and LPS

A. Duzgun, A. Bedir, T. Ozdemir, R. Nar, V. Kilinc, O. Salis. *Ondokuz Mayıs University, Samsun, Turkey,*

Glucocorticoids are potent anti-inflammatory and immunosuppressive agents that are known to affect T cell-mediated inflammation by the inhibition of cellular proliferation and cytokine production. The literature is lacking in explaining the mode of action of such agents on the epithelial cells. Therefore epithelial cells (HEp-2) were used to determine the effects of dexamethasone (Dex) administration or Dex in the presence of LPS on the unfolded protein response (UPR).

Cells were treated with 0.1 μM and 1.0 μM concentrations of Dex or Dex + LPS for periods of 6, 12, 24, and 48 hours. After each phase cell number and qPCR analyses were performed to follow the expression changes of CHOP, Grp78 and Grp94 genes and cell ELISA was done to show the cell surface expression of GRP78 protein. The results indicated that 0.1 μM Dex alone or in the presence of LPS up-regulated CHOP gene expression, typical marker of apoptotic branch of UPR, at the earliest phase of incubation ($p<0.05$), whereas both combinations of 1.0 μM Dex down-regulated the expressions of CHOP, Grp78 and Grp94 all through the incubation periods ($p<0.05$). Consistent with this result, we did not detect any increment in the cell surface expression of GRP78 protein in any group ($p<0.05$).

These observations suggest that Dex with or without LPS may trigger apoptosis on HEp-2 cells dose- and time-dependently, as evidenced by the up-regulation of CHOP gene expression. Overall, the results indicate that lower dose of Dex has different effects on HEp-2 cells in a time-dependent manner compared to higher dose. In cancer, up-regulation of apoptotic branch of the UPR is thought to provide a growth disadvantage to tumor cells.

C-123

Rods And Rings: A New Fluorescence Pattern In Hep-2 Cells With Strong Association With Hepatitis C Infection And Interferon Therapy

C. L. P. Mangueira[1], M. G. L. Valim[2], M. J. A. Oliveira[2], P. R. Estelha[2], V. A. Santos[2]. *[1]Immunology Section - Central Laboratory Division - Hospital das Clinicas - University of Sao Paulo School of Medicine; Clinical Laboratory - Hospital Albert Einstein, Sao Paulo, Brazil, [2]Immunology Section - Central Laboratory Division - Hospital das Clinicas - University of Sao Paulo School of Medicine, Sao Paulo, Brazil,*

Aims: Since the introduction of HEp-2 cells as the standard substrate for the search of antinuclear antibodies (ANA), new fluorescence patterns have been described and associated with various clinical conditions. Recently, a new cytoplasm pattern, called "Rods & Rings" (RR), was recognized and eventually associated with Hepatitis C Virus (HCV) Infection. Our aim was recognize this new pattern in a routine of a Clinical Laboratory, establish the frequency of occurrence with three different HEp-2 commercial substrates and check the real association with HCV infection.

Methods: One month of the ANA routine from a hospital-based, universitary Clinical Laboratory was monitored for the occurrence of RR pattern. All the positive serum samples for the pattern were tested with three different commercial substrates: Biocientifica (Argentina), Bion (USA) and TBS (UK). HCV serology was performed in all the positive RR samples. The clinical records of the patients presenting the RR pattern were reviewed.

Results: We found 13 RR positive serum samples in a group of 2324 analyzed specimens. All RR positive samples also had a positive serology for HCV. Two of the commercial substrates used in the study (Biocientifica and Bion) recognized the pattern in all the 13 samples. TBS substrate failed to disclose the RR pattern in three samples that showed positivity on the other two substrates. Ten of the patients who presented the RR pattern were under Interferon therapy for treatment of HCV infection.

Conclusions: Our results showed a strong association of the RR pattern with HCV infection and Interferon therapy. This new association could be clinically useful in some instances and help clinicians in the diagnosis of HCV infection.

C-124

Lipid hidroperoxide as oxidative stress marker in patients with recent-onset rheumatoid arthritis.

K. Santos-Rey, E. Melguizo, M. Navarro, B. Hernández-Cruz, F. Navarro, F. Fabiani, C. González-Rodríguez. *HUVM, Sevilla, Spain,*

Background: Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs. It is associated with multiple extra-articular lesions and presence of autoantibodies which affects the joints of hands and feet symmetrically. Oxidative process appears to be associated with the disease, and the disbalance between oxidant/antioxidant mechanisms may generate free radicals and lipid peroxidations. Thus, lipid hidroperoxide (LPO) could be used as a marker of oxidative status in patients with RA. The aim of the present work was to demonstrate an increase of lipid peroxidation in patients with recent-onset RA.

Methods: A total of 39 patients with recently diagnosed active RA and 22 healthy controls were included in this study. Population was selected from the Rheumatology Service of the 3rd Level Teaching Hospital. The samples were analyzed in the Biochemistry Service of the University Hospital Virgen Macarena. Whole blood was obtained from every individual and colected in tubes containing K_3-EDTA. Samples were centrifuged immediately at 0º C to obtain fresh plasma. An antioxidant cocktail was added to every sample to avoid oxidation. Samples were stored at - 80 °C until analysis. Fatty peracids were extracted with chloroform before being tested and subsequent LPO was measured by chemical oxidation of ferrous thiocyanate to ferric thiocyanate and measurement of absorbance at 500 nm (Cayman Chemical, Ann Arbor, USA). Statistical analysis was performed using the SPSS statistical software 15.0 (SPSS, Chicago, USA).

Results: Patient and control group were similar regarding age ($p= 0.67$) or sex ($p = 0.97$) and presented a mean LPO value of 37.67 ± 15.34 μM, and 34.31 ± 18.37 μM,

respectively. A statistically significant increase ($p < 0.05$) was observed for LPO levels in patients with recent-onset RA.
Conclusion: Changes were observed in oxidative stress involving lipid peroxidation in a group of patients with recent-onset RA, demonstrated by an increase in the LPO concentration when compared with healthy controls. Differences found in LPO concentration between RA patients and healthy controls can not be attributed to age or sex.

C-125

Clinical application of solid-phase antiphospholipid antibodies in the diagnosis of Hughes syndrome.

C. González-Rodríguez, E. Melguizo, K. Santos-Rey, J. Toyos-Sáenz de Riera, M. Navarro, R. Goberna. *HUVM, Sevilla, Spain,*

Background: The antiphospholipid syndrome (APS) or Hughes syndrome is a rheumatic disease characterized by thrombotic events, pregnant morbility and antiphospholipid antibody positive. The aim of present work was to analyze the clinical application of the measurement of antiphospholipid antibodies in the diagnosis of Hughes syndrome in the routine samples of Biochemistry Service of a 3rd Level Teaching Hospital.
Methods: Samples were collected, selected, and analyzed by the Biochemistry Service of the Virgen Macarena University Hospital, Sevilla, Spain. Anticardiolipin (aCL) IgG and IgM antibodies and anti-β2-glycoprotein I (aβ2GPI) IgG and IgM were measured. aCL antibodies and aβ2GPI were determined by standardized ELISA (European Antiphospholipid Forum 2001 and 2004 respectively). We studied the association between aβ2GPI and aCL antibodies of IgG and IgM isotopes. The cutoff points were 40 MPL units (aCL IgM antibodies), 40 GPL units (aCL IgG antibodies) and 20 U/mL (aβ2GPI) as recommended in APS classification criteria.
Results: A total of 710 samples were analized. The contingency table between aCL-IgM antibodies and aβ2GPI IgM shows 95.2% of concordance. To evaluate the association between both parameters, we calculate $\chi 2=190$, which was statistically significant ($p <0.0001$), and κ index of agreement ($\kappa=0.47$), whereas the contingency table between aCL-IgG antibodies and aβ2GPI IgG showed 96.8% of concordance, $\chi 2=219$ ($p < 0.0001$) and $\kappa=0.55$. These results indicate a moderate agreement between the two markers in both cases.
Conclusion: aCL antibodies and aβ2GPI showed a good agreement in the samples selected from aPL study according to clinical suspicious of APS.

C-126

Evaluation of IgAκ and IgAλ nephelometric immunoassays for the detection and quantification of monoclonal gammopathies.

P. S. Steele[1], D. M. Hoefner[1], S. Alvi[2], S. Harding[2]. *[1]Marshfield Clinic, Marshfield, WI, [2]The Binding Site, San Diego, CA,*

INTRODUCTION: Detection and quantification of IgA monoclonal proteins by serum protein electrophoresis (SPE) can be difficult depending upon the concentration of the paraprotein and whether it co-migrates with other proteins on the gel. Immunofixation (IFE) alleviates some of these issues, but is not quantitative. The Binding Site has developed nephelometric assays for the Siemens BN™II analyser which quantifies IgAκ and IgAλ in serum. *Our objective was to evaluate the utility of these reagents in our laboratory*.
METHODS: Assay validation was completed and 69 SPE samples (49 IFE negative, 20 IFE positive) were tested using IgAκ/IgAλ nephelometric assays.

Assay	IgAκ	IgAλ
Regression Statistics	y = 0.983x + 0.463 R^2 = 0.994	y = 1.030x + 0.100 R^2 = 0.989
Intra-assay precision %CV (mean g/L n=10)	3.80 (1.74) 1.72 (7.82)	1.07 (1.59) 5.09 (5.90)
Inter-assay precision %CV (mean, g/L, n=3)	0.41 (1.72) 0.81 (7.88)	9.18 (1.54) 11.43 (5.51)
Median normal value, g/L, n=25 (range)	0.925 (0.497-1.45)	0.84 (0.452-1.28)
Median ratio, n=25 (range)	1.08 (0.877-1.78)	
Agreement with Standard Panel*	y = 1.047x + 0.043 R^2 = 0.997	y = 1.122x - 0.151 R^2 = 0.999

* 4 IgAκ, 4 IgAλ and 3 normal sera
RESULTS: Normal IgAκ/IgAλ ratios were found in 40/49 IFE negative samples, while 7 of the remaining ratios fell outside 2 SD and 2 outside 3 SD. Concordance between IFE and IgAκ/IgAλ ratios for typing monoclonal immunoglobulins was achieved in 19/20. The remaining sample had a normal IgAκ/IgAλ ratio but a minor IgAλ band by IFE; quantification of the monoclonal protein was below the sensitivity of SPE.
CONCLUSION: We have demonstrated that serum IgAκ/IgAλ assays precisely measure IgA serum immunoglobulins and have acceptable linearity, intra and inter-assay precision. Furthermore, IgAκ/IgAλ ratios correctly identified all significant monoclonal gammopathies. The clinical significance of the discordant results between the ratios and IFE remains to be elucidated.

C-127

Clinical Significance of Discrepant Results Between Enzyme Immunoassays and Luminex Bead-based Assays in Anti-Nuclear Antibody Testing

K. Murata, M. W. Ettore, A. K. Saenger, M. R. Snyder. *Mayo Clinic, Rochester, MN,*

Background: Anti-nuclear antibodies (ANA) are autoantibodies directed against a variety of intracellular antigens and are used to aid in the diagnosis of connective tissue diseases (CTD). While enzyme immunoassays (EIA) and indirect immunofluorescence assays (IFA) have been traditional mainstays in the detection of ANAs, bead-based multiplex assays have recently emerged as a new method for ANA detection. Unlike EIA or IFA, these multiplex assays are able to detect antibodies to multiple nuclear antigens simultaneously and identify the antigenic target(s) of the ANA. Many laboratories use EIA or IFA methods as a screening test, with follow up testing for specific ANAs for samples that screened as positive. Theoretically, a positive result for antibodies to one or more individual nuclear antigens by the bead based assay should correlate with a positive ANA result by EIA. Periodically, however, discrepant results can arise, with certain patients testing positive for individual antigens while testing negative by EIA. The frequency of such occurrences has not been documented, and the clinical significance of such results is unknown.
Objectives: To quantitate the frequency by which specimens testing positive for 1 or more individual nuclear antigens on the Bioplex 2200 result in negative findings by EIA, and determine the clinical significance of such results
Methods: Serum specimens were collected from a total of 357 outpatients identified as having a CTD by board certified rheumatologists at our institution between 2005 and 2009. ANA results were obtained on all patients by both EIA (Helix EIA ANA Screen, Bio-Rad) and Bioplex 2200 ANA Screen (Bio-Rad).
Results: Of 357 patients, 54% were found to be positive for ANA by EIA (>1 IU), while 38.1% were found to be positive for 1 or more nuclear antigens on the Bioplex. In this cohort, we identified 50 patients who tested positive for ANA by EIA as well as a single nuclear antigen on the Bioplex. We identified an additional 17 patients who also tested positive for a single nuclear antigen on the Bioplex, but tested negative for ANA by EIA. A comparison of these two groups revealed that the nuclear antigens most frequently found to test negative by EIA were SCL-70, U1RNP, SS-B , Centromere, SS-A, and dsDNA. The proportion of Bioplex single positive, EIA negative patients, to patients positive for a single particular nuclear antigen, were calculated for each of these 6 nuclear antigens, and found to be 67%, 42%, 33%, 33%, 24%, and 6%, respectively. These 17 Bioplex single positive, EIA negative patients were ultimately diagnosed with diseases not typically associated with the nuclear antigens for which they tested positive, primarily rheumatoid arthritis.
Conclusion: A significant proportion of specimens testing positive on the Bioplex show negative results by EIA. These discrepancies appear to be more prevalent in certain nuclear antigens, such as SCL-70, and U1RNP. Furthermore, a positive result for a nuclear antigen on the Bioplex in the absence of a positive ANA by EIA appears to have limited diagnostic value.

C-128

The inflammatory response in stroke

A. Kost, B. Lutsyk, L. Lapovets. *Danylo Halytsky National Medical University, Lviv, Ukraine,*

Background. Inflammatory mechanisms play an important role during the acute period of brain ischemia, which contributes to functional outcome of patients. Resent works in the area of stroke and brain ischemia has demonstrated the significance of the inflammatory response accompanying necrotic brain injury. Inflammation has been implicated as a secondary injury mechanism following ischemia and stroke. Our objective was to evaluate differences in the inflammatory response of acute period between ischemic stroke (IS) and intracerebral hemorrhage (ICH).
Methods. Eighty two acute stroke patients (ischemic, n=44; hemorrhagic,n=38) were included in this study. All of them were admitted to hospital within 24 h after stroke onset. We studied inflammatory markers (<24 h and 10 day) in this patients. Serum samples for hight-sesitivity C-reactive protein (hsCRP) and cytokine measurements were collected on admission. Plasma levels of hsCRP, IL-1beta, IL-6, TNF-alpha were determined by

an enzyme-linked immunosorbent assay (ELISA) method. The increase/decrease of the parameters between both dates was analyzed.
Results. Plasma levels of IL-1beta, IL-6, TNF-alpha decrease is significantly higher in IS than ICH (p=0,044; p=0,037; p=0,041). There was no evidence of time trend in levels of hsCRP during 10 days of follow-up. Although mean hsCRP in the ICH group increased and decreased in the IS group from day 1 to day 10, this was not statistically significant (p > 0,05).
Conclusions. The observation suggests that the inflammatory response in the acute period is different for ICH and IS. A better understanding of the inflammatory response may help to a better design of clinical trials. Inflammation is an important avenue of therapeutic research in stroke.

C-129

Diagnostic Usefulness Of Transglutaminase Antibodies In Celiac Disease.
R. Ramos, J. Carretero, L. Rodelgo, A. Garcia-Claver, C. Tapia Ruano, M. Gomez-Serranillos. *Hospital Virgen De La Salud, Toledo, Spain,*

INTRODUCTION Celiac Disease (CD) is an inflammatory disorder of the proximal small intestine caused by a permanent intolerance to gluten prolamins in genetically predisposed subjects (haplotype DQ2 or DQ8). Diagnosis is based on the finding of histopathological alterations in small bowel biopsy when the patient has a normal diet and the clinical and histological normalization after gluten withdrawal. As serological tests support the diagnosis are IgA class transglutaminase antibodies (attg) and IgA endomysial.
OBJETIVE Evaluate the clinical usefulness and diagnostic yield of attg in diagnosing CD in our area.
MATERIAL AND METHODS We analyzed 449 patients between March 2008 and May 2009. 84 were diagnosed with CD by biopsy, 207 were healthy subjects and the remainder have heterogeneous group of diseases such as diabetes mellitus, hypothyroidism, diarrhea and abdominal pain. The attg were determined by fluoroinmunoassay by inmunocap 250 (Phadia). ROC curve analysis was performed using the MedCalc program.
RESULTS The area under the curve (AUC) for attg was 0,952 (CI = 95%, 0.928-0.970). The resulting optimal cutoff point by ROC curve analysis was attg = 3.2 with a sensitivity of 89.2% (CI = 95%, 80.4-94.9) and a specificity of 92% (CI = 95%, 88.7-94.6).
CONCLUSION Attg show good sensitivity and specificity in our area that is consistent with that is described in the literature although the cutoff point obtained is somewhat lower than proposed by Phadia. Therefore, studies are needed to set the cutoff point based on each population.

Wednesday AM, July 28

Poster Session: 10:00 am – 12:30 pm
Nutrition/Trace Metals/Vitamins

C-130

Nutritional implications and flour functionality of defatted wheat germ

M. U. Arshad[1], F. M. Anjum[2], T. Zahoor[3], H. Nawaz[2]. *[1]University of Sargodha, Sargodha, Pakistan, [2]University of Agriculture, Faisalabad, Pakistan, [3]Washington State University, Pullman, WA,*

Purpose: The present study aims to explore the physicochemical, sensory and functional properties of defatted wheat germ (DFWG) flour blends. Furthermore, the nutritional implications and baking suitability of flour blends were to be investigated through their utilization for the preparation of chapattis.
Design/methodology/approach: Defatted wheat germ (DFWG) was blended into wheat flour (WF) at levels of 0, 5, 10, 15, 20 and 25% with the purpose to investigate its effect on physicochemical and functional properties of blends. The flour blends were prepared by adding up DFWG upto 25%. After the physicochemical and functional evaluation of flour blends, chapattis were prepared from these blends. DFWG supplemented chapattis were subjected to sensory evaluation to a trained panel of judges for the point of consumer acceptance. Furthermore, the protein quality of the chapattis was assessed through weanling albino rats by feeding a diet of chapattis for 10 days, which was formulated to supply 10% protein, with a casein diet as a control.
Findings: The crude protein content of DFWG was as high as 27.80% with highly valuable amino acid profile, especially lysine (2.324 g/100g). The chemical composition of flour blends revealed that protein, fiber as well as ash content were increased as a function of DFWG addition. Likewise, calcium, potassium and iron content of flour blends were also improved. The water and oil absorption capacities of the DFWGF/WF blends improved progressively with increased amount of DFWG flour in the blends, however the bulk density did not differ significantly ($p > 0.05$) among the blends. Similarly, the foaming properties of the blends were also improved by the incorporation of DFWG flour; However the emulsion capacity and stability of the flour blends seemed to be low as compared to the wheat flour. The sensory characteristics of chapattis revealed that substitution of wheat flour with 24 DFWG flour up to 15% produced acceptable chapattis that are similar to the control (100% wheat flour) chapattis. The chapattis containing 15% DFWG were best regarding protein bioavailability in rats. The protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV) and true digestibility (TD) differed significantly among diets containing chapattis with 0-10% DFWG, and casein diet when fed to rats. Diets containing 15% DFWG have values, of these parameters, similar to the casein diet. In the nutshell, defatted wheat germ can be used as a functional ingredient to enhance the nutritional quality of chapattis.
Originality: This research paper is original in evaluating the new proteinous source i.e. DFWG for its functional and organoleptic suitability in the preparation of nutritionally enhanced cereal based baked products.

C-132

Folic acid and vitamin B_{12} deficiency as a cause of higher prevalence of hyperhomocysteinemia in North Indian patients of vascular disease

S. Bhargava[1], A. Ali[2], A. Manocha[1], M. Kankra[1], S. Das[1], S. Bora[3], L. M. Srivastava[1]. *[1]Sir Ganga Ram Hospital, New Delhi, India, [2]Jamia Milia Islamia, New Delhi, India, [3]Mount Kisco Medical Centre, New York, NY,*

Background: Global burden of vascular disease is increasing exponentially with 80% of the patients being from developing countries. Traditional markers are often within the biological reference interval in these patients and hence are inadequate for identification of the population at risk. In the quest for other markers, hyperhomocysteinemia [hhcy] has emerged as a significant marker of vascular disease, especially in patients of Asian origin. The prevalence of hhcy has been observed to be higher in Indians than in western populations. It is known to be caused by genetic polymorphisms as well as dietary deficiencies of the vitamins B_{12}, B_6 and folate. We attempted to identify the association of hhcy with vitamin B_{12} and folate in Indian patients of vascular disease.
Methods: Homocysteine [hcy], vitamin B_{12} and folate levels were estimated by chemiluminescent immunoassay in 100 controls and 100 patients of vascular disease, of which 35 had coronary artery disease [CAD], 35 had cerebrovascular disease [CVD] and 30 had peripheral vascular disease [PVD]. The data was subject to analysis by SPSS verion 14.
Results: The mean levels of B_{12} and folate are shown in the table below. Hcy exhibited a significant negative correlation with B_{12} only in PVD patients [p=0.010], and with folate in CAD [p=0.040] and CVD [p=0.025] patients as well as controls [p=0.016].Conclusion: Low B_{12} levels could contribute to the higher incidence of PVD, and low folate levels could account for the higher prevalence of CAD and CVD in North Indian urban population. Moreover, since it is known that hcy levels can be reduced by administration of folate irrespective of the cause of hhcy, large-scale corrective measures like food supplementation with folate might benefit the Indian population and reduce the incidence and morbidity of vascular disease.

Homocysteine, vitamin B12 and folate in controls and vascular disease patients

Analyte	Controls(n=100)	CAD(n=35)	CVD(n=35)	PVD(n=30)
Homocysteine [µmol/L]	12.423 ± 0.85	27.169 ± 4.160***	19.303 ± 2.408***	20.213 ± 3.705***
Vitamin B_{12} [pg/mL]	505.69 ± 29.979	516.743 ± 50.891	588.857 ± 58.269	397.4 ± 41.655*
Folic Acid [ng/mL]	13.04 ± 0.709	8.4 ± 0.707**	9.486 ± 1.281**	10.84 ± 1.107*

(All values are Mean ± SEM; *p<0.05; **p<0.005; ***p<0.001.)

C-133

A Comparative Study of Visceral Fat and anthropometric measurements of waist, hip and waist-to-hip ratio among Diabetic and Hypertensive Patients at the Komfo Anokye Teaching Hospital, Kumasi, Ghana

E. Togbe[1], B. Eghan[1], N. Korsah[2], J. Kwame[3], R. Adjei Frimpong[3]. *[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [2]Metro Health Center, Cleveland, OH, [3]Komfo Anokye Teaching Hospital, Kumasi, Ghana,*

Introduction: In clinical practice and epidemiological surveys anthropometric measurements represent simple, convenient, rapid, noninvasive and an inexpensive way to assess nutritional status. Visceral fat or intraperitoneal fat, which cannot be measured by anthropometric means, is composed of mesenteric and omental fat masses. Excessive visceral fat (VF) is associated with insulin resistance and other metabolic risk factors for coronary heart disease and diabetes mellitus.
Objective: In this project we set out to do a comparative study between visceral fat and the common anthropometric measures of circumferences of waist and hip and waist-to-hip ratio.
Methods and materials: 398 subjects who were known to be both NIDDM diabetic and hypertensive patients attending the diabetic clinic at Komfo Anokye Teaching Hospital, Kumasi, Ghana of mean age 58.5 years were recruited for the exercise. Waist (W) and hip (H) circumferences were measured in centimetres to the nearest 0.1cm by a tape measure and waist-to-hip ratio (WHR) was calculated as Wcm/Hcm. The intra- and interobserver % CV for W and H of ten measurements each were, 3.6%, 4.8%, and 4.0%, 3.8% respectively. Visceral fat measurements employing bioelectrical Impedance (BI) were done using the Omron® Body Composition Monitor (model HBF-500). Pregnant women, children and people who did not meet manufacturer's criteria were excluded. All other measurements were done according to the manufacturers' instructions by a well trained health worker.
Results: Our results showed the following correlations VF and W, $r = 0.65$ ($p<0.001$); VF and H, $r = 0.55$ ($p<0.001$); VF and WHR, $r = 0.095$ ($p<0.001$).
Conclusion: Waist circumference measurement promises to be the best surrogate for visceral fat estimate among the three parameters in our patient population. In addition W measurement is easy to understand and track and will encourage self management and awareness about changes in waist circumference.

C-134

Target Groups for Nutritional Intervention in Ghana.

E. Togbe, F. A. Yeboah, M. T. Agyei-Frempong. *Kwame Nkumah University of Science and Technology, Kumasi, Ghana,*

Introduction and objective: Food security even in the absence of war is threatened in many developing countries by rapid population growth and widening poverty. Children are especially susceptible to the devastating effects of Protein Energy Malnutrition (PEM). In adults Chronic Energy Deficiency (CED) is also more often a food security problem. CED is defined as a "steady state" where an individual is in energy balance, i.e. the energy intake equals the energy expenditure, despite the low body weight and low body energy stores. The objective of this study is to select individuals at the highest risk of PEM and CED as priority groups for nutritional intervention. 305 children aged 1-to-12 years and 307 adults from age 50 to 80 years were recruited for the project.
Methods and materials: An attempt was made to select the group of children who had

the highest prevalence of Protein Energy Malnutrition i.e. wasted, using WHZ - scores, underweight, using WAZ - scores and stunted, using HAZ - scores according to NCHS standards. All children who met any of the three criteria were described as Protein Energy malnourished. A Z - score cut-off point below 2 SD indicated malnutrition in each case. The children were stratified into the following age groups of 1-to-3 years, 4-to-5 years, 6-to-9 years and 10-to-12 years. The adults were also stratified into the following age groups of 50-to-60 years, 61-to-70 years, and 71-to-80 years. In adults BMI was determined as Weight (kg)/Height (m) [2]. BMI was used to define 3 grades of chronic energy deficiency (CED) in the adult sample. Severe CED as BMI, < or = 16.0, moderate CED 16.1 - 17.4 and mild CED as BMI 17.5 - 18.4. BMI > or = 18.5 was considered normal. Weight was determined using a Seca balance (Seca, Germany) to the nearest 1g and height to the nearest 1cm with microtoise (Stanley Tools, New Britain, CT, USA). All measurements were performed according to standard WHO criteria. An experienced nurse screened all the subjects and eligibility was based on the absence of any obvious chronic disease.

Results: Two hundred and twenty-three (223 i.e. 73.1%) of the 305 children were found to be malnourished. Out of these the 6-to-9 year group was found to have highest prevalence of one, two, or all the 3 forms of malnutrition i.e. 72.2% were wasted, 64.1% were underweight and 52.7% were stunted. In the adults, the total prevalence rate of any of various degrees of CED was 23.8% of the 307 (i.e. 73 adults). Among these, the 50-to-60 year group had 20.1%, the 61-to-70 year group had 26.2% and the 71-to-80 group had 30.4% prevalence rates of CED, which makes the latter age group the most malnourished.

Conclusion: Protein-energy malnutrition is more prevalent among children than adults. The most nutritionally deprived age groups of children and adults are 6-to-9 years and 71-to-80 years respectively. It would be most welcome if governmental, non-governmental and international aid donors prioritize these groups as target groups needing immediate nutritional attention.

C-136

Detection and Analysis of Trace element of Peripheral blood in 6562 Children in Jingmen City

C. Liang[1], L. Zuo[2]. *[1]Wuhan University Zhongnan Hospital, Wuhan, China, [2]The first People's of Hospital of JingMen, Wuhan, China,*

Objectives To investigate the current status of the content level of internal trace element in children of Jingmen City of Hubei Province, and explore the rate of lack and exceeding between blood levels of trace element Zn, Fe, Cu Ca, Mg, so as to provide the basis for children's health and clinical direction.

Methods Blood samples of 6562 children (from 0 to 5 years-old) , randomly selected from health examination in The 1st hospital in Jingmen City were analyzed . Levels of Zn, Fe, Cu, Ca and Mg were measured with atomic absorption graphiteingle method.

Result The blood level of Zn, Fe, Cu Ca, Mg in male and female, the difference were not statistically significant (P>0.05) . The blood level of the five trace element in different ages were statistically significant, respectively(P<0.001). The difference of rate of lack and exceeding between blood levels of trace element Zn, Fe, Cu Ca, Mg were statistically significant(P<0.001).

Conclusions The incidence of lack blood level of essential trace element was low in Jingmen City. he blood level of the five trace element in different ages were statistically significant, and so as to explore the reconsider the reference range.

C-137

Selenium Status in Mammals and its Role for Health Care

A. Gross[1], L. Schomburg[2], K. Renko[2], T. Behrends[2]. *[1]Bruker Nano GmbH, Berlin, Germany, [2]Institute for Experimental Endocrinology, Charite Berlin, Berlin, Germany,*

This presentation will highlight the physiological role of selenium (Se) and its quantitative detection in different tissues by total reflection X-ray fluorescence (TXRF) spectrometry.

Se represents one of the few essential elements for humans. It is the only trace element for which the position within proteins is defined precisely by the genetic code. Among the intensively studied selenoproteins, Selenoprotein P (SePP; up to 10 Sec per protein) controls serum Se concentration and transport in rodents and humans [1]. The individual Se status, dietary Se intake and SePP expression are interrelated and become increasingly recognized as an important pathophysiological parameter for disease risk and prognosis [2]. While the role of SePP for storage and transport of Se have convincingly been proven by respective analyses with transgenic mice [1, 3], similar direct evidence on the physiological importance of SePP for other metabolic functions is missing.

Recently, we have established transgenic mouse models of modified Se metabolism and SePP expression including SePP-knock out and transgenic SePP overexpressing mice.. For the analysis of Se and other element concentrations we developed protocols and improved sample handling for TXRF spectrometry.

In pilot studies, TXRF allowed the parallel quantification of several trace metal elements from minute amounts of murine starting material without laborious sample preparation. Tissue-specific preparation procedures were developed and first results of metal concentrations in different matrices were obtained. Reliable Se determination and quantification was achieved with as little as 1 thyroid gland lobe of an adult mouse (wet weight of only 1-2 mg per gland). Here, the importance of perfusion became obvious especially in poorly Se supplied mice to separate the tissue Se content from the circulating fractions. For serum analyses, as little as 10 µl of serum sufficed for the simultaneous duplicate analyses of Se, Zn, Cu and Fe. Preanalytical precipitation of serum proteins allowed for a convenient separation of proteins and high MW material from small soluble metal-containing factors, e.g. selenosugars, free seleno-amino acids or anorganic Se forms. The advantage of TXRF becomes obvious when different trace metals are compared and physiological meaningful correlations are deduced. To this end, we are currently studying the importance of Se status for heavy metal detoxification, the potential interrelation of Se with other physiological relevant trace elements (Zn, Cu, Fe) in male fertility and the metabolic changes that take place upon the acute phase response in mice. In this context, we observed a sexual dimorphic Se redistribution between blood and cellular selenoproteins.

We conclude that TXRF-based analysis in combination with biochemical methods and molecular biology techniques opens new doors for a fast and sensitive multi-element analysis of a variety of biological matrices. Our analyses clearly demonstrate that selenoproteins represent important endogenous factors controlling male fertility, heavy metal metabolism and acute phase response offering new opportunities in prevention and health care.

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C-138

Validation of a candidate reference measurement procedure based on ID-LC/tandem MS for 25-hydroxyvitamin D3 and D2 in human serum.

H. C. M. Stepman, A. Vanderroost, K. van Uytfanghe, L. M. Thienpont. *University of Ghent, Ghent, Belgium,*

Context: Increased clinical awareness of the prevalence of vitamin D deficiency and insufficiency has led to a dramatic rise in 25-hydroxyvitamin D_3 and D_2 ($25OHD_3$, $25OHD_2$) testing. However, the poor between-assay comparability as shown from, e.g., the Vitamin D External Quality Assessment Scheme (www.deqas.org) emphasizes the need for reference measurement procedures (RMPs) to assist in the establishment of calibration traceability and method validation.

Objective: Our aim was to develop and validate 2 candidate isotope dilution-liquid chromatography-tandem mass spectrometry RMPs for separate quantification of serum $25OHD_2$ and $25OHD_3$, the latter with sufficient chromatographic resolution between $25OHD_3$ and the 3-epi-metabolite.

Method: Serum (250 µL) is sampled and diluted to 1 mL with 0.9% (g/g) sodium chloride solution; an appropriate amount of either hexa-deuterated $25OHD_3$ (d_6-$25OHD_3$) or d_6-$25OHD_2$ (Medical Isotopes Inc.) (dissolved in ethanol) is added; after equilibration, the mixture is alkalinized and extracted with n-hexane (750 µL). The organic layer is evaporated and redissolved in a mixture of methanol/chloroform/cyclohexane (1:4:8, v/v/v) prior to fractionation on a Sephadex LH-20 column. The $25OHD_2$ or $25OHD_3$ collection window is evaporated and reconstituted prior to LC/MS analysis with an UPLC column-switching system coupled to a TQD mass spectrometer (Acquity®, Waters). In the $25OHD_2$ procedure the first and second dimension are an Acquity UPLC BEH300 C4 (2.1 x 50 mm, 1.7 µm) and an UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm); in that for $25OHD_3$, the first dimension is identical, but the second is a Zorbax SB-CN column (2.1 x 250 mm, 5 µm) (Agilent). All columns are gradient eluted by mixing phase A, i.e., water/methanol/formic acid (50:50:0.025, v/v/v) and B, i.e., water/methanol/formic acid (5:95:0.025, v/v/v). The elution time for $25OHD_2$ was ~ 13 min, for $25OHD_3$ and the 3-epi-form ~ 17 and 18 min, respectively. For tandem MS detection of $25OHD_2$ (d_6-$25OHD_2$) in the positive electrospray ionization mode the transitions from m/z 413.4 (419.4) > 159.4 were monitored, of (3-epi-)$25OHD_3$ (d_6-$25OHD_3$) from m/z 401.3 (407.3) > 159.3. The procedures were validated for trueness and precision with the NIST standard reference material (SRM) 972 (duplicate analysis during 5 days). The substances tested for interference were a kind gift of Immunotech-Beckman Coulter and Prof. H. DeLuca (University of Wisconsin-Madison).

Results: Our candidate RMPs had an average bias of 1.2% ($25OHD_2$) and 0.8% ($25OHD_3$). Quantification of 3-epi-$25OHD_3$ deviated by 1.6% (level 4 of SRM 972). The mean within- and between-run CVs were 2.8% ($25OHD_2$) and 2.0% ($25OHD_3$). The limits of quantification (LoQ) were set at 1.5 nmol/L (CV = 5.3%, bias = 0.3 nmol/L, $25OHD_2$) and 1.2 nmol/L (CV = 6.3%; bias 0.1 nmol/L, $25OHD_3$). None of the tested substances interfered. Absence of interference was additionally demonstrated from analysis of multiple serum samples without added d_6-analogues.

Conclusion: From the good agreement of our measurement results with the SRM 972

certificate, sufficient precision, specificity, LoQ and resolution between $25OHD_3$ and the 3-epi-metabolite, we conclude that we achieved our objective of 2 state-of-the-art candidate RMPs for serum $25OHD_3$ and $25OHD_2$.

C-139

Semi-Automated Solid Phase Extraction and Measurement of Vitamin-D in Serum

K. Foley, D. Knotts, M. Ivie, S. Welch. *Kaiser Permanente, Portland, OR,*

The quantitation of vitamin D2 and D3 in serum remains a high-volume test for many clinical laboratories. Our laboratory previously performed an *n*-heptane extraction followed by vortexing, centrifugation and freezing in an acetone dry-ice bath. With the aqueous layer frozen, the organic layer is poured off and dried under nitrogen. Dried samples were then reconstituted in ethanol and vitamin-D measured using LC-MS/MS. Given our weekly volume of approximately 1200 specimens, this manual extraction procedure became unmanageable and resulted in slow processing, repetitive motion injuries and excessive reagent waste. We recently validated a new semi-automated technique for vitamin-D extraction and analysis. Using solid-phase, disposable, ion-exchange columns (SPEware) and a robotic pipetting system (Tecan), we can now process and run specimens in a 96-well plate format with analysis on a Waters UPLC Acquity LC-MS/MS. A low volume Acquity BEH 2.1x50mm 1.7µm phenyl chromatography column with pre-filter was used. This column achieved good separation of D2 and D3 peaks. Use of the solid-phase columns required a change in the standard matrix. The best results were seen when standards (5, 30 and 100 ng/ml) were made in 30% isopropyl alcohol rather than in BSA as done previously. Standard concentrations were validated against the now available NIST SRM 972 vitamin-D materials. Precision, recovery and method comparison studies are summarized in the Table. Linearity of both D2 and D3 is 5 ng/mL-200 ng/mL. The method showed no significant lipid, hemoglobin or bibirubin interference. To gauge accuracy, CAP, NIST SRM972 and DEQAS samples were tested and found to be within 10% of target or peer-group values.

Conclusion: This new assay is approximately 25% faster than the previous method but more importantly, requires less hands-on technologist time and significantly lower volumes of organic reagents.

	Vitamin D2	Vitamin D3
Method Comparison Deming Regression (D2 n=253, D3 n=257)	y= 1.026x -0.9 r^2 = 0.984	y= 1.137x -3.5 r^2= 0.987
Average Bias	-0.5	0.3
Total Imprecision (Level 1)	Mean: 32.0 SD: 2.8 CV: 8.7%	Mean: 31.7 SD: 1.9 CV: 6.0%
Total Imprecision (Level 2)	Mean: 78.1 SD: 7.3 CV 9.3%	Mean: 82.0 SD: 7.2 CV 8.8%
Low-end Precision (within run)	Mean: 7.35 SD: 0.51 CV: 6.9%	Mean: 4.49 SD: 0.40 CV: 8.7%
Average Recovery	97%	110%

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Vitamin D: an evaluation of the Elecsys and Liaison Immunoassay analyzer

Y. H. Othman[1], G. Dhatt[1], M. Agarwal[2], B. Bishawi[1], E. Valenciano[1]. *[1]Tawam Hospital, Al-Ain- Abu Dhabi, United Arab Emirates, [2]United Arab Emirates University, Al-Ain- Abu Dhabi, United Arab Emirates,*

Background: To meet the increasing demand, Vitamin D (Vit D) assay had to be done in the hospital laboratory. The aim of this study was to evaluate the 1) Elecsys 25-OH Vitamin D3 electrochemiluminescence immunoassay (Cobas e 411, Roche Diagnostics,Indianapolis, USA) and 2) DiaSorin 25-OH vitamin D chemiluminescence immunoassay (Liaison, DiaSorin, Crescentino,Italy).

Methods: The Elecsys and DiaSorin are chemiluminescence immunoassays; Elecsys measures only Vit D3 while the DiaSorin assay measures the total Vit D (D2 + D3). The assays were compared to a) each other at two different sites and b)an high performance liquid chromatography (HPLC) total Vit D assay; c) The Elecsys assay was compared to external quality assurance material from the Royal College of Pathologists of Australia (RCPA) with the Vit D3 results validated by Gas Chromatography Isotpe Dilution Mass Spectroscopy (GCMS). The precision was established by running at least two different levels of quality control with each run. The results of the paired comparisons are shown in the table.

Results: The intra assay CV for both, the Elecsys and DiaSorin, was <12%; however, they overestimated the Vitamin D when compared to either HPLC or GCMS. The inter analyzer comparison (R2) was 0.8 for Elecsys and 0.9 for DiaSorin. The correlation of Elecsys or DiaSorin to HPLC was unacceptable (R2 = 0.31), though there was reasonable correlation between the Elecsys and GCMS (R2 = 0.83).

Conclusions: Both, DiaSorin and Elecsys achieved an acceptable precision, within each analyzer and between the same analyzer (at the two sites). The results for Vit D were not transferable among the various methodologies. Assays measuring total Vit D are preferable; they can be used for the diagnosis of Vit D deficiency and for the follow up of Vit D treatment.

Comparison studeis

Instruments	Number of samples	Regression equation	R^2	$S_{y/x}$	p
Elecsys Vs HPLC	88	Y = 25.29 + 0.22x	0.31	10.68	<0.0001
DiaSorin Vs HPLC	43	Y = 22.99 + 0.41x	0.31	13.86	<0.0001
Elecsys Vs GCMS	43	Y = 37.2 + 0.64 x	0.83	18.67	<0.0001
Elecsys Vs Elecsys	44	Y = -3.64 + 0.99 x	0.8	9.15	<0.0001
DiaSorin Vs DiaSorin	90	Y = 6.93 + 0.9 x	0.9	6.3	<0.0001

C-141

An LC-MSMS Analytical Method for Steroids and Mono-Hydroxy-vitamin D3 Analysis in Dried Blood Spots

T. Lee, S. Leonard, H. Liu. *AB Sciex, Foster City, CA,*

Background An over or under secretion of one or more steroid can cause a steroid imbalance resulting in various types of diseases, hence steroid analysis is important in medical diagnosis. However analytical challenges often occur when analyzing steroids, due either to the low concentrations and/or the structural similarities between the different steroids.

Dried blood spot screening (DBS) is the concept that blood is easily and conveniently obtained from a prick of the finger, or heel and blotted onto filter paper. DBS is an ideal method since it is straight forward, easy to obtain and utilizes only a small amount of blood from the patient. The goal is to have a simple extraction method (without derivatization) without sacrificing good sensitivity or specificity.

Methods: The following steroids including cortisol, 17-alpha hydroxy hydroxyl progesterone, progesterone, testosterone and mono-hydroxy metabolites of vitamin D3 are extracted from one whole blood spot using the liquid liquid extraction method, prior to analysis with LC-MS/MS. The challenge of calibration curve generation for endogenous level analyte analysis will be examined, and will be a key factor in this experiment.

Results: The method development included: the evaluation of different dry blood spot sample preparation procedures; calibration standard curve construction for endogenous steroids; HPLC interferences and separation; the reproducibility of the assay. The dried blood spots calibrators curve were was constructed with a 1:1 ratio of stripped serum and red blood cells, spiked with compounds, spotted onto fresh filter paper (Whatman 903) in 60µL aliquots, left to dry in room temperature for 24 hours, and stored in -20°C. until needed.

The optimal sample preparation procedure was utilized for sample analysis. A whole spot (~60µL) is cut and soaked in water for around 60 min, followed by liquid-liquid extractions with a mixture containing hexane and ethyl acetate. The organic supernatant was transferred to glass tube and then evaporated to dryness with nitrogen gas and reconstituted with a solution of 50:50 methanol: water before loading onto the LC-MS/MS system.

The detection limits of all steroids and mono-hydroxy metabolites of vitamin D3 achieved the requirement of endogenous steroids testing in general population screening. The low limit of quantitation for cortisol, 17-alpha hydroxyl progesterone, progesterone, testosterone, and mono hydroxy vitamin D_3 is as follows respectively: 5ng/mL, 100pg/mL, 100pg/mL, 100pg/mL, and 10 ng/mL. The reproducibility of the assay is under 15% accuracy ±100 in real patient sample set.

C-142

Increase of serum 25-hydroxyvitamin D after a trip to south during winter in a healthy adult population.

G. D. Fink[1], J. Baillargeon[2], M. Berthier[1], D. Cyr[1], M. Gingras[3]. *[1]CHUS, Sherbrooke, QC, Canada, [2]Université de Sherbrooke, Sherbrooke, QC, Canada, [3]CSSS Richelieu-Yamaska, St-Hyacinthe, QC, Canada,*

Introduction: It is well-known that sunlight is required for vitamin D synthesis in humans. However, the extend of and the conditions needed for 25-hydroxyvitamin D augmentation following exposition to sun are still unclear. Whether vitamin D status could be improved

while using sunscreen is also of major interest considering the newly recognized virtues of this vitamin.

Material and Methods: We conducted a prospective study in a group of 45 healthy adult living in the region of Sherbrooke Canada (latitude = 45° north). The project was approved by the ethics committee and all participants gave an informed consent. Using LC-MS/MS, we measured their serum level of 25-hydroxyvitamin D before and after a trip to south during the months of December to April. Questionnaires were obtained from all participants and allowed to investigate sun exposition and nutritional habit influencing the level of the serum 25-hydroxyvitamin D.

Results: The 25-hydroxyvitamin D mean level before departure was 63 nmol/L, while it was raised to 81 nmol/L upon arrival. The extent of 25-hydroxyvitamin D change was determined in each subject and resulted in a significant augmentation with a mean of 18 nmol/L. Subjects having a lower level of 25-hydroxyvitamin D before the trip were the ones that presented higher increases. We found that the level of 25-hydroxyvitamin D before the trip was positively and significantly correlated with the consumption of supplements of vitamin D but not with alimentation containing vitamin D. Also, increases in 25-hydroxyvitamin D level were significantly associated with the destination latitude, higher increases found near equator. Sunscreen use did not prevented the increase in 25-hydroxyvitamin D and no difference in the level of increase was found among subjects using a solar protection factor of 30 compared to 15, suggesting that vitamin D status can be improved with solar protection. Increase in 25-hydroxyvitamin D was not associated with the number of travel days or days spent outside. Increase in the number of sunbathing hours also had no effect. This is in accordance with the current knowledge that synthesis of vitamin D is achieved rapidly and that any amount of vitamin D made in excess is destroyed. Finally, we found no influence of age and sex on the vitamin D status or increase.

Conclusion: This study suggests that, along with the use of supplements, a trip to a sunny country during winter is a good way to improve the vitamin D status during winter. Moreover, increase in serum 25-hydroxyvitamin D can be achieved even when using sunscreen.

C-143

Method comparison between two automated immunoassay platforms which measure total 25-hydroxyvitamin D

A. Chin[1], C. Ramsey[2], W. Roberts[3], J. Ritchie[1]. *[1]Emory University Hospital, Atlanta, GA, [2]Emory University, Atlanta, GA, [3]ARUP Laboratories, Salt Lake City, UT,*

Background: Deficiency in vitamin D is a common phenomenon worldwide. While previous research has well established that adequate vitamin D levels prevent skeletal deformities in children and reduce the risk of osteopenia and osteomalacia in adulthood, more recent evidence suggests that vitamin D may play a more extensive role in preventing the onset of many chronic illnesses. Given this renewed interest, there has been a rapid escalation in laboratory testing and the subsequent development of high throughput methods to measure 25-hydroxyvitamin D (25OHD) which is the major circulating product of vitamin D metabolism. Vitamin D consists of either D2 or D3 forms which are derived from plant sources or from cutaneous UV-B irradiation, respectively. Although methods exist that can distinguish D2 from the more active metabolite D3, current immunoassay platforms measure both forms and report total 25OHD. While standardization and harmonization of 25OHD methods is likely to occur in the near future, current studies that address possible differences in assay performance and how they may affect medical decisions, especially at the generally accepted vitamin D deficiency cutoff of <20 ng/mL are urgently needed.

Objective: To compare the performance of two commercially available automated total 25OHD platforms.

Methods: Clinical plasma specimens which were destined for 25OHD measurement by Diasorin Liaison (ARUP) were aliquoted and measured by Immunodiagnostic Systems IDS iSYS (Emory). Intra- and interassay precisions were determined using respective manufacturer-provided assay controls. Method comparisons were assessed on 215 plasma samples and BSA spiked with varying concentrations of purified 25-hydroxyvitamin D2 or D3. The IDS iSYS method was also evaluated according to a Vitamin D External Quality Assessment Scheme (DEQAS) peer group.

Results: IDS interassay precisions using accompanying assay controls were 19.5% (6.5 ng/mL), 12.4% (27.3 ng/mL), and 15.9% (64.9 ng/mL), while DiaSorin intraassay precisions were 3.5% (10.9 ng/mL), 5.0% (40.4 ng/mL), and 3.4% (69.2 ng/mL). Initial evaluations between the IDS (y) and the DiaSorin (x) methods on plasma samples yielded a linear correlation of $y = 0.76x+6.32$, $r^2 = 0.59$. Furthermore, 25OHD2 recovery for both assays was lower than expected. The two assays were in 83.3% concordance in identifying patients with or without vitamin D deficiency according to the <20 ng/mL cutoff. All samples (366-370) fell within 1 standard deviation of the All Laboratory Trimmed Mean (ALTM) in the January 2010 DEQAS survey.

Conclusion: The IDS assay exhibits good performance characteristics regarding inter- and intraassay precision, and acceptable correlation with the Diasorin assay. The fair concordance between the two assays further underscores the importance of standardization and harmonization of 25OHD assays for the correct identification of vitamin D deficiency.

C-144

Automated measurement of 25-OH Vitamin D_2 and D_3 in serum and plasma by high turbulence-flow liqhid chromatography and tandem mass spectrometry

W. Wu, H. Wang. *Cleveland HeartLab, Cleveland, OH,*

Background Vitamin D is a fat-soluble prohormone, the two major forms of which are vitamin D_2 and Vitamin D_3. The major role of vitamin D is to maintain normal levels of calcium and phosphorus in the blood. Vitamin D deficiency results in bone diseases and has also been implicated in the development of other diseases such as autoimmune diseases. More recently, it has been linked to increased risk for cardiovascular disease. Within a large cohort of subjects without prevalent cardiovascular or kidney disease, multivariable adjusted hazard ratios demonstrated an increased risk when Vitamin D levels were below 15 ng/ml.

The purpose of this study is to develop and validate a method to assess Vitamin D levels by measuring 25-OH Vitamin D_2 and D_3 (Vit D_2 and D_3). Here we report an automatic, rapid, and sensitive liquid chromatography-tandem mass spectrometry (LC/MS/MS) method combined with High Turbulence Liquid Chromatography (HTLC) online extraction for quantification of Vit D_2 and D_3 in human serum and plasma.

Methods We developed and validated an assay for Vit D_2 and D_3 determination in human serum and plasma based on LC/MS/MS using HTLC online sample extraction. Patient samples, calibrators, or controls, were spiked with isotope labeled internal standard and thoroughly mixed. After centrifugation, 30 ul of supernatant was was introduced into LC/MS/MS for online extraction and analysis.

Results This assay is validated according to the CLSI guidance procedures. The accuracy of the assay was conducted by spiking recovery study, sample correlation study, and running SRM 972 from NIST. The overall recovery of Vit D_2 and D_3 in serum and plasma are 100%, 101%, 94.4%, and 98.5%, respectively. This method correlates very well with another LC/MS/MS method. 4 serum specimens in the SRM 972 package have also been analyzed using this method. The average deviation of measured value compared to target values for the 25-OH Vitamin D_2 and D_3 are only 5% and 6%, respectively. The overall reproducibility for Vit D_2 and D_3 in both serum and plasma are less than 6 % and 5 %. The analytical reportable range was from 1.25 ng/ml to 200 ng/ml and the clinical reportable range was from 1.25 ng/ml to 1000 ng/ml. Linearity study suggests that the anyalyte response is linear across the range of the calibration curve. The lower limit of detection of Vit D_2 and D_3 was determined at 0.07 ng/ml and 0.23 ng/ml, respectively. Vit D_2 and D_3 in serum is stable for at least 2 days at room temperature, 3 month at 4 °C, and 3 freeze-thaw cycles. Vit D_2 and D_3 levels are almost identical in paired specimens (serum vs plasma) from same individual (n=20).

Conclusion This LC/MS/MS method combined with HTLC online sample extraction provides an automatic, rapid, and accurate way to determine Vit D_2 and D3 in human serum and plasma. This method has been standardized using SRM 972 from NIST and used for routine clinical sample analysis.

C-145

Holotranscobalamin in the diagnosis of Vitamin B12 status in comparison to tB12 and MMA

A. Khajuria[1], J. Jan Dayton[1], P. Jansen[1], P. Hall[2], D. Lehotay[2]. *[1]DynaLIFEDx, Edmonton, AB, Canada, [2]Inborn Screening Laboratory, Regina, SK, Canada,*

Holotranscobalamin (HoloTC) is the fraction of circulating vitamin B12 (tB12) that contains the biologically available cobalamin. Its uptake is driven by specific receptors and represents the functionally active component of vitamin B12. HoloTC has been proposed to be a better marker to predict the vitamin B12 status. The objective of the study was to evaluate the diagnostic performance of HoloTC in an adult population and to determine if HoloTC could be a better marker for vitamin B12 status than tB12 when compared to methyl malonic acid (MMA), the current standard for vitamin B12 status. *Method*: The study samples comprised of 199 subjects (Females 66%; Males 34%) submitted for vitamin B12 testing in our reference laboratory. Normal (n=121; >150 pmol/L tB12) and deficient groups (n=78; <150 pmol/L tB12) were selected on the basis of tB12 values from Siemens tB12 assay (in-house assay) with normal renal and kidney function and normal folate. All specimens were then analyzed for tB12 assay with Abbott Architect, ActiveB12 (HoloTC) (Abbott AXSYM), homocysteine (Siemens) and MMA (LC/MS). *Results:* Thirty-three percent of the samples (n=65) were not concordant with respect to Siemens tB12, ActiveB12 and MMA, indicating discrepancies across the vitamin B12 methods. Siemens tB12 to ActiveB12 concordance was 71%; Siemens tB12 to MMA concordance was 72%. ActiveB12 to MMA was 91%. ActiveB12 correlated better with ARCHITECT

tB12 (85%) than Siemens tB12. In terms of diagnostic performance with respect to MMA as reference (cut-off 0.4 µmol/L), AUC for HCY was 0.66, Centaur tB12 being 0.85 and Active B12 and tB12 Architect had similar AUC of 0.90 and 0.91 respectively. Conclusion: ActiveB12 was determined to be an accurate measure of vitamin B12 status in a normal, healthy adult population relative to MMA versus Siemens tB12to MMA.

C-146

Development of a Rapid and Simple 25-Hydroxy-Vitamin D Assay on the LCMSMS

B. C. Yu[1], P. A. Andrews[1], H. K. Lee[2]. *[1]Dartmouth Medical School, Lebanon, NH, [2]Dartmouth Medical School & Dartmouth-Hitchcock Medical Center, Lebanon, NH,*

Background: Vitamin D plays many important functions in the human body, and vitamin D deficiencies have been linked to higher incidence of certain diseases. There are several clinical platforms used to measure vitamin D, including radioimmunoassays, liquid chromatography-tandem mass spectrometry (LCMSMS), HPLC, and competitive protein binding assays. LCMSMS is considered the most sensitive and specific technology for quantifying 25-hydroxy-vitamin D (25OHD), the best biomarker for determining vitamin D levels in patients.
Objectives: This study aimed to develop a rapid and simple LCMSMS assay for analyzing 25OHD in patient serum.
Methods: Serum was manually extracted with methanol containing hexadeuterated 25-hydroxyvitamin D_3 (d_6-25OHD_3) as an internal standard. The supernatant was injected into a C18 pre-column and then a C18 analytical column. Serum vitamin D levels were quantitated by the mass spectrometer, using the positive atmospheric pressure chemical ionization mode. Patient comparison with our current reference laboratory was performed using 40 samples containing vitamin D_2 and 107 samples containing vitamin D_3. Intra-day precision was determined by running three levels of control 20 times in one day. Inter-day precision was determined by running the same controls in duplicate for 20 times over 10 days. Using serial dilutions of known vitamin D_2 and D_3 concentrations, linearity and recovery were determined by comparing measured values to expected values. Analytical sensitivity was determined by running a zero calibrator over 20 separate runs. Patient serum pools with low 25OHD levels were used to determine the functional sensitivity. Carryover and ion suppression studies were also performed.
Results: The intra-day precision study showed a CV that ranged from 2.8% to 9.6% for vitamin D_2 and D_3 analytes over three concentration levels. Inter-day precision showed a CV that ranged from 4.3% to 13.0% for the analytes over the same three concentration levels. Linearity testing showed the method was linear over the range of 0-100 ng/mL for both analytes. Recovery over the same linear range varied from 95.2 to 102%. Patient comparison for vitamin D_2 (n=40) showed a regression slope of 0.90, intercept of -0.82, and r^2 of 0.98. Patient comparison for vitamin D_3 (n=107) showed a regression slope of 1.01, intercept of -1.28 and r^2 of 0.98. Analytical sensitivity and functional sensitivity were determined to be 1.5 ng/mL and 5 ng/mL respectively for both analytes. No carryover or ion suppression was observed. The analytical time for one sample was 2 minutes, while the manual sample extraction time was 30 minutes.
Conclusions: The LCMSMS method using offline manual protein extraction is a rapid, simple and accurate method for clinical testing of serum 25OHD levels.

C-147

Development and evaluation of an HPLC tandem mass spectrometric assay for the simultaneous determination of 25-OH vitamin D3 and D2

Y. Zhu, B. R. Slay. *Medical University of South Carolina, Charleston, SC,*

Background: 25-hydroxyvitamin D (D3/D2) is frequently tested in clinical laboratories due to its importance in the management of patients with bone disease and the association between 25-hydroxyvitamin D (25-OH-D) deficiency/insufficiency and certain cancers, autoimmune disorders, infectious diseases, cardiovascular diseases, and many other conditions. Current methodologies for 25-OH-D testing in clinical laboratories include radioimmunoassay, enzyme-linked immunosorbent assay, high-performance liquid chromatography (HPLC), HPLC coupled mass spectrometry (LC-MS), and chemiluminescent immunoassays. Most LC-MS methods for 25-OH-D testing are home-brew assays; therefore, careful evaluation is needed prior to clinical application. The objective of this study is to develop and evaluate an LC tandem MS (LC-MS/MS) assay for simultaneous quantification of D3/D2.
Methods: 150 µL of serum, standards, or controls were mixed with methanol-isoproponol containing internal standard (IS, 25-OH D3-d6), and ZnSO4 reagent was added followed by vortex mixing. Then methanol was added and mixed to precipitate proteins. Hexane was added to extract the D3/D2. After centrifugation, the hexane layer was dried under nitrogen. The residue was reconstituted in methanol-water and chromatographed on a SunFire C8 column on Alliance HPLC 2795 XE Separation module (Waters). D3/D2 and IS were detected by electrospray ionization MS/MS in multiple-reaction monitoring mode using mass (m)/charge (z) transitions of 401.35>159.10 (D3), 413.35>83.10 (D2), and 407.35>159.10 (IS) on Micromass Quattro Micro® mass spectrometer (Waters). Performance characteristics including intra- and inter-assay impression, carry-over, analytical measurement range (AMR), lower limit of quantification (LLOQ), interference, and the accuracy were evaluated.
Results: The retention time was 5.33 min for D3, 5.41min for D2, and 5.32 min for IS. The intra-assay CVs for D3 were 3.7%, 3.5%, and 2.0% at 11.1, 27.6, and 65.1 ng/mL, and for D2 were 3.7%, 2.7%, and 2.2% at 13.6, 39.4, and 97.2 ng/mL (n = 20). No carry-over was observed over the above ranges. The inter-assay CVs were 7.3%, 5.4%, and 5.2% for D3 and 7.5%, 7.2%, and 7.2% for D2. The LLOQs were 3 ng/mL and 4 ng/mL with AMRs up to 150 ng/mL and 90 ng/mL for D3 and D2, respectively. 1α-hydroxyvitamin D3 and D2 up to 200 ng/mL didn't show any interference; however, 3-epi-25-OH-D3 co-elutes with D3. Compared with the National Institute Standards and Technology SMR 972, the recoveries were 110%, 112%, and 108% at 12.3, 18.5, and 23.9 ng/mL for D3, and 106% at 26.4 ng/mL for D2. No significant difference was found between the present assay and a reference laboratory (Mayo) LC-MS/MS assay for D3, D2, and total 25-OH-D with P values of 0.00002 (D3, n=99), 0.00749 (D2, n=21), and 0.00283 (total 25-OH-D, n=99). Two methods showed excellent correlation: y = 0.974x + 0.99 (r2 = 0.979, D3), y = 1.066x + 0.756 (r2 = 0.968, D2), and y = 1.013x + 0.581 (r2 = 0.958, total 25-OH-D).
Conclusions: The performance characteristics of this robust LC-MS/MS assay for the simultaneous determination of D3 and D2 in human serum are acceptable for clinical use. However, caution should be taken when testing infant samples because these samples may contain 3-epi-25-OH-D3, which interferes with this assay.

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Analysis of serum tryptophan, kynurenine and kynurenic acid by HPLC

y. Z. xiang, g. A. tang, p. Y. ren, x. Q. zhou, b. X. luo. *Department of Clinical Laboratory, Second Xiangya Hospital, Central South University, Changsha, P.R., changsha, China,*

Background and objective: L-Tryptophan (TRP) is an essential amino acid that is required for the biosynthesis of proteins and is the precursor for several important biological compounds. In mammals, TRP is mainly metabolized via the kynurenine (KYN) pathway in which TRP is converted to KYN, KYN is further catabolized into kynurenic acid (KYNA). Serum TRP and its metabolites KYN and KYNA levels have been found to be altered in such diseases as mental disorder, kidney disease, immune system disease, abnormal pregnancy, which could be biomarkers for the progression of these diseases. So far, few HPLC methods have been described the simultaneous determination of TRP, KYN and KYNA .We developed a simple and specific high performance liquid chromatography (HPLC) for simultaneously quantitative determination of serum TRP, KYN and KYNA with fluorescence detection (FD) setting programmed wavelength and using on-column fluorescence derivatization.
Methodology: It employed a Hypersil C18 column and a mobile phase of 0. 20 mol/L zinc acetate-8.3 mmol/L acetic acid containing 2.5% (v/v) acetonitrile at a flow rate of 1.5ml/min, excitation and emission wavelength of fluorescence detector, respectively, were 365nm and 480nm in 0-11min for KYN, 344nm and 404nm in 11-15.5 min for KYNA, 254nm and 404nm in15.5-22min for TRP, Serum samples were first precipitated with 0.624 mol/L perchloric acid solution, then centrifuged to remove protein residue and finally analyzed by HPLC.
Results: The retention time of KYN, KYNA and TRP were 8.5 min, 13.7min and 17.6 min, respectively. For TRP, the assay was linear from0.245 to196µmol/L and the detection limit was 0.001µmol/L. For KYN, the linear range form 0.049 to 98µmol/L and the detection limit was 0.0245µmol/L. For KYNA, the linear range form 1.05 to 2093nmol/L and the detection limit was 0.05nmol/L. Its precision and recovery are satisfactory.
Conclusions: The method is simple and rapid, providing satisfactory precision, sensitivity and accuracy. It is well suited for clinical routine analysis of diseases with possible TRP metabolism consequences.

C-149

Development and Validation of a Tandem Mass Spectrometry Method to Measure Nicotinamide and Nicotinamide Mononucleotide in Plasma

T. Isbell, P. Gilmore, C. Lichti, R. Townsend, J. Milbrandt. *Washington University in St Louis School of Medicine, Saint Louis, MO,*

Nicotinamide mononucleotide (NMN) is synthesized from nicotinamide (Nam) and phosphoribosyl pyrophosphate (PRPP) in the rate limiting step of the NAD salvage pathway. The biosynthesis is catalyzed by nicotinamide phosphoribosyltransferase (Nampt). Discovery of an extra-cellular circulating form of Nampt (eNampt) has opened up a novel field of systemic NAD biology. Increased concentrations of plasma eNampt have been reported to be associated with many human disease processes; however, little is

known about the corresponding plasma NMN and Nam concentrations in these disorders. Here we describe a robust, novel and sensitive method for the measurement of Nam and NMN in plasma using liquid chromatography-mass spectrometry (LC-MS) with multiple reaction monitoring. To lay the foundation for measurement of these analytes in the clinical lab an assay was developed and validated according to FDA guidelines for small molecules which included determination of the following: sensitivity, accuracy, precision, reproducibility, matrix effects, and stability. Data presented herein report an LOD for aqueous standards of NMN and Nam at 0.025 pmol (1.0 nmol/L) and 0.2 pmol (8 nmol/L), respectively with linear responses (R2 = 0.9932 for Nam and R2 = 0.9927 for NMN) over 3 orders of magnitude. Based on initial measurements of Nam and NMN in murine plasma an operating range of 0.2-20 pmol for Nam and 0.05-5.0 pmol for NMN was chosen. QC samples covering this range demonstrate acceptable performance (%RE and %CV less than or equal to 20%). NMN and Nam calibrators ran over three consecutive days show good reproducibility (not statistically different by ANOVA). Plasma samples spiked with calibrators demonstrated a linear response (R2 =0.99). Mean percent recovery for Nam was 57% and for NMN 110%. End spike experiments yielded Matrix Factors (MF) for Nam of 0.76 and NMN of 1.42. Aqueous standards were found to be stable over a 5 h analytical run time with %CV 2-15. Collectively, these results demonstrate a method to measure Nam and NMN in plasma. Future plans include measurement of Nam and NMN in patient samples to 1) establish a reference range for circulating Nam and NMN and 2) to test the clinical utility of circulating Nam and NMN in a variety of human diseases. Moreover studies indicate this method to be amenable to other samples including cell culture extracts, which will be useful for understanding the regulatory network of NAD signaling pathways.

C-150

Determination of trace elements concentrations in serum samples by neutron activation analysis from a healthy elderly population of São Paulo city, Brazil.

N. M. Sumita[1], M. E. Mendes[2], O. Jaluul[3], W. Jacob Filho[3], M. Saiki[4].
[1]Central Laboratory Division & Laboratories of Medical Investigation (LIM-03) of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) and Fleury Medicina e Saúde, São Paulo, Brazil, [2]Central Laboratory Division & Laboratories of Medical Investigation (LIM-03) of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil, [3]Clinical Geriatric Discipline of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil, [4]Instituto de Pesquisas Energéticas e Nucleares (IPEN), Neutron Activation Analysis Laboratory, São Paulo, Brazil,

Introduction: The trace elements perform functions indispensable to maintenance of life, growth, and reproduction. Inadequate concentrations may impair cellular and physiological function and often cause illness.
Objective: The aim of this work was to evaluate some trace elements concentrations in serum samples from healthy elderly people attended at the Clinical Hospital of Medicine School of São Paulo University.
Patients and methods: The Ethic Committee at the Medicine School of São Paulo University approved this research and, the selection of the individuals was based on the SENIEUR protocol (*SENIor EURopean Protocol*). The samples of 137 elderly people, 43 men aging 74±8 years and 94 women aging 72±8 years, without significant cronic diseases, were analyzed. The blood, after 12 hours fast, was collected through the venipuncture using sterile metallic needle. The blood was collected in the evacuated (Vacutainer Systems - Becton Dickinson, EUA), without heparin, specific for trace elements analysis. An aliquot of the serum (3.0 mL) was transferred to a flask (Nalgene) using a micropipet. The neutron activation analysis (NAA) were performed using about 200 mg of sample weighed in clean polyethylene bags, and irradiated at the IEA-R1 research nuclear reactor together elemental standards. Short irradiations of 30s for Cl, and Na determinations were carried out by using a pneumatic transfer system facility under a thermal neutron flux of 1.4×10^{12} n cm^{-2} s^{-1}. Longer irradiations of 16 hours under a thermal neutron flux of about 5×10^{12} n cm^{-2} s^{-1} were performed for Br, Fe, Rb, Se and Zn determinations. After adequate decay times, the irradiated samples and standards were measured using a hyperpure Ge detector Model GX2020 coupled to Model 1510 Integrated Signal Processor, both from Canberra. Each sample and standards were measured at least twice for different decay times. Counting times from 200 to 50 000 seconds were used, depending on the half-lives or activities of the radioisotopes considered. The radioisotopes measured were identified according to their half-lives and gamma-ray energies. The concentrations of elements were calculated by comparative method. The certified reference material, NIST 1566b Oyster Tissue was analyzed for the evaluation of the accuracy and precision of the method.
Results: The mean concentration values obtained by NAA for men were: Br: 3.00± 0.67 mg/L, Fe: 134.13 ± 33.82 µg/L, Rb: 332.57± 67.70 µg/L, Se: 66.69 ± 16.05 µg/L and Zn: 94.19 ± 14.27 µg/L. The mean concentration values obtained by NAA for women were: Br: 3.57± 0.85 mg/L, Fe: 108.97 ± 27.68 µg/L, Rb: 318.20± 51.57 µg/L, Se: 79.44 ± 26.53 µg/L and Zn: 95.97 ± 14.55 µg/L.
Conclusion: This group of eldery population did not present any deficiency of trace elements analysed. There was no statistical difference among all trace elements concentrations between men and women.

C-151

Measurement of Vitamin D Using the IDS-iSYS: A New Co-Specific 25-OH D_2 and 25-OH D_3 Assay

L. Bage[1], C. Arrowsmith[1], A. Barnes[1], M. Garrity[1], B. Larsson[2].
[1]Immunodiagnostic Systems Ltd., Boldon, United Kingdom, [2]Laboratory Medicine Skåne, Malmö, Sweden,

The purpose of this study is to assess the co-specificity and general assay performance of the 25-Hydroxy Vitamin D (25-OH D) assay on the IDS-iSYS. The IDS-iSYS is a closed, fully automated random access system that will enable clinical laboratories to determine 25-OH D and other analytes from a single specimen tube. The measurement of Vitamin D is becoming more widely used as part of a panel of tests for a variety of conditions including bone diseases, autoimmune diseases, cancer, diabetes, heart & circulatory diseases, and nervous system disorders. The importance of being able to measure both 25-OH D_2 and 25-OH D_3 is evident as both are available as supplements to improve Vitamin D status. Exclusive measurement of 25-OH D_3 can lead to a patient's Vitamin D status being underestimated, leading to unnecessary supplementation and further expensive testing.
The measurement of 25-OH D on the IDS-iSYS analyser is performed as a competitive chemiluminescent immunoassay. The assay includes an on-board sample pre-treatment step to denature the Vitamin D Binding Protein (VDBP), followed by neutralisation and the addition of a specific anti 25-OH D antibody labelled to acridinium. After a further incubation, 25-OH D coated magnetic particles are added; these coated particles compete with the endogenous 25-OH D for the antibody-acridinium. The magnetic particles are 'captured' using a magnet and a wash step is performed. Addition of trigger reagents produces a signal in Relative Light Units (RLU) which is quantified using a luminometer, with a time to first result of 38 minutes. RLU signal is inversely proportional to the concentration of 25-OH D present in the sample.
The IDS-iSYS 25-OH D assay has a reportable range of 5-140ng/mL. The analytical sensitivity for this assay is 1.8ng/mL and Limit of Quantification (LoQ) is 5.5ng/mL. Precision within run and within device (NCCLS-EP5-A2*)* determined using a low sample (9.7ng/mL) gave a CV between 8.2% and 8.6%, and with a high sample (133.1ng/mL) a CV between 6.1% and 7.8% was observed. Linearity performed by diluting a high sample with a low sample gave a mean observed/expected (O/E) of 103.9%, and a high sample with zero calibrator buffer gave a mean O/E of 100.6%. The specificity was assessed using the 50% binding of B_0, and was calculated as 100% for both 25-OH D_3 and 25-OH D_2. Correlation to RIA with serum and plasma samples (n = 223) gave an r value of 0.95, with an intercept of -3.1ng/mL and a slope of 1.0. Correlation to LC/MS Malmö (n = 120) including some samples with a high proportion of 25-OH D_2 gave an r value of 0.87, with an intercept of 1.4 and a slope of 1.0.
This assay is the first of a comprehensive bone panel on the IDS-iSYS analyser that will be essential for the clinical laboratory. The assay can provide a rapid and accurate automated measurement of 25-OH D_2 and 25-OH D_3 with excellent correlation to existing globally recognised assays, in conjunction with good linearity, sensitivity and precision.

C-152

Development and Validation of a Gas Chromatography-Mass Spectrometry Method for the Analysis of 3-o-methylglucose in Serum and Urine.

J. Rood, F. Xu, V. Hymel, B. Toth, J. Schwarz. *Pennington Biomedical Research Center, Baton Rouge, LA,*

Background: 3-o-methylglucose (3OMG) is a non-metabolizable glucose analogue that is used as a marker to assess glucose transport. 3OMG is absorbed similarly to glucose but is not phosphorylated. Following an oral dose of 3OMG, it's measurement in blood and urine gives an index of active intestinal transport. The objective of this study was to develop and validate a GC/MS method for the analysis of 3OMG in human serum and urine.
Methods: 3OMG is extracted from 200 uL of human serum or urine using acetone. Samples are then combined with 5% hydroxylamine hydrochloride in pyridine, and heated. Immediately prior to analysis, acetic anhydride is added to complete the final step in preparing an aldonitrile derivative. Samples are analyzed on an Agilent 7890 gas chromatograph using an HP5-MS column with mass spectrometry detection using the positive chemical ionization mode. An isotopically labeled compound (U ^{13}C glucose) is used as an internal standard (IS). Retention times for 3OMG and the IS are 5.60 and 5.72 minutes respectively.

Results: The assay is linear from 25-400 ug/mL and the standard curve is generated using the following quadratic equation: $y=ax^2 + bx +c$. The standard curve consistently yielded an r^2 value of 0.999. Within run precision is ≤ 1.68% and between run precision is ≤ 6.5%. Recovery averaged 98.7%. Samples are stable for 96 hours at room temperature and there was no significant decay in samples that were subjected to 3 freeze-thaw cycles. Samples which were higher in concentration than the highest standard could be diluted up to 10 fold with acceptable results.
Conclusions: This method is a sensitive and accurate measure of 3OMG in human serum and urine samples. Measurement of glucose transport can easily be examined using this novel approach.

C-153

Tandem mass spectrometry, involving no-extraction, method for the measurement of 25-hydroxy vitamin D2 and D3
U. Garg, A. Munar, D. Scott, C. C. Frazee. *Children's Mercy Hospital, Kansas City, MO,*

Background: The major biologic function of vitamin D is to maintain normal blood levels of calcium and phosphorus. In addition to its well known role in bone health, vitamin D plays important roles in physiology and pathophysiology of many other body functions. Vitamin D role has been implicated in diabetes, cardiovascular diseases, cancer, multiple sclerosis and immune system. It is now well established that vitamin D deficiency is a common problem in the general population. The best markers for vitamin D nutritional deficiency are vitamin D metabolites 25-OH vitamin D2 and D3. In recent years due to increase in awareness of vitamin D deficiency, clinical laboratories have seen significant increase in the testing of 25-OH-vitamin D2 and D3. One of the commonly used methods for the measurement of 25-OH-vitamin D2 and D3 is tandem mass spectrometry (MS-MS). Current MS-MS methods for the measurement of 25-OH-vitamin D2 and D3 involve liquid-liquid or solid-phase extractions. We describe a MS-MS method for the measurement of vitamin D metabolites which involves no extraction.
Method: To 200 µL serum (blood collected in plain red top tube), 200 µL of acetonitrile containing 100 ng/mL estriol and 50 ng/mL deuterated internal standard 25-OH- vitamin D3 (26, 26, 26, 27, 27, 27-d6) was added. The samples were vortexed and left at room temperature for 10 minutes. The samples were centrifuged at 10,000 g for 10 minutes. The supernatants were transferred to autosampler vials. The analysis involved injection of 30 µL supernatants, 5 cm x 46 mm 5 µm C18 column, Shimadzu UFLC and Applied Biosystems 4000 QTrap MS-MS. Atmospheric pressure chemical ionization (APCI) and multiple reactions monitoring (MRM) mode were used for MS-MS analysis. MRM transitions were: 25-OH vitamin D2 (395.5/377.5 and 395.5/269.5), 25-OH vitamin D3 (383.4/257.3 and 383.4/365.5) and 25-OH vitamin D3-d6 (389.3/211.5). Calibrators were prepared in 7% BSA in phosphate buffered saline and ranged from 4.6 to 74 ng/mL for 25-OH-vitamin D3 and 3.9 to 62 ng/mL for 25-OH-vitamin D2. UTAK quality controls were performed with each run.
Results and Conclusions: The method was evaluated for reportable range, accuracy, within and between run imprecision and limit of quantification. Reportable range (linearity) of the method was from 2- 120 ng/mL for 25-OH vitamin D2 and D3 with recovery of ~100%. Between run imprecision of 25-OH vitamin D3 for 11 and 65 ng/mL controls were 7 and 6% respectively. Within run imprecision was <5%. Limit of quantification (inaccuracy of <20% of target value) was 2 ng/mL. Method comparison using 81 samples with a reference laboratory using LC-MS-MS showed a regression equation of y = 0.97x - 0.19. Ion suppression, as judged by comparing the peak areas of internal standard of precipitating reagent with that of samples, was <10%. In conclusion we present a, direct no-extraction, LC-MS-MS method for the quantification of 25-hydroxy vitamin D2 and D3. The method has clinically acceptable reportable range, accuracy, imprecision and limit of quantification.

C-154

Low serum vitamin D is associated with AMI and inflammatory and molecular markers
L. G. L. Neto[1], R. D. C. Hirata[2], A. Largura[3], A. D. Luchessi[2], V. N. Silbiger[4], D. Cavichioli[4], M. F. Sampaio[5], D. Armaganijan[5], E. B. Sousa[6], S. R. S. Sprovieri[6], S. Q. Doi[7], N. Y. Nguyen[1], A. A. Rezende[8], E. S. dos Santos[5], M. P. Pereira[5], A. G. M. R. Sousa[5], M. H. Hirata[4]. *[1]University of São Paulo, São Paulo, Brazil, [2]University of Sao Paulo, São Paulo, Brazil, [3]Laboratorio Alvaro, Cascavel, Brazil, [4]University of Sao Paulo, Sao Paulo, Brazil, [5]Instituto Dante Pazzanese de Cardiologia, Sao Paulo, Brazil, [6]Irmandade da Santa Casa de Misericordia de Sao Paulo, Sao Paulo, Brazil, [7]Uniformed Services University of the Health Sciences, Bethesda, MD, [8]Universidade Federal do Rio Grande do Norte, Natal, Brazil,*

Background: Increased inflammatory markers have been associated with risk for cardiovascular diseases. Vitamin D deficiency has been found in inflammatory conditions and it may play an important role in the early events of acute myocardial infarction (AMI). The aim of this study was to investigate the relationship of the vitamin D status with mRNA expression of TLR2, TLR4, IL-6 and TNF-alpha in peripheral blood leukocytes (PBL) and inflammatory and biochemical markers in AMI patients.
Methods: One-hundred AMI patients and 52 non-AMI individuals (Control group), aged 32 to 73 years, were selected at the emergency room of the Instituto Dante Pazzanese de Cardiologia, Sao Paulo, Brazil. Peripheral blood samples were collected to measure inflammatory (IL-6, CRPus, fibrinogen), glucose, lipids, apoAI, apoB and 25-hydroxyvitamin D (vitamin D) levels. Biochemical analytes were measured by standard methods using Hitachi 912 System and CRPus and IL-6 were determined by immunoassyas. Serum vitamin D was measured by a chemiluminescence immunoassay method (DiaSorin Stillwater, MN, USA). RNA was extracted from PBL using a QiaAmp RNA Blood Mini Kit® (Qiagen, Germany). TLR2, TLR4, IL6 and TNF-alpha mRNA expression was measured by real-time PCR using *GAPD* as reference gene.
Results: Individual with AMI showed lower vitamin D levels (32.25±17.5nmol/L) than Control group (52.75±28.25nmol/L, $p<0.001$). Individuals with vitamin D lower than (75nmol/L) had 15 timer higher risk for AMI (OR:15.07; CI:1.8-126.1, $p=0.026$) than those with higher vitamin D values. Vitamin D concentrations were negatively correlated with serum CRPus ($r=-0.233$, $p=0.0271$) but not with other inflammatory markers (IL-6: $r=-0.171$, $p=0.170$; fibrinogen: $r=-0.138$, $p=0.315$) in AMI patients. Moreover, vitamin D levels were negatively correlated with TLR4 mRNA expression ($r=-0.374$, $p=0.00213$) in PBL. On the other hand, no correlations between vitamin D and inflammatory or biochemical markers were found in control group ($p>0.05$).
Conclusion: The relationship between serum vitamin D and inflammatory and molecular markers in AMI patients is suggestive that this vitamin plays an important role in cardiovascular diseases, and its deficiency seems to be an important risk factor for AMI.

C-155

Development of the ADVIA Centaur® Systems Vitamin D Total Assay
J. Freeman[1], S. Jafary[1], B. Bossen[1], S. Lin[1], Y. Ken[1], R. Schnaidt[1], R. Evangelista[2], N. Sahakian[2], Q. Liao[2], M. Robles[2]. *[1]Siemens Healthcare, Tarrytown, NY, [2]Siemens Healthcare, Los Angeles, CA,*

An assay* for total vitamin D (25-hydroxyvitamin D) with a range up to 250 ng/mL that measures both 25(OH)D2 and 25(OH)D3 on the ADVIA Centaur systems is being developed by Siemens Healthcare Diagnostics.
Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. Vitamin D is essential for the formation and maintenance of strong, healthy bones.
Vitamin D deficiency can result from inadequate exposure to the sun, inadequate alimentary intake, decreased absorption, abnormal metabolism, or vitamin D resistance. Recently, many chronic diseases such as cancer, high blood pressure, osteoporosis, and several autoimmune diseases have been linked to vitamin D deficiency. Whether consumed or produced, both forms of the vitamin (D2 and D3) are metabolized by the liver to 25(OH)D and then converted in the liver or kidney into 1,25-dihydroxyvitamin D. Vitamin D metabolites are bound to a carrier protein in the plasma and distributed throughout the body. It is generally accepted that 25(OH)D is the metabolite that is the most reliable clinical indicator of vitamin D status because serum 25(OH)D levels reflect the body's storage levels of vitamin D and correlate with the clinical symptoms of vitamin D deficiency.
The Siemens ADVIA Centaur Vitamin D Total assay design is based on a sequential competitive immunoassay format. Sample is added to the reaction cuvette followed by displacement buffer and allowed to react for 4.5 minutes. Monoclonal antibody conjugated to acridinium ester is added and allowed to react for 5.5 minutes to bind 25(OH) vitamin D in the sample. A 25(OH) vitamin D analog conjugated to bovine serum albumin and

fluorescein is added along with antifluorescein-coated paramagnetic particles and allowed to react for 3.75 minutes. The reaction cuvette is washed, and acid and base reagents are added to initiate the chemiluminescent reaction. The time-to-result is 18 minutes. An inverse relationship exists between the amount of 25(OH) vitamin D in the patient sample and the amount of relative light units (RLUs) detected by the system.

The data obtained with the ADVIA Centaur Vitamin D Total assay demonstrated equimolar detection of 25(OH)D2 and 25(OH)D3 and showed traceability to LC-MS/MS. Cross-reactivity to 25(OH)D2 was determined to be 105% at 50 ng/mL. The assay demonstrated a limit of detection (LoD) of less than 3.0 ng/mL, a functional sensitivity (20% dose total CV) of less than 4ng/mL, and an upper limit of 250 ng/mL. Total assay CVs were 6.4%, 7.1%, 4.2%, and 3.7% for samples at 22.1, 52.3, 121, and 153 ng/mL, respectively. Linearity up to 240 ng/mL was demonstrated. A correlation study against LC-MS/MS was performed with 150 serum samples, yielding a slope of 0.96, intercept of 1.0, and regression coefficient of 0.97.

The Siemens ADVIA Centaur Vitamin D Total assay may be a valuable tool in clinical laboratories for the accurate measurement of vitamin D deficiency in human sera.

* For investigational use only. The performance characteristics of this product have not been established.

C-156

Relationship of Functional T436K Polymorphism in Vitamin D Binding Protein (DBP) with Serum 25-hydroxyvitamin D [25(OH)D], DBP Concentration and Binding Capacity: A Pilot Study

L. Fu[1], C. Cade[2], S. S. Holm[3], T. Just[3], F. Yun[4], B. Y. L. Wong[2], M. Li[2], R. Vieth[5], D. E. C. Cole[6]. *[1]Department of Clinical Pathology, Sunnybrook Health Sciences Centre; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, [2]Department of Clinical Pathology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada, [3]Dako Denmark A/S, Glostrup, Denmark, [4]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, [5]Department of Laboratory Medicine and Pathobiology, University of Toronto; Department of Pathology and laboratory Medicine, Mount Sinai Hospital, Toronto, ON, Canada, [6]Department of Clinical Pathology, Sunnybrook Health Sciences Centre; Departments of Laboratory Medicine and Pathobiology, Pediatrics (Genetics), Medicine, University of Toronto, Toronto, ON, Canada,*

Introduction: DBP is a multifunctional and highly polymorphic serum protein synthesized by the liver. We have previously reported that a functional T436K SNP in the DBP gene predicts serum 25(OH)D.

Objectives: To assess the correlation between DBP concentrations and binding capacity and their association with T436K genotypes at baseline and after one year high-dose (4000IU/d) vitamin D supplementation.

Methods: Subjects were selected from a cohort who received high-dose vitamin D supplement for one year. Serum 25(OH)D was measured by standard RIA, serum DBP concentration was measured by an immunoturbidimetric assay, and the T436K SNP by sequence-specific PCR-RFLP.

Results: Mean baseline 25(OH)D (±SD) in all subjects (54 ± 6 yr) was 61 ± 24 nmol/L. Subjects with the 436KK homozygous genotype had a significantly lower 25(OH)D (36 ± 7 nmol/L) than TK heterozygotes (65 ± 22 nmol/L) or TT wild type (70 ± 24 nmol/L, $p < 0.02$, linear ANOVA); as well as the lowest DBP concentration (318 ± 23 mg/L) than heterozygotes (370 ± 58 mg/L) and wild type (413 ± 59 mg/L, $p = 0.01$, linear ANOVA). There was a significant correlation between DBP concentration and binding capacity at baseline and after one-year supplement ($p < 0.005$). However, there was no significant change in mean DBP concentration between baseline and one-year, despite the substantial and significant increase of 25(OH)D (mean 261%).

Conclusion: T436K was significantly associated with both serum 25OHD and DBP concentrations at baseline. This gene association and its physiological significance in response to vitamin D loading deserve further study and confirmation in large controlled cohorts.

C-157

Comparison of methods for measurement of 25-hydroxy-vitamin D

J. E. Whittington[1], M. M. Kushnir[2], J. A. Ray[2], P. Middleton[2], R. J. Singh[3], A. L. Rockwood[1], A. W. Meikle[1]. *[1]University of Utah School of Medicine, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]Mayo Clinic, Rochester, MN,*

Background: Vitamin D deficiency results in impaired formation of bones, rickets in children and osteomalacia in adults. Poor agreement between radioimmunoassays (RIA), chemiluminescent immunoassays (CIA), and liquid chromatography tandem mass spectrometry assays (LC-MS/MS) measuring 25-hydroxy-vitamin D (VitD) has been reported. *Objective:* To compare all three VitD assay methods, including three reference laboratories' LC-MS/MS methods. Seasonal variation was considered in the comparison. *Methods:* A total of 150 patient samples were collected in the winter (2008, N=30 and 2009, N=20) and summer (2009, N=100), deidentified, and 5 aliquots of each were made for use in method comparison. VitD concentrations were determined, ranging from 5-142 ng/mL, by laboratory A using RIA, CIA, and LC-MS/MS assays; and measured by laboratories B and C using LC-MS/MS VitD assays. *Results:* All results were compared to the LC-MS/MS VitD assay results of laboratory A (*see table*). Deming regression analysis gave a range of slopes (0.45 to 1.07) and correlation coefficients (r, 0.822 to 0.988). The CIA method used in Lab A showed marked improvement from winter of 2008 to summer/winter of 2009. Simultaneously, a marked improvement in the overall performance of RIA was observed over the same time period. Improvements may be due to differences in the sample sets being tested or due to small changes made by the manufacturer of the commercially available assays. In addition to patient samples, NIST standard reference material (972) was tested by CIA and LC-MS/MS and measured within 10% of the expected concentration of VitD. *Conclusion:* Overall, a negative bias is observed when RIA is compared to CIA and LC-MS/MS methods. The CIA method is in reasonable agreement with LC-MS/MS VitD assays. The introduction of NIST material, specifically level 1 made using human serum, provides a great measure of accuracy in evaluating VitD assays.

Method Comparison of VitD Assays by Deming Regression Analysis

	Deming regression statistics	Winter 2008 (N=30)	Summer 2009 (N=97)	Winter 2009 (N=20)	2008-2009 (N=147)
Lab A, RIA	**slope**	**0.45**	**0.70**	**0.90**	**0.72**
	intercept	4.8	4.6	-1.9	4.0
	r	0.826	0.936	0.974	0.939
Lab A, CIA	**slope**	**0.78**	**0.99**	**0.97**	**0.99**
	intercept	-0.1	-1.2	-1.8	-1.3
	r	0.884	0.822	0.960	0.839
Lab B, LC-MS/MS	**slope**	**0.93**	**0.93**		**0.93**
	intercept	-2.3	1.4		1.5
	r	0.980	0.988		0.988
Lab C, LC-MS/MS	**slope**	**1.07**			
	intercept	-2.9			
	r	0.963			

C-158

Quantification of serum methylmalonic acid by liquid chromatography-mass spectrometry

V. Gatautis, A. Hughes, S. Wang. *Cleveland Clinic, Cleveland, OH,*

BACKGROUND Serum methylmalonic acid (MMA) is clinically measured for macrocytic anemia workup. OBJECTIVE To develop and validate a liquid chromatography-mass spectrometry (LC-MS) method to quantify MMA in serum.

METHODS 500 µL serum was mixed with 25 µL internal standard (d^3-MMA, 0.2 mmol/L in 20 mmol/L sodium bicarbonate) and 500 µL water. The resulted mixture was loaded on a solid phase extraction column (Bond Elute SAX, Varian, Lake Forest, CA). After washed with distilled water, methanol, and formic acid (10 mol/L), MMA and d^3-MMA were eluted with 125 µL formic acid (18 mol/L in water). 3 µL of the eluate was injected onto a Symmetry analytical column (2.1 x 50 mm, Waters Corporation, Milford, MA) in a Agilent 1100 LC system coupled with MSD single quadrupole, (Agilent, Santa Clara CA). The column was eluted at 0.25 mL/min with an isocratic mobile phase composed of 3% methanol and 97% formic acid solution (1.0% in water). The mass spectrometer was set at negative atmospheric pressure chemical ionization and selective ion monitoring mode. Quantification was based on peak area ratios of MMA (m/z 117) to internal standard (m/z 120).

RESULTS The analytical cycle time was 4 minutes per injection. Linearity and analytical measurable range were assessed by triplicate serial dilution in phosphate buffered saline and found to be 44.4-23022.7 nmol/L with analytical recovery ranging from 88.7-115.1% and coefficient of variation ranging from 0.59-8.26%. No significant carryover was observed from samples with concentration of 200,000 nmol/L. Succinic acid, which has the same molecular weight, had a baseline resolution from MMA.

CONCLUSIONS We have developed a reliable LC-MS method for quantification of MMA with a simple sample preparation and free of interference.

C-159

Effect Of Ginkgo biloba extract On Selected Markers Of Oxidative Stress

A. L. Welch, G. B. Kudolo. *University of Texas Health Science Center at San Antonio, San Antonio, TX,*

There is increasing evidence that oxidative stress (OS) may lead to development of chronic diseases such as diabetes mellitus and its complications. Antioxidant therapies, such as using large doses of vitamins C and E are now common. The specific aim of this pilot study was to determine if the ingestion of *Ginkgo biloba* extract will result in reduced levels of the markers of oxidative stress in plasma (malondialdehyde, MDA) and urine (8-Isoprostane, 8-iso-PGF-2-alpha) and whether the ingestion will lead to an increase in the total antioxidant capacity of the individual. Twelve healthy non-diabetic volunteers (confirmed with the 2-hour oral gucose tolerance test) underwent a double-blind placebo-controlled crossover study, in which the subject ingested either Ginkgo (120 mg/day) or a vegetable-based placebo for each 3-month arm. At the end of each arm, plasma and 24-hour urine specimens were collected stored at -86 C until measurements of the analytes. MDA was measured in the plasma using a modification of the TBAR assay for the microtiter plate reader (530 nm wavelength) and 8-isoprostane and total antioxidants were measured using commercially available kits (EIA, Assay Designs, Inc., Ann Arbor, MI). Paired t-tests were performed and $p<0.05$ was considered significant.

Results:	Subjects, N = 12 (M/F; 4/8)	
Values=mean±SD (range)	PLACEBO	GINKGO
Age (years)	44±9	
Compliance (%)	85±15	87±16
Body Mass Index (kg/m^2)	28.9±5.2	29.3±5.3
Blood pressure (Systolic, mm Hg)	126±14	121±10
Blood pressure (Diastolic, mm Hg)	75±12	73±8
Fibrinogen (mg/dl)	320±80	306±37
PT (sec)	13.3±0.6	13.3±0.7
PTT (sec)	30.7±3.1	29.9±0.8
Fasting glucose (mg/dl)	93±13	93±8
Fasting insulin (µU/ml)	5±5	9±1
Fasting C-peptide (ng/ml)	3.9±2.5	3.4±1.5
Total cholesterol (mg/dl)	187±46	187±41
Triglycerides (mg/dl)	130±143	129±118
HDL-cholesterol (mg/dl)	48±7	50±9
LDL-cholesterol (mg/dl)	118±33	113±29
Malondialdehyde, MDA (uM)	4.6±1.0	4.3±0.6
8-iso-PGF-2-alpha (ug/mg creatinine)	0.30±0.13	0.43±0.34
Total Antioxidant Capacity (mM)	0.52±0.04	0.55±0.05

Conclusion: Unlike the total antioxidant capacity, the ingestion of *Ginkgo biloba* extract did not significantly alter the plasma MDA or urinary 8-Isoprostane production. This may be due to the choice of markers - both MDA and 8-isoprostane are metabolic products of arachidonic acid, located in the plasma membranes. The appropriate choice of OS markers is thus very important and may explain the inconsistencies in the OS field.

Wednesday PM, July 28

Poster Session: 2:00 pm – 4:30 pm
Point-of-Care Testing

D-01

How Useful is Point-of-Care Testing [POCT] for INR?

C. Puza[1], D. Lantz[2], E. S. Pearlman[3]. *[1]South Boston Community Health Center [SBCHC], Boston, MA, [2]East Boston Neighborhood Health Center (EBNHC), Boston, MA, [3]Boston Medical Center, Boston, MA,*

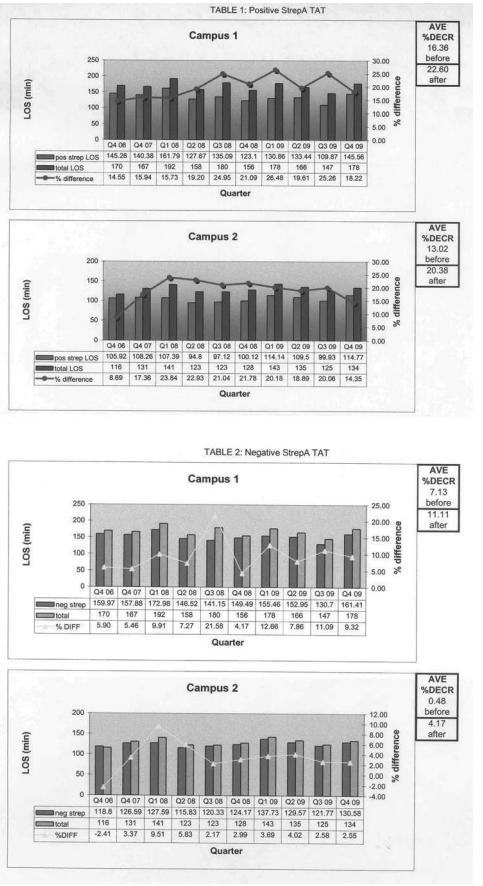
Coumadin Clinics (CCs) attempt to enhance the efficacy of oral anticoagulant therapy (OAT) through patient education, increased patient responsibility for his/her own health care and intensification of monitoring using the INR. Vendors of POC equipment and reagents for determination of the INR argue that the POC approach enhances the quality of care for patients on OAT by encouraging more frequent testing and providing "instant" result feedback to patients and providers. We attempted to assess the validity of these claims by examining data from two CCs one of which used POCT for INR assay while the other determined INR in the central lab. . A CC was begun at SBCHC in late November, 2008. Allowing a four month recruitment and learning curve period data from 51 patients who participated in the CC between April 1 and July 31 2009 and had more than one INR determination in the electronic medical record [EMR] was analyzed Of these 22 had data from the entire period, 17 from 3 months and the remainder from 2 months. INRs were determined according to manufacturer's instructions on a Sysmex-530 coagulation analyzer [Siemens; Newark, DE] using a thromboplastin reagent with a manufacturer's rated ISI of close to 1.0. The SBCHC group was 56.9% female with a median age of 67 YO. The CC situated at EBNHC was larger and older than that at SBCHC and had used POCT for INR determination starting in November, 2006. In the four month period under consideration there were 195 CC patients with more than one INR determination in the EMR. INR determinations were performed using a Coaguchek POC system (Roche Diagnostics; Indianapolis, IN). Based on a randomly chosen sample (n=51) from the CC population at EBNHC, the median age was 60 YO and the group was 35.3% female. The EBNHC group was significantly less female (z score = 2.19; p<.015) than the SBCHC group but the difference in median ages did not reach statistical significance (median test; p=.067). **SBCHC copied the protocols from EBNHC in starting up its' CC with the exception that the latter used POCT while SBCHC did INR assays in the central lab.** The median number of INR determinations was 7 in both locations with a range of [2-17] at SBCHC and [2-22] at EBNHC. The proportion of INR results within the therapeutic range (2-3.5) was .564 at EBNHC and .543 at SBCHC (z score = .7; p=NS). Although the comparison took place early in the history of the CC at SBCHC and although the gender composition of the two populations appeared to be different at the two locations, the current data, even given these limitations, does not appear to support the claims that POCT for INR improves the quality of patient care commensurate with the increment in costs associated with POCT.

D-02

Implementation of POC testing in the ED: A study on decresed throughput times for patients being seen for rapid strep testing

K. Halverson[1], D. Milner[2]. *[1]Children's Hospitals & Clinics of Minnesota, Minneapolis, MN, [2]Children's Hospitals & Clinics of Minnesota, St. Paul, MN,*

OBJECTIVE: To determine if implementation of Point Of Care Testing (POCT) Rapid StrepA Test in a pediatric Emergency Dept by nursing staff will lead to increase in patient throughput. Secondary benefits: Anticipate that improving throughput times will benefit patient care by improving safety with prompt diagnosis and treatment, minimize exposure to other ill contacts in the ED, and improve satisfaction by timely service.
METHODOLOGY: Patient throughput will be measured by average length of stay (LOS) in the ED and comparison of LOS for overall ED patients in ratio comparison to those on whom POCT is performed. Those with positive results will be compared to those with negative POCT results to determine overall benefit in throughput for positive test results.
RESULTS: LOS for patients in the emergency room was collected fourth quarter 2006 through fourth quarter 2009. POCT Rapid StrepA testing was implemented in first quarter 2008 in Campus 2 and third quarter 2008 in Campus 1. Table 1 shows average LOS measured in minutes by campus for positive strepA results. Table 2 shows shows average LOS measured in minutes by campus for negative strepA results.
CONCLUSION: Implementation of POCT by nursing and nursing aid staff does improve throughput in the Emergency Department, with a 7.64% decrease in LOS overall for those on whom strepA testing was performed and 21.49% decrease on those patients seen with positive strepA test results.



D-03

Estimating bias and imprecision using patient samples for markers of lipid metabolism using a POCT instrument

O. V. Chernichuk[1], L. A. Khorovskaya[2], A. Kallner[3]. *[1]Khanty-Mansiysk Okrug Clinical Hospital, Khanty-Mansiysk, Russian Federation, [2]Pavlov State Medical University, St.-Petersburg, Russian Federation, [3]Karolinska Hospital, Stockholm, Sweden,*

Objective. Screening, diagnosis and monitoring of cardiovascular disease (CVD) is a practical problem in medicine. Development of instruments for Point Of Care testing (POCT) allows non- laboratory staff to make measurements and supply results for immediate consideration by the clinician. The aim of our present study was to verify the performance of POCT instrument for measurement of Triglycerides-, Cholesterol- and HDL concentrations compared with a reference method.
Method. A POCT instrument CardioChek (Polymer Technology Systems, US) was compared with Synchron 9 (CX-9,Beckman Coulter, US) by measuring 60 paired patient routine sample in duplicates. Samples were chosen to cover the measuring intervals of the quantities. Capillary blood samples in two portions were collected in outpatient department in Khanty-Mansyjsk Okrug Clinical Hospital. The first portion was measured in CardioChek in outpatient department. The second portion was used to prepare serum and measure in CX-9 in core laboratory in the same hospital. Special software was developed to estimate the bias and imprecision from duplicates, make a regression analysis of the results and display the absolute and relative differences between the methods. The statistical significance of a difference between the methods was evaluated by Student's dependent *t*-test. The mean bias is the mean of the differences between each sample result obtained by the two methods. Additionally, the bias was estimated in partitions of concentrations that are clinically significant for each quantity.
Results. Cholesterol- , Triglycerides- and HDL concentrations show no significant difference between the two methods at any concentration (p>0.05) in the measuring interval A more interesting quantity than the statistical significance is the absolute and relative difference (bias) between results. An estimate of the analytical imprecision was based on the difference of the duplicate results. Except for the high third of the cholesterol results the results seem close to homoscedactic and therefore the estimate of the

imprecision is applicable. Thus, the reference change value (RCV) was estimated to 1.95 x sqrt(2) x *sd* indicating the least significant difference between results. The coefficient of determination was close to 1 except for cholesterol (0.77).
Conclusion. We found no significant difference in the results of the two methods. Results from POCT and reference method were comparable for measurement of studied quantities and the RCV values acceptable for screening and diagnosis. The POCT method could be used for diagnosis and screening of CVD for measurement of lipid metabolism in Primary Health Care.

D-04

Accu-Chek Performa system with advanced chemistry test-strips: maltose interference eliminated?

W. Y. Ng, C. C. Tiong, E. Jacob. *Singapore General Hospital, Singapore, Singapore,*

Background: Maltose interference with the glucose dehydrogenase pyrroloquinoline-quinone (GDH-PQQ) chemistry has been an unsettling concern with Accu-Chek test-strips. Elevated sugar levels wrongly inferred as glucose have been misinterpreted in renal patients using Extraneal peritoneal dialysis solutions containing icodextrin, a starch-derived maltose polymer. To overcome this limitation, a new modified glucose test-strip (with recombinant GDH-PQQ) has been introduced for the Accu-Chek Performa system. This report is a performance evaluation of the modified test-strips and the effect of interfering sugars - maltose, xylose and galactose.
Methods: Parameters evaluated include imprecision, limit of detection, linearity and a comparison with the laboratory method (Roche MODULAR-glucose oxidase). Venous whole blood specimens (from ICU/ED) for routine glucose testing were immediately determined with the test-strips prior to laboratory testing. Imprecision was determined with control solutions (daily triplicate testing over 5 days - within/total CV) and whole blood (tested repeatedly 5 times - within CV). Meter-to-meter reproducibility was evaluated with three meters by testing blood specimens simultaneously. For specificity studies, heparin-blood specimens (2.0 and 5.0 mmol/L glucose) were spiked with various levels of maltose, xylose and galactose (up to 500 mg/dL) followed by testing with modified Performa, Advantage II, Accutrend glucose test-strips and laboratory method.
Results: Glucose test imprecisions were typically <7% (total CV); within-run CVs were 3.8-4.1% at 2.4, 2.5 mmol/L and 2.7-4.3% at 16.3, 16.5 mmol/L using controls and two test-strip lots (#370054, #370071), and 2.8-5.4% at 4.4, 11.0, 17.0 and 19.7 mmol/L with whole blood (strip lot #370071). Linearity and limits of detection were verified with blood samples - 0.6-29.1 mmol/L and 0.8 mmol/L respectively. The comparison with laboratory results achieved 0.960 correlation (Spearman's r^2) with a Deming regression y=0.95x - 0.11 from 0.8-27.6 mmol/L (n=120; SEy|x 0.06 mmol/L). The Bland-Altman plot produced a slight bias of -0.5 mmol/L (95%CI= -0.41 to -0.67 mmol/L). Clarke error grid analysis showed no values outside critical clinical limits. The ANOVA test for variance between three meters' glucose results from 40 samples (mean 10.4 mmol/L; range 0.6-30.6 mmol/L) was insignificant, p=0.98. Tests with added sugars (up to 500 mg/dL for maltose (13.9 mmol/L) and xylose (33.3 mol/L), and 250 mg/dL for galactose (22.2 mmol/L)) showed no significant interference (<20% difference from laboratory) with maltose and xylose. As mentioned in the test-strip inserts, galactose at 1.1-2.2 mmol/L had a significant impact. The Advantage II test-strips began to show increased readings at low levels of the three added sugars but Accutrend test-strips were not affected at all. Under ISO 15197:2003 criteria, 99% of results determined by the modified Performa test-strips were within the minimal acceptable performance. At <=4.1 mmol/L glucose, 0/14 showed >0.83 mmol/L difference from laboratory; at >4.1 mmol/L glucose, only 1/106 had an error >20% difference from laboratory.
Conclusions: The modified and improved specificity Performa test-strips showed no significant interference from maltose and xylose (tested to 500 mg/dL). Close correlation with laboratory results was shown with a slight bias (-0.5mmol/L) across 0.8-27.6 mmol/L glucose levels. The Accu-Chek Performa system with modified test-strips meets ISO 15197:2003 requirements.

D-05

Assessment of the Afinion™ HbA1c Assay in the Diagnosis of Diabetes

T. L. Brett, D. Furtado. *Axis-Shield, Norton, MA,*

Purpose: The purpose of this study is to assess the suitability of the Afinion HbA1c assay as a reliable and accurate method to measure HbA1c in the diagnosis of diabetes. It has been suggested by clinical experts that the ideal marker to measure HbA1c for diagnosis would have CVs of <2%. In reviewing recent studies, this abstract will show that the Afinion HbA1c assay has mean CVs that meet this criteria.
Background: The ADA Standards of Medical Care in Diabetes (2010) recommends the use of HbA1c in the diagnosis and screening of diabetes. Previous concerns have been raised about the quality of both laboratory and point of care HbA1c assays. The introduction of standardization by NGSP and IFCC has resulted in a major improvement in the accuracy and precision of HbA1c assays. That being said, the optimal goal of having all HbA1c assays achieve CVs of <2% is an ambitious one and it has been suggested that an imprecision target of <3% may be more realistic.
Methods: Data from all independent, published Afinion HbA1c studies were analyzed and compared to imprecision goals set by clinical experts.
Results: The mean CVs for low, mid and high patient samples across the studies were 1.62%, 1.61% and 1.33% respectively.

Study Site	Patient Sample		
	Low	Mid	High
Baystate Medical Center, MA	1.8%	1.3%	1.7%
University of Missouri, MO	1.97%	1.79%	1.51%
SKUP, Univ. of Malmo, Sweden	1.1%	1.7%	1.3%
Univ. of Chicago Medical Center, IL		1.2%	1.1%
Ewha Woman's Univ., Rep. of Korea		1.54%	1.06%
University of Milan, Italy		1.0%	
Virgen Macarena Hosp., Spain		1.95%	
European Reference Lab, Netherlands		2.4%	
Siemens Healthcare, MA		1.3% 2.65%	
Sandwell and West Birmingham Hospitals, UK		0.9% 2.0%	
IFCC Standardization Certificate		1.2%	
Mean CVs	1.62%	1.61%	1.33%

Conclusion: The Afinion HbA1c assay shows precision well within the suggested imprecision target of <3%. In addition, the mean CVs fall within the optimal goal of <2% as proposed by clinical experts for the diagnosis and screening of diabetes.

D-06

A Novel Point-of-Care Test for the Simultaneous Detection of Non-Treponemal and Treponemal Antibodies in Patients with Syphilis

A. R. Castro[1], J. Esfandiari[2], S. Kumar[3], M. Ashton[2], S. E. Kikkert[1], M. M. Park[4], R. C. Ballard[1]. *[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]Chembio Diagnostic Systems, Medford, NY, [3]Chembio Diagnosticb Systems, Medford, NY, [4]Georgia Department of Community Health, Atlanta, GA,*

We describe a point-of-care (POC), immunochromatographic test for the detection of both non-treponemal and treponemal antibodies in the sera of patients with syphilis. The assay is based on the principle of a dual path platform (DPP) with two antigens and control material striped onto the surface of a nitrocellulose membrane within the device. The assay is able to detect these two antibodies simultaneously within 20 minutes, therefore acting as both a screening and confirmatory test that can be performed before the patient leaves the clinical venue. A total of 1601 banked serum samples were examined by the dual DPP-POC test and the results compared to those obtained using a quantitative rapid plasma reagin (RPR) test and the Treponema pallidum passive particle agglutination assay (TP-PA). When compared to the RPR test, the overall sensitivity and specificity of the dual DPP-POC nontreponemal line were 88.6% and 98.6% respectively. However, the sensitivity was 98.4% when the RPR titers of sera were ≥1:2. The sensitivity and specificity of the treponemal line were 96.5% and 95.5% when compared to the TP-PA test. Equivalent results were also obtained when sera obtained from 105 patients of known stage of syphilis, 179 sera from patients with conditions other than syphilis and 14 sera known to produce false positive reactions in reagin-based tests were tested by the dual DPP-POC and comparator tests. These results indicate that the dual DPP-POC test could be used for the serological diagnosis of syphilis primarily in primary health care clinics or resource poor settings and therefore improve rates of treatment where patients may fail to return for their laboratory results.

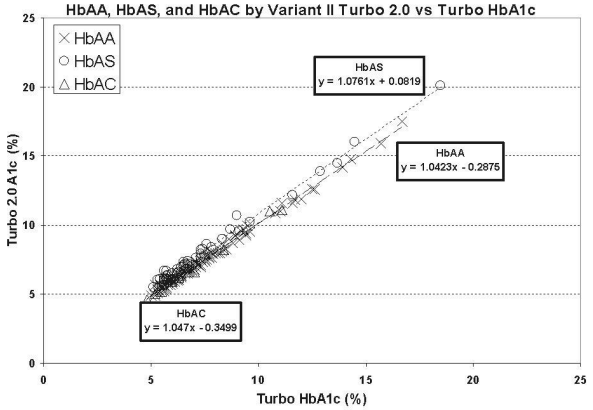
D-07

Variant II Turbo 2.0 and In2it HbA1c Measurements Facilitate Workflow

J. Zajechowski[1], D. Swiderski[2], C. Chmielicki[2], C. Feldkamp[2], J. Carey[2], V. I. Luzzi[2]. *[1]Henry FordHospital, Detroit, MI, [2]Henry Ford Hospital, Detroit, MI,*

Ion-exchange chromatography VariantII Turbo® 2.0 measures HbA1c% in the presence of HbF up to 25% . However, HbAS variants are no longer individually identified. Since HbAS may be present in a large percentage of our patient population we were interested in comparing HbA1c% results with our current method. In addition, we looked into using In2it®, a boronate affinity chromatography point of care device, as an alternative method

to measure HbA1c% in the presence of other unknown variants or when HbF is higher than 25%. To examine the HbA1c results in normal and variant Hb specimens by Variant II Turbo 2.0 and In2it methods, we measured HbA1c% by the Variant II Turbo 2.0, or the In2it methods in normal or variant Hb specimens and compared the results with the Primus® boronate affinity chromatography or the Turbo methods. Within and between run precision, linearity, and reportable ranges for the Turbo 2.0 kit were examined using human whole blood specimens or commercially available control material. EP evaluator or Microsoft Excel was used to analyze the data. Linear regression showed that HbA1c% measured in the presence of HbF using the Variant II Turbo 2.0 or the In2it methods correlated well with the Primus method (Turbo 2.0: r = 0.99; In2it: r =0.97). The Turbo 2.0 kit also correlated well with the Turbo in normal and HbAS or HbAC specimens (figure). Within run precision demonstrated a CV of 0.6 % at 5.7 HbA1c%, and 0.4% at 9.9 HbA1c%. Between run precision was 1.96%, and 1.46%, at 5.7 and 9.9 HbA1c% respectively. The average percent recovery between 3.9 % and 18.8 % was 103 %. The Turbo 2.0 and Turbo methods correlate well even in the presence of Hb variants. The In2it device is also a good complement when measuring HbA1c in the presence of HbF.



D-08

The TearLab - a Lab-on-a-Chip POC Nanoliter Tear Collection and Analysis Platform

M. S. Berg[1], S. Zmina[1], M. Senchyna[2], R. Williams[2], C. G. Chen[3], S. Garst[4], E. C. Harvey[4], J. P. Hayes[4], M. D. Solomon[4], L. Gamble[5], A. Knaack[5], V. Kosheleff[1], M. A. Lemp[1], B. D. Sullivan[1]. *[1]TearLab, Corp., San Diego, CA, [2]Alcon Laboratories, Fort Worth, TX, [3]A*STAR, Data Storage Institute, Singapore, Singapore, [4]MiniFAB, Scoresby, Australia, [5]Invetech, Mt. Waverley, Australia,*

Objective: The use of tear fluid as a matrix for in vitro diagnostics has been largely constrained by the challenges of specimen collection and the technical complexity of nanoliter (nL) assays. The purpose of this current study was to evaluate the performance of the TearLab, a novel lab-on-a-chip platform designed for point-of-care tear osmolarity analysis.

Methods: The analytical accuracy and precision of 50 nL aliquots of NIST traceable sodium chloride solution was established using 32-chip samples from 259 lots (8,288 TearLab chips in total). To establish the repeatability of the relationship between the impedance and osmolarity of tear fluid, two pools of human tear samples were collected from unique sets of 31 people under IRB approval, three months apart. Pools were split into fractions and partially lyophilized to increasing levels of osmolarity, and measured in parallel on a Wescor 5520 Vapro® vapor pressure osmometer and a reference TearLab.

Results: The analytical accuracy of the TearLab chips resulted in a standard deviation of 0.84 mOsms/L, or 0.25% at 338 mOsms/L. Precisions of each lot ranged from 0.4% to 1.5% coefficient of variation. The repeatability of the impedance-osmolarity transfer function used within the TearLab was shown to have a 0.7% difference in slope and 0.2% difference in intercept between two distinct pools of human tears.

Conclusion: These studies have demonstrated the validity of a new impedance-based, microfluidic technology for the direct collection and simultaneous analysis of nanoliter volumes of tear fluid on a lab-on-a-chip platform for use in the point of care.

D-09

Fully Integrated, Automatic, and Rapid Molecular Detection and Identification of 20 Clinically Relevant HPV types using the CARD™ Platform

G. Spizz[1], L. Young[1], G. Mouchka[1], W. Honey[1], Z. Chen[1], D. Mahoney[1], R. A. Montagna[2], Y. Gu[3], P. Zhou[1]. *[1]Rheonix, Inc., Ithaca, NY, [2]Rheonix, Inc., Grand Island, NY, [3]Haidian Maternal & Children Health Hospital, Beijing, China,*

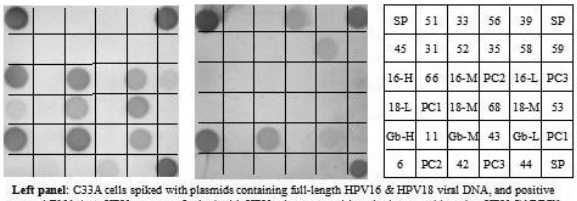
Objective: To rapidly, easily and automatically detect and distinguish at least 20 types of clinically relevant human papilloma virus (HPV) directly from clinical samples on CARD(Chemistry and Reagent Device).

Relevance: Cervical cancer is the leading cause of cancer-related deaths among women in low-income countries and is the second leading cause of cancer-related deaths for women on a worldwide basis. Among currently FDA-approved molecular diagnostics, none are capable of distinguishing the various HPVs other than to classify them as "high" or "low" risk types. Our CARD is capable of detecting and specifically distinguishing 20 HPV types.

Methodology: A vaginal swab is collected in our proprietary transport media which allows for extended room temperature storage, if necessary. An aliquot is applied to the CARD sample reservoir and the run initiated. Without any further intervention by the analyst, all the following steps are automatically performed: cell lysis, nucleic acid purification, PCR amplification and multiplexed end-point detection on a low density microarray.

Validation: Analysis of 69 clinical specimens was directly compared on the HPV CARD and an FDA-approved product. Of those specimens, 48 were found to be HPV negative by both tests, 14 were determined to be HPV positive by both tests, and 7 specimens, while negative on the comparison test, were positive on the HPV CARD. The presence of HPV in the discordant samples was confirmed by amplicon sequencing, thus indicating the accuracy of the CARD method. A representative microarray (Figure 1) demonstrates the ease of detection and the presence of appropriate controls to validate proper assay performance.

Conclusions: The HPV CARD provides a fully automated system for the rapid and reliable molecular detection of 20 clinically relevant HPV types Furthermore, due to the portability of CARD platform, this test has widespread application in both industrialized and developing nations.



Left panel: C33A cells spiked with plasmids containing full-length HPV16 & HPV18 viral DNA, and positive control DNA (non-HPV sequence flanked with HPV primer recognition sites) were subjected to HPV CARD™ assay. Following CARD purification, amplification, and RDB, Globin, HPV 16, HPV 18, and PC are detected as anticipated.

Middle panel: Clinical samples, originally demonstrated to positive for high risk HPV by the FDA approved product, were subjected to HPV CARD assay. Results from CARD assay indicate a mixed infection with high risk types 56 and 58 and demonstrate the presence of the human beta-globin gene used as a control for nucleic acid purification from vaginal/cervical cells.

Right panel: Filter key: *SP*: Spotting Control- biotin labeled poly-A; *PC 1, 2, 3*: Positive control. All probes spotted at 50 uM; Globin (Gb), 16 and 18: spotted at 2 (L), 20 (M), and 50 (H) uM.

D-10

CARD™ Technology for Rapid and Automatic Determination of SNP Profile for Pharmacogenomics

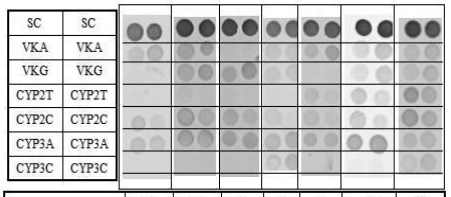
R. Yasmin[1], W. Honey[1], G. Mouchka[1], B. Thomas[1], R. A. Montagna[2], P. Zhou[1], G. Spizz[1]. *[1]Rheonix, Inc., Ithaca, NY, [2]Rheonix, Inc., Grand Island, NY,*

Objective: To develop a rapid, fully integrated and automated genotyping CARD (Chemical and Reagent Device) for individualized dosing of Warfarin.

Relevance: Inaccurate dosing of anti-coagulants resulting from current methods is associated with a significant number of life-threatening thrombolytic events. Since appropriate dosing could be faster and more safely achieved by genetic-based testing, the FDA has approved nucleic acid tests to analyze the specific single nucleotide polymorphisms (SNPs) associated with warfarin response. However, the cost and expertise required to perform these tests prevents their routine use. Our CARD technology significantly reduces the required skill level and the cost of equipment and materials needed to perform genotype testing.

Methodology: A buccal swab is collected from an individual's cheek and transferred to our proprietary transport media that can be either stored at room temperature or immediately applied to the CARD. Once applied to the CARD, all required steps are automatically performed without intervention by the analyst (cell lysis, DNA purification, PCR amplification, denaturation, annealing of amplicons to filter-linked primer

extension probes, primer extension in the presence of biotinylated dUTP, incubation with streptavidin-coupled HRP and color detection). Only an exact match between the immobilized primer-probe and the amplicon template will allow an extended product to be generated.
Validation:. We have tested and confirmed the known genotypes of commercially available individual genomic DNAs as well as successfully genotyping 20 individuals with previously unknown genotypes, and confirmed the CARD results via sequencing (representative results shown in figure). In all cases, the CARD called the correct genotype.
Conclusions: The CARD SNP assay provides a "turn key" solution to automatically perform rapid, point-of-care testing for warfarin dosing. The reduced complexity and cost of the CARD SNP assay will help to make personalized medicine a reality and further improve the health and wellbeing of individuals on a worldwide basis.



	1	2	3	4	5	6	7
VKORC1	AA	AG	GG	AG	AA	AG	AG
CYP2C9*2	CC	CC	CC	CC	TC	TC	TC
CYP2C9*3	AA	AA	AA	AC	AA	AA	AC

Buccal swabs were obtained from 20 previously non-genotyped individuals and were subjected to SNP CARD™ assay. Seven of the 12 genotypes known to be present in the population were identified within this group. Representative results are shown from each of the 7 identified genotypes. The primer extension results were all confirmed with sequencing. The table below the filters indicates the genotypes.
The filter key in the upper left corner indicates the genotype being targeted by the primer-probe. There are 4 individual probes for each SNP, 2 for each allele targeting both strands of DNA. SC: spotting control; covalently linked biotinylated poly-A.

D-11

The RATPAC Trial (Randomised Assessment of Treatment using Panel Assay of Cardiac markers): A randomised controlled trial of point-of-care cardiac markers in the emergency department

P. O. Collinson[1], S. Goodacre[2], M. Bradburn[2], P. Fitzgerald[2], L. Cross[2], A. Gray[3], A. Hall[4]. *[1]St George's Hospital, London, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom, [3]Edinburgh Royal Infirmary, Edinburgh, United Kingdom, [4]Leeds University, Leeds, United Kingdom,*

Background: To determine whether measurement of cardiac troponin I, CK-MB and myoglobin at 0 and 90 minutes by point of care testing (POCT) using the Stratus CS would increase the rate of successful discharge home after emergency department assessment.
Methods: A multicentre randomised controlled trial comparing diagnostic assessment using POCT to standard care for patients with acute chest pain at six hospitals. Patients were excluded if they had ECG changes for myocardial infarction or high-risk acute coronary syndrome (>1mm ST deviation or >3mm inverted T waves), known coronary heart disease presenting with prolonged (>1 hour) or recurrent episodes of cardiac-type pain, proven or suspected serious non-coronary pathology, an obvious non-cardiac cause or more than 12 hours since their most significant episode of pain. Participants were then randomly allocated to receive either: a) Diagnostic assessment using the point-of-care biochemical marker panel, or b) Standard care without the panel, but including routine measurement of cardiac biomarkers according to the local protocol.
The point-of-care cardiac marker panel comprised creatine kinase MB mass (CK-MB), myoglobin and troponin I (cTnI), measured at presentation and 90 minutes later, using the Siemens Stratus CS analyser. The analytical characteristics of the assays were as follows: cTnI detection limit 0.02 µg/L, l range 0.02 to 50 µg/L, CV 4.3-5.1% (0.03 to 0.22 µg/L), 99th centile 0.07 µg/L. Myoglobin: range 1-900 µg/L; CV 1.9-12.7% (56 to 308 µg/L). CK-MB: range 0.3-150 µg/L; CV 0.15-1.27% (3.7-39.3 µg/L).
Hospital admission was recommended if any CK-MB level exceeded 5µg/L, if the CK-MB gradient exceeded 1.6µg/L or if the myoglobin gradient exceeded 25% of the initial level or if either cTnI level exceeded 0.07ng/ml or if the first sample was less than 0.03ng/ml and the second was between 0.03 and 0.07ng/ml.
The primary outcome was successful discharge home by four hours after attendance and no major adverse events during the following three months. Secondary outcomes included use of the coronary care unit, cardiac medications and cardiac interventions, re-attendance at and/or re-admission to hospital over the following three months and adverse events (death, non-fatal myocardial infarction according to the universal definition, emergency revascularisation or hospitalisation for myocardial ischaemia).
Results:2263 participants were recruited across 6 hospitals between 30th January 2007 and 2nd June 2008.(1125 POCT, 1118 to standard care).Point-of-care assessment was associated with an increased rate of successful discharge (358/1125 (32%) versus 146/1118 (13%); odds ratio 3.59; 95% CI 2.86 to 4.52; p<0.001), reduced median length of initial hospital stay (8.8 versus 14.2 hours, p<0.001) and greater use of coronary care (50/1125 (4.0%) versus 31/1118 (3.0%), p=0.041), but no difference in mean length of the initial stay (28.6 versus 31.7 hours, p=0.317), mean inpatient days over follow-up (1.8 versus 1.7) or major adverse events (36 (3%) versus 26 (2%), odds ratio 1.31; 95% CI 0.78 to 2.20; p=0.313).
Conclusions: Point-of-care assessment increases successful discharge home and reduces median length of stay, but does not alter overall hospital bed use.

D-12

Coaguchek XS Plus evaluation for point-of-care INR measurement in two anticoagulotherapy clinics

S. Dugre-Brisson[1], M. Provencal[1], S. Leblanc[1], L. Marquis[1], G. Honos[1], G. Leclerc[1], M. Gannon[2], N. Rathel[2], J. Lambert[3], C. Savaria[3], C. Milante[3], L. Labrecque[1]. *[1]Centre Hospitalier de l'Universite de Montreal, Montreal, QC, Canada, [2]Roche Diagnostics, Laval, QC, Canada, [3]Hopital Pierre-Boucher, Longueuil, QC, Canada,*

Background: An adequate measurement of International Normalized Ratio (INR) is crucial in the context of a therapy with oral anticoagulants. The follow-up of these therapies is usually carried by measuring INR with a laboratory analyzer from a venous puncture. However, for some years, portable meters allowing INR measurement in a point-of-care (POC) setting from a capillary blood sample are available.
Objectives: To carry a correlation study comparing the INR measurement of a portable meter (CoaguChek XS Plus, Roche Diagnostics) with two different laboratory analyzers (BCS from Dade Behring and Sysmex CA-1500 from Siemens). To evaluate the amount of time spent at the hospital while attending their scheduled appointment for patients whose INR is measured at the laboratory or with the CoaguChek XS Plus.
Methods: At Pierre-Boucher Hospital (PBH), we kept track of the patients with a document indicating the time of the events occurring during their visit (time of arrival, blood sampling, meeting with the doctor, making an appointment and departure). At the Centre Hospitalier de l'Université de Montréal (CHUM), a simulation was carried using the Med/IQ™ model for the different options available for the anticoagulotherapy clinic. Correlation of the CoaguChek XS Plus with laboratory analyzer was evaluated both at PBH (n = 155) and CHUM (n = 196).
Results: The correlation of the CoaguChek XS Plus with the laboratory analyzers is acceptable in regard to the ISO 17593:2007 norm at PBH (R^2 = 0,9368) as well as at the CHUM (R^2 = 0,9560). In average, the visit at PBH was 54.5% (46.92 minutes) longer for the patients whose INR was measured in the laboratory compared to those who were followed using the CoaguChek XS Plus. At the CHUM, an important reduction in the amount of time spent by the patient at the clinic could be achieved by using the CoaguChek XS Plus. Furthermore, the institution of a collective prescription, allowing the nurses to discharge patients with INR in the appropriate range, would greatly enhance the efficiency of the anticoagulotherapy clinic.
Conclusion: The correlation studies results show that the portable meter CoaguChek XS Plus is adequate to measure the INR in the context of the follow-up of an oral anticoagulant therapy. Moreover, the use of this monitor is an improvement for the patients who have to attend the anticoagulotherapy clinic several times a year; the visits are shorter and the blood sampling is less invasive.

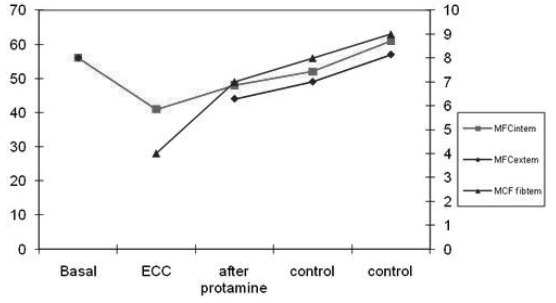
D-13

Administration of fibrinogen concentrates guided by The Movil Laboratory Unit for treatment exessive perioperatory bleeding in grown-up congenital heart disease surgery.

J. Noval Padillo, C. Serra-Gomez, A. Leon Justel, J. Luis-Navarro, R. Hinojosa, J. Guerrero Montavez. *Universitary Hospital Virgen Del Rocio, Sevilla, Spain,*

INTRODUCCTION AND AIMS:Patients who have undergone cardiac surgery with extracorporal circulation (ECC) are under a high risk of excessive loss of perioperatory blood and require high amounts of allogeneic blood products. The reoperation, the prolongation and the eventual necessity of hipothermya in complex grown-up congenital heart (GUCH) disease surgery, aggravate the risk of coagulation disorders. The Movil Laboratory Unit in the cardiac surgery area, give detailed information and in the real time of the patients hemostatic process, so helping the clinicians in decision making non-empiric therapeutics. This laboratory consit of a gasometer (Cobas b221), a hematologic conter (Sysmex poch-100i) and a thromboelastogram (ROTEM). The efficacy of post ECC intraoperatory administration of fibrinogen concentrates during the elective GUCH surgery is observed. METHOD: The clinically relevant widespread bledding afer ECC was treated with alogenic hemiderived products (platelet concentrates followed by freshly frozen plasma) in a retrospective group of patients (grupo A, n=9) who underwent elective

surgery on congenital therapeutic or palliative cardiopathies. In a prospective group (group B, n=6) a first therapeutic step with fibrinogen concentrates according to the maximum clot firmness (MCF) provided by (ROTEM).RESULTS: In group B, the administration of 5 gramos of fibrinogen concentrates stabilized the haemostatic system, completely according intraoperatory transfusion of freshly frozen plasma and platelets concentrates. Transfusion of freshly frozen plasma after ECC and during the first 24 horas after surgery was markedly inferior in group B than in group A. (39 vs 6).In the group B, 4 patients de 6, did not required transfusion of hemoderivates.CONCLUSIONS: Administration of fibrinogen concentrates guided by post ECC Rotem, improved the intraoperative control of bleeding due to haemostatic alterations in GUCH surgery and reduced blood transfusions in the first 24 hours.



D-14

Analytical evaluation of Precision XceedPro point-of-care glucose assay

Y. Zhu, R. P. Rogers, S. B. Barrett, N. A. Epps. *Medical University of South Carolina, Charleston, SC,*

Background: Monitoring blood glucose levels is essential to maintain normoglycemia in patients with diabetes mellitus and prevent the progression of complications from this disease. Blood glucose point of care tests are widely performed because of their ease of use and rapidity of results. However, the performance characteristics of glucose point of care assays should be carefully evaluated. We report the analytical evaluation of Precision XceedPro blood glucose test. The objective of this study is to validate the performance characteristics of the assay.

Methods: Glucose dehydrogenase and NAD+ were employed in the strips to measure glucose in whole blood. Within-run imprecision was evaluated by testing two whole blood samples with glucose levels of approximately 100 mg/dL and 200 mg/dL 20 times on 4 glucose meters, and between-run imprecision was assessed by running 2 levels (97 mg/dL and 260 mg/dL) of Glucose Calibration Verification Controls (RNA MEDICAL) twice per day for 10 days on 4 glucose meters. The linearity was determined by measuring a 5-level (25, 95, 271, 363, 467 mg/dL) Glucose Calibration Verification Control (RNA MEDICAL), and 4 replicates of each level were performed. The Accuracy of the assay was evaluated by comparing with DxC 800 plasma GLUCm (Beckman Coulter) and i-STAT whole blood glucose assays (Abbott). The results of 49 whole blood samples were obtained over the course of several days, at a rate of 5 to 15 per day. The hematocrit of each sample was determined by an i-STAT analyzer for the evaluation of hematocrit interference. Maltose interference was evaluated by spiking maltose (100 mg/dL and 200 mg/dL) into 4 whole blood samples with various glucose concentrations, and the glucose recovery of each spiked specimen was calculated.

Results: The within-run CV varied from 4.2% to 4.6%, and the between-run CV varied from 4.1% to 5.1%. Linearity was demonstrated by passing a straight line through all five points and the linearity range of 20 - 500 mg/dL was confirmed, and the linear equation was $y = 1.06x - 4.3$ ($r2 = 1$, $n = 5$). The comparison between Precision XceedPro and Beckman Coulter GLUCm method, as well as i-STAT method demonstrated high agreement: $y = 0.92x + 9.49$ ($r2 = 0.993$, $n = 49$, Precision XceedPro vs. GLUCm; $y = 0.91x + 11.11$, $r2 = 0.992$, $n = 49$, Precision XceedPro vs. i-STAT) over a range of 20 - 500 mg/dL. Hematocrit between 25 - 50% did not show significant interference. No significant interference was found in samples containing maltose up to 200 mg/dL.

Conclusion: Although Precision XceedPro just requires minimal sample size of 0.6 µL, the assay demonstrates acceptable within-run and between-run impression, broad linearity, and excellent agreement with Beckman Coulter GLUCm plasma glucose and i-STAT whole blood glucose assays. The assay is not significantly interfered by hematocrit in a wide range, and maltose up to 200 mg/dL does not significantly interfere with the assay.

D-15

Sample selection and accuracy evaluation of point-of-care highly sensitive C-reactive protein detection

A. Y. Bao, Y. Li, H. Shao. *Renmin Hospital of Wuhan University, Wuhan, China,*

Objective Blood concentration of highly sensitive C-reactive protein(hsCRP) was considered to have a significant risk predictive function in occurrence of cardiovascular event. More and more convenient point-of-care testing (POCT)analyzers were applied in clinic wards and emergency department, while few systematic evaluation was made on these POCT analyzers. In this study, we performed an accuracy evaluation of highly sensitive C-reactive protein(hsCRP) detection at point-of-care through the comparison with laboratory reference system, and to get the correlation and bias between POCT and laboratory system. Also, we intended to investigate the difference of results with serum, plasma and whole blood samples at the determination of hsCRP. Methods In-patient individuals at departments of pediatrics, cardiology and neurology, etc in a comprehensive teaching hospital were involved in the study. Venous whole blood, serum and plasma samples were obtained from patients with serum tube and EDTA-K2 added vacuum tube, and the samples were determined by POCT instrument i-CHROMA(Bodi Tech Med Inc, South Korea) and biochemical analyzer Olympus AU2700 (Olympus Corporation, Japan) for hsCRP concentration. The testing principles of POCT and biochemical system were immunofluorescence and immunoturbidimetric method, respectively. Pearson correlation analysis, paired-t test, Bland and Altman bias plot and Passing and Bablok regression were used to observe the correlation and difference between POCT and laboratory reference system. ANOVA analysis was used to observe the difference among three types of sample for hsCRP concentration. Results hsCRP concentration detected by POCT instrument i-CHROMA correlated well with biochemical analyzer. hsCRP concentration in serum, plasma and whole blood sample detected by POCT analyzer did not differ significantly with that of biochemical analyzer (for each paired group, $P>0.05$), respectively. The Passing and Bablok regression equation indicated that the actual bias was clinically acceptable with the criteria of biological variation range. Bland and Altman bias plot didn't show a significant change of difference with the increasing hsCRP concentration. Furthermore, the sample type--serum, plasma and whole blood--did not make a significant influence on POCT result of hsCRP level.

Conclusion: hsCRP concentration determined by POCT instrument showed good correlation and comparability compared to laboratory reference system. Because of the shorter turn around time (TAT), POCT system for hsCRP determination can be well applied in clinical wards.

D-16

A Novel Automatic Check for Humidity-Compromised Urine Strips on the CLINITEK Status®+ Analyzer

L. S. Schulman, G. W. Rheinheimer, C. T. Zimmerle, M. J. Pugia. *Siemens Healthcare Diagnostics, Elkhart, IN,*

Urine strip quality and integrity are essential in obtaining accurate urinalysis test results. The improper handling of strips, regardless of brand, can lead to false results and delays in correct diagnosis. If strip bottles are not recapped or are not tightened completely, the contents can be exposed to humidity, potentially compromising strip integrity and leading to reagent degradation and false results. A novel feature of the Siemens CLINITEK Status+ Analyzer is its algorithm for humidity-compromised reagent strip detection. The study analyzed Siemens' MULTISTIX® 10SG urine reagent strips tested side by side with Roche Diagnostics' CHEMSTRIP 10 MD and Diagnostic Test Group's Clarity UROCHECK 10SG, to determine product quality and integrity. Strip testing was conducted at 18 test points over a 54-day period. For each test point, clinical results were obtained using one strip from each reagent bottle product, exposed and unexposed to humidity. These were tested with three test solutions containing the following analytes: leukocytes, nitrite, protein, ketone, and/or glucose. The study was conducted in a climate-controlled laboratory with an average temperature of 21.8°C and relative humidity of 46.3%. Conclusion: Siemens' MULTISTIX 10SG strips were the least moisture sensitive, with only two out of five analytes (leukocyte and glucose) giving a false-negative result when exposed to humidity. The Roche CHEMSTRIP 10 MD strips had false results for four analytes; and with the Clarity UROCHECK 10SG strips, all five of the tested analytes gave false results, as shown in the table. The Siemens algorithm for detecting humidity-compromised strips was able to detect degradation in as little as 5 days and was highly accurate at 54 days. Using the CLINITEK Status+ Analyzer, many of these false results would be prevented using MULTISTIX 10SG with Auto-Checks.

Exposure	Product	Failure	Leukocyte		Nitrite		Protein	Glucose	
Unexposed Bottle	Siemens	Day	n/a		n/a		n/a	n/a	
		Type	n/a		n/a		n/a	n/a	
	Roche	Day	n/a		n/a		n/a	n/a	
		Type	n/a		n/a		n/a	n/a	
	Clarity	Day	1		1		n/a	1	
		Type	FN		FN		n/a	FN	
Exposed Bottle	Siemens	Day	11		n/a		n/a	20	
		Type	FN		n/a		n/a	FP	
	Roche	Day	34		11		32	n/a	
		Type	FN		FP		FN	n/a	
	Clarity	Day	5	20	1	32	54	1	54
		Type	FN	FP	FN	FP	FP	FN	FP

n/a = no false results obtained FN = false negative FP = false positive

D-17

Regression Models for Methods Comparisons. A Practical Example with i-STAT® and CELL-DYN® Sapphire™ Hematocrit Methods.

V. M. Genta[1], W. Teng[1], L. Wyer[2], D. Graubner[1], J. White[1], Y. Shen[1], D. Greiber[1]. *[1]Sentara Virginia Beach General Hospital, Virginia Beach, VA, [2]Sentara Healthcare, Norfolk, VA,*

Introduction In hospitals, tests for Hematocrit (HCT) are used by physicians to evaluate the patient's need for a blood transfusion and the response to it. In presence of active bleeding a rapid estimate of the patient's HCT may prompt the physician to promptly initiate appropriate transfusion therapy. When several methods to determine the HCT are used in a hospital, these should be harmonized for their interchangeable use in evaluating patients conditions. We studied the relationship of several lots of i-STAT® Chem 8 cartridges versus the Laboratory method for several months. To evaluate this relationship, we used regression analysis models for assessing linearity and the Clinical Laboratory Improvement Amendments' (CLIA) criterion of target value ± 6% for assessing the relative bias.

Materials and Methods Sixty one patient specimens were assayed in parallel and within 30 minutes with the i-STAT method (Chem 8, lots P08328B, P08323A, P090090, P09070, P09033, P09103, A09166B, A09150C, Abbott), and the CELL-DYN® Sapphire™ (Abbott) Laboratory method. The paired observations were transferred manually to Minitab® (version 15, Minitab Inc.) statistical software for statistical analysis.

Results The ordinary least squares (OLS) (i-STAT = 0.23 + 1.03 Sapphire), the three-group regression line (3GRL) (i-STAT = -2.07 + 1.09 Sapphire), and the locally weighted scatter plot smoother (Lowess) regression analysis models showed a similar quasi-linear relationship in the interval 20-50%. Furthermore, the standardized deleted residuals of the OLS model showed a quasi-normal distribution, and their plot versus the HCT values as determined by the laboratory method, showed a quasi-linear relationship. However, 82 % of the observations obtained with the i-STAT method showed a positive relative bias versus the paired observations obtained with the Sapphire method. For 15% of these observations the bias exceeded the CLIA's criterion.

Discussion and conclusions This positive bias makes it difficult for physicians to use the two methods interchangeably. In some clinical situations the higher values obtained with the i-STAT method may give a false sense of security. This positive bias was also reflected in a CAP survey (AQ-B-2009); the results obtained with the i-STAT method exceeded the expected upper value of the acceptable range. Furthermore, this bias may be compounded by faulty operator's manual technique (an excess of specimen applied to the cartridge gives falsely elevated results). In conclusion, while the i-STAT method for HCT in emergency situations offers the convenience of obtaining rapid results at the patient bedside, due to the cartridge's design, calibration cannot be adjusted by the operator and no corrective action can be implemented to harmonize the i-STAT HCT method with the Laboratory HCT method.

D-18

Evaluation of the GEM® Premier4000 for urea and creatinine analysis

C. Rees, C. Brownlie, R. Spooner. *Department of Clinical Biochemistry, Glasgow Royal Infirmary, Glasgow, United Kingdom,*

Introduction Instrumentation Laboratory (IL) has under development a Basic Metabolic Panel (BMP) cartridge for the GEM Premier4000 blood gas analyser that, in addition to Na^+, K^+, Ca^{++}, Cl^-, glucose, lactate and haematocrit includes urea and creatinine measurements on a whole blood sample and will in the future include a bicarbonate/tCO_2 measurement to provide fast reliable results for diagnosis/treatment of renal disease. This evaluation aimed to assess the analytical performance of urea and creatinine in patient samples on the IL GEM 4000 compared to current laboratory methods.

Method 103 random waste arterial blood gas samples were obtained from ITU, Renal and Emergency wards. Whole blood (WB) samples were analysed on the GEM 4000 and plasma, obtained after centrifugation, on a c8000 analyser (Abbott) using the Abbott urea method and both the Abbott Jaffe and Roche enzymatic creatinine methods. The plasma was also analysed for urea and creatinine on the GEM 4000 to allow comparison with WB.

Results Results were compared using Passing Bablok analysis.

Urea WB urea analysis compared well with the Abbott method:

GEM 4000 WB urea = 0.97 (Abbott plasma urea) + 1.00. Bland Altman analysis revealed slight positive bias on the GEM 4000 at lower concentrations and slight negative bias at higher concentrations (>20 mmol/L).

Comparison of WB and plasma urea on the GEM 4000 showed good agreement:

GEM 4000 WB urea = 1.01 (GEM 4000 plasma urea) - 0.57. The measurements showed a greater variability at concentrations >20 mmol/L with the majority of plasma measurements significantly positively biased.

Creatinine WB creatinine analysis compared well with both the enzymatic and the Jaffe methods:

GEM 4000 WB creatinine = 1.05 (enzymatic plasma creatinine) - 9.64,

GEM 4000 WB creatinine = 1.02 (Jaffe plasma creatinine) - 12.35. Bland Altman showed random scatter for both methods.

Plasma and WB creatinine also agreed well on the GEM 4000:

GEM 4000 WB creatinine = 1.06 (GEM 4000 plasma creatinine) + 11.96. Bland Altman revealed a positive bias on the plasma measurement which increased at higher concentrations.

84/103 patients were classified into the same CKD stage group (after eGFR MDRD4 calculation) across each method.

Negative interference by creatine on creatinine measurement was observed in one patient (creatinine results were (umol/L) 115 (GEM WB), 318 (GEM plasma) 264 (Jaffe) and 218 (enzymatic). Serum creatine was 1334 µmol/L (reference range 10-100µmol/L, analysed by LC-MS/MS). This patient had septic shock post resection of an ischaemic bowel and was receiving renal support. Rhabdomyolysis was present (serum CK 48,900 U/L) on the same day the blood gas sample with the high creatine was taken. Creatine interference is detected by the GEM 4000 via an onboard creatinase based electrode.

Conclusion Good correlation was observed between the IL GEM 4000 and current laboratory methods. This development is in the early stages and not all calculation algorithms have been finalised.

The BMP cartridge for the GEM Premier4000 shows promise for use in both the laboratory and POCT environments.

D-19

Clinical Impacts of Differences in Intra-Operative Parathyroid Hormone Assays.

C. Lee, P. Angelos, E. L. Kaplan, S. J. Zibrat, L. Toney, J. Helke, N. Babic, K. T. J. Yeo. *University of Chicago, Chicago, IL,*

Objective: Intraoperative Parathyroid Hormone (IOPTH) monitoring helps guide a surgeon's decision-making by confirming successful removal of adenomatous parathyroid tissue. It is widely accepted that IOPTH concentration decreases of ≥50% from baseline (T_{50}), are indicative of successful removal of the lesion. We compared the rates of IOPTH decline using the Siemens Immulite® Turbo and Roche STAT Elecsys® 2010 PTH in 100 consecutive surgical patients and evaluated differences in total turn-around times (TAT) between testing performed in the operating room (OR) and central laboratory.

Methods: Serial blood specimens from 19 patients were collected and measured in the OR using the Immulite assay; identical aliquots were transported to the central laboratory in real-time to compare the PTH measured on the Elecsys assay and the total TAT. For the remaining 81 patients, leftover EDTA plasma from these surgeries were collected and frozen at -20°C for batch analysis on the Elecsys 2010 at a later time. For the determination of sensitivity (of successful removal of tissue), a true positive test is defined as PTH decline to ≥50% baseline value during the intra-operative procedure which is ultimately confirmed by a post-surgical (overnight and/or 2 weeks) sample that showed low/normal PTH and normal calcium. The Wilcoxan matched pairs t-Test was used to assess for statistical differences between the two methods. This study was approved by the Institutional Review Board.

Results:In all serial samples, the Immulite assay showed IOPTH values declining to T_{50} more rapidly than the Elecsys assay (median 42 min vs 48 min, $p < 0.0001$). At 5 and 10 minutes post-excision, declines from baseline were greater with the Immulite vs the Elecsys assay (medians, 33.0% of baseline vs 46.5% of baseline for T_{5min}, and 19.0% of baseline vs 35.5% of baseline for T_{10min} respectively, $p<0.0001$). Overall, the Immulite PTH assay demonstrated a higher sensitivity in predicting successful tissue removal than the Elecsys PTH assay for both the T_{5min} (sensitivity, 67.0% vs 51.0%) and T_{10min} (sensitivity, 80.0% vs 63.0%) samples, respectively. There was a statistically significant difference in TAT (median OR 20 min vs central laboratory 26.5 min, $p<0.0001$) measured

in 19 concurrent patients.
Conclusion: The analytical differences between the Siemens Immulite and Roche Elecsys PTH assays will have clinical and economical impacts since the assay that demonstrates a greater sensitivity and a reliably faster decline to <50% baseline (Immulite) has the advantage of potentially reducing time and costs of the surgical procedure. However the total TAT difference between the testing performed in OR versus the central laboratory, although statistically significant, is unlikely to be clinically significant-suggesting that the service can be provided by the central laboratory if the dedicated OR service becomes unavailable or cost-prohibitive in the future.

D-20

Evaluation of the CoaguChek S and CoaguChek XS Plus for Point of Care INR Testing
L. K. Beyer, A. M. Wockenfus, R. D. McBane, R. Chaudhry, B. S. Karon, P. J. Santrach. *Mayo Clinic, Rochester, MN,*

OBJECTIVE: The Point of Care (POC) international normalized ratio (INR) is an increasingly common procedure to monitor warfarin real-time and adjust warfarin dosage. This evaluation was conducted to determine the accuracy of the CoaguChek S and CoaguChek XS Plus by comparing whole blood INR on these devices to plasma INR values using a reference laboratory method.
METHODS: 52 INR measurements were performed in parallel using two separate capillary punctures on the CoaguChek S and CoaguChek XS Plus (Roche Diagnostics, Indianapolis IN) devices, compared to venous plasma INR drawn within two hours and analyzed on a Stago Evolution (Diagnostica Stago, Parsippany NJ) coagulation analyzer using Dade Innovin reagent (Dade Behring, Newark DE). The CoaguChek S mixes the capillary whole blood with rabbit thromboplastin to activate clotting. Tiny iron particles on the test strip are moved within the sample by alternating magnetic fields, and the endpoint is reached when the blood clot stops the movement of the iron particles. The CoaguChek XS Plus is a new generation of the CoaguChek device in which the strip electrochemically measures PT following the activation of blood coagulation with human recombinant thromboplastin.
RESULTS: Mean bias (± standard deviation) for the CoaguChek S capillary whole blood compared to lab plasma INR was 0.1 ± 0.2 INR units. Mean bias on the CoaguChek XS Plus was 0.1 ± 0.1 INR units. 47 of 52 (90%) CoaguChek S results were within 0.5 INR units of the reference result; while 51 of 52 (98%) CoaguChek XS Plus results were within 0.5 INR units of the reference plasma result.
CONCLUSIONS: Overall agreement between POC and laboratory plasma INR was very good, as neither device demonstrated significant systematic bias compared to plasma INR. The CoaguChek XS Plus showed greater accuracy than the CoaguChek S as demonstrated by fewer outliers (≥ 0.5 INR units) when capillary whole blood was compared to plasma INR. The performance of the CoaguChek XS Plus will allow the vast majority of warfarin dosing decisions to be made accurately at the point-of-care.

D-21

A Bed-Side Mobile Laboratory Unit Monitoring The Hemostatic Defects Of Adults With Congenital Heart Disease Undergoing Cardiac Surgery. A Pilote Study.
C. Serra-Gomez, J. Noval Padillo, A. Leon Justel, F. Garcia Torrado, R. Hinojosa, A. Hosseinpour, J. Luis-Navarro, J. Guerrero Montavez. *Universitary Hospital Virgen Del Rocio, Sevilla, Spain,*

OBJECTIVE: To assess the usefulness of a mobile laboratory unit within the operating area for the monitoring of coagulation defects in the context of complex cardiac surgery.
RELEVANCE: Patients Undergoing cardiac surgery with extracorporeal circulation are at risk of excessive perioperative blood loss and the need for the transfusion of blood products. Such therapy is often given empirically. Consequently, its effects are not always predictable. This risk is particularly high amongst adults with congenital heart disease as they have often been operated on before, and also due to the protracted nature of their procedures. Under such conditions, we started to use a mobile laboratory unit within our operating area in order to monitor the coagulation defects more specifically and rapidly, in order to implement more targeted therapy.
METHODOLOGY: We present seven cases operated on at our Adult Congenital Heart Disease Unit. Blood samples were taken at induction of anesthesia, on bypass, and after bypass once protamine had been given. These samples were analysed on site, by our mobile laboratory unit, to assess the coagulation profile, full hematological profile and gasometry of each patient in each of the three surgical phases.
VALIDATION: The coagulation defects are monitored using rotational thromboelastometry (ROTEM®) using different reagents including heparinase to assess the state of coagulation while the patient is heparinized. Therefore, the coagulation profile and both the intrinsic and extrinsic pathways (inTEM®, exTEM®) were assessed. The hematologic study is done with a hematologic counter (Sysmex® poch-100i). The gasometry is done with astandard gasometry machine (Cobas® b 221).
RESULTS: In only two of the seven cases therapy with fibrinogen proved necessary. These were both very long and complex procedures. In both cases ROTEM® indicated the relevant defect within 15 minutes of the onset of bypass. Platelet transfusion was frequently avoided based on the objective data provided. Such measure would have been taken empirically previously. The hemostatic results have been satisfactory in all cases.
CONCLUSION: The use of a mobile laboratory unit within the operating area provides rapid and specific information regarding the coagulation state of the patients during protracted and complex cardiac surgery for congenital heart disease. The knowledge of the specific defects within the coagulation system facilitates an informed rather than empiric choice of therapy and blood products.

D-22

Evaluation of Measuring Pleural Fluid pH and Glucose on the Siemens RAPIDPoint® 400 Series Blood Gas Analyzers
E. Cowden, S. Fennell, K. LaRock. *Siemens Healthcare Diagnostics, Norwood, MA,*

Background: The measurements of pH and glucose in parapneumonic effusions have proven to be practical tools in the diagnosis of several medical conditions. The combination of the two measurements can be used in the diagnosis of rheumatoid pleurisy, pleural infection (including pneumonia and tuberculosis), esophageal perforation, and empyema. The Siemens RAPIDPoint 400 series analyzers (RP400 and RP405) are intended for near-patient and laboratory testing of blood gases, electrolytes, metabolites, total hemoglobin, and hemoglobin derivatives in whole blood samples. In an ongoing experimental study, pleural fluid was recently assessed on RAPIDPoint analyzers for pH and glucose performance characteristics and pleural fluid sample carryover effects. The carryover protocol was designed to assess the effect that a pleural fluid sample may have on a subsequent whole blood analysis, while the performance aspect of the study was intended to measure pH and glucose precision, glucose correlation to a glucose hexokinase method, calibration drift rate, and general errors due to sample path obstruction issues.
Methods: The tension of pCO_2 in 20 clinical pleural fluid samples was altered to achieve five different pH levels in the range of 6.9-7.6, and 30 clinical pleural fluid samples were spiked with D-(+)-glucose or diluted with a bovine serum albumin solution to achieve six different glucose concentration levels in the range of 50-700 mg/dL. The performance of pleural fluid pH and glucose was assessed by calculating total imprecision at each of the different pH and glucose levels on four RP405 analyzers. Pleural fluid glucose performance was also measured by comparing glucose results generated on two RP400 and two RP405 analyzers to results generated on the Siemens ADVIA 1200 chemistry system. To evaluate pleural fluid sample carryover effects, 10 pleural fluid samples were analyzed in a contrived pattern among 60 unaltered whole blood samples on two RP400 and two RP405 analyzers. Statistical analyses were performed comparing the whole blood analytes before and after each pleural fluid sample.
Results: The CVs for pleural fluid pH ranged from 0.12%-0.35% across the 5 different pH levels, while the CVs for pleural fluid glucose ranged from 3.32%-6.61% across the six different glucose levels. The correlation of glucose on four RAPIDPoint analyzers to the ADVIA 1200 generated a slope of 0.983, with an intercept of -3.11 mg/dL and a correlation coefficient of 0.996 (n=360). Pleural fluid samples analyzed prior to a whole blood sample did not induce a pattern of analyte failures consistent with a sample carryover mechanism. Finally, the calibration failure rate for each analyte was <3% over the course of the study, meeting the acceptance criteria specified in the product design requirements for whole blood, and no obstruction errors due to pleural fluid integrity were observed during the length of the study.
Conclusion: On the basis of these data, it is technically feasible to assay pleural fluid for pH and glucose on the RAPIDPoint 400 series; however, no claims for this have been made for the RAPIDPoint 400 and 405 blood gas analyzers.
Confidential

D-23

Drugs of Abuse: Detection in a Cup
D. N. Greene, H. Carlisle, C. J. Clark, C. D. Thompson, C. M. Lehman, G. A. McMillin. *University of Utah/ARUP laboratories, Salt Lake City, UT,*

Background: Over the last decade, the availability of point of care (POC) medical devices for drug testing has surged. Reduction in turn around time, and hence, rapid results are attractive, particularly to rehabilitation facilities and pain management clinics. Here we describe our validation results for the Integrated E-Z Split Key® Cup II, a low cost rapid test for urine that employs competitive immunoassay technology to detect 12 classes of commonly abused drugs (calibrator, published cutoff): amphetamines (d-amphetamine, 1000 ng/mL), barbiturates (secobarbital, 300 ng/mL), benzodiazepines (oxazepam, 300 ng/mL), cocaine (benzoylecognine, 150 ng/mL), marijuana (11-nor-Δ^9-THC-9 COOH, 50 ng/

mL), methadone (methadone, 300 ng/mL), methamphetamine (d-methamphetamine, 300 ng/mL), methylenedioxymethamphetamine (d,l MDMA, 500 ng/mL), opiates (morphine, 300 ng/mL), oxycodone (oxycodone, 100 ng/mL), propoxyphene (propoxyphene, 300 ng/mL), and buprenorphine (buprenorphine, 10 ng/mL). Positivity is based on the absence of a colored band at a labeled portion of the detection strip, while a negative result produces a distinct colored band. Each strip has a control band to assure the reader that urine was properly added to the cup.

Methods & Results: Using reagent grade standards, the apparent cutoff for each of the drugs was challenged with 5 replicates spiked with target analyte at 25%, 50%, 100%, 125%, and if needed, 150% of the published cutoff concentrations. At the 100% cutoff concentration, 5/5 cups generated positive results for 33% of the drug classes (4/12); an additional 6 drug classes were positive at 125% of the published cutoff; the final 2 drug classes were consistently positive at 150% of the cutoff. The results were stable up to an hour, but at time points beyond one hour, the accuracy began to diminish and false negative results were likely; cups left overnight were highly inaccurate. Applying these cutoffs, 90 patient samples confirmed positive by GC/MS or LC-MS/MS for several drugs and/or drug metabolites claimed to be detected by the test (at least 5 positive samples for each drug category) were tested. We chose positive urines that could account for many of the possibilities within a drug class. For example, to validate barbiturate detection we tested individual urine samples positive for either phenobarbital or butalbital. Five urine samples known to be negative for all drug categories were also tested. The identities of the samples were blinded to the reader. Negative results were obtained for all drug-free urine samples. One of the urines analyzed generated a reproducible false positive result for benzodiazepines. One false negative result was identified, but when the urine was poured off into a new cup the presence of the drug was confirmed. The product labeling suggests that many commonly prescribed, over the counter, and social drugs do not cross react at high concentrations. However, certain commonly prescribed medications, such as hydrocodone, have very low cross reactivity, suggesting that there are limitations to using the cups to verify compliance with prescribed opioid therapy.

Conclusion: The cups demonstrated excellent sensitivity and specificity at 150% of the published cutoff concentrations, for all drugs represented.

D-24

Locally-smoothed (LS) median and maximum absolute difference (MAD, MaxAD): theory, application, and boundary conditions.

H. Singh, N. K. Tran, G. J. Kost. *University of California, Davis, Davis, CA,*

Objective. Point-of-care (POC) glucose meters are crucial for maintaining blood glucose within an acceptable range in both diabetics and critically ill patients. Standards such as ISO 15197 now are inadequate for performance assessment. We propose the use of a new approach to improve existing guidelines for licensing POC devices.

Methods. We introduce the locally-smoothed maximum absolute difference (LS MaxAD) curve to identify maximum errors. This approach complements locally-smoothed median absolute difference (LS MAD) curves (*Clin Chim Acta* 2008;389:31). We implemented these methods using Matlab (Natick, MA). Data were extracted from multi center (n=2,767) and multi system (n=613, n=100) studies of glucose meter system (GMS) performance. We use an error tolerance limit (ETL, 5mg/dL) for the LS MAD curve, 95% non-parametric confidence intervals, "breakout points", "knockout intervals", and Class I and II discrepant values. Here, we also introduce a maximum error tolerance limit (METL) to the maximum error envelope and apply it to the LS MaxAD curve.

Results. The GMS from the multi center study performed satisfactorily up to the breakout point at 179 mg/dL by the LS MAD and the ETL conditions. Analysis from the modified Bland-Atlman plot illustrated a mostly negative locally-smoothed mean bias (LS MB). From the multi system study, LS MAD curves showed that GMS 1a and 2a performed best around the tight glycemic control (TGC) interval than in the higher measurement range. The LS MAD curve for GMS 5 remained below the ETL throughout the measurement range, except for two outliers present at the reference values of 358 and 406 mg/dL. For all systems, we propose an ETL_{MAX} of 20 mg/dL above which due consideration must be given to assigning knock-out ranges.

Conclusions. The LS MAD and LS MaxAD curves with error tolerance limits when combined with the modified Bland-Atlman plot provide an integrated package for clinically relevant assessment of POC devices.

D-25

The effect of plasma cholesterol and triglycerides concentrations on glucose assays using amperometric and photometric point-of-care analyzer

B. Solnica[1], J. W. Naskalski[1], W. Gernand[2]. *[1]Jagiellonian University Medical College, Krakow, Poland, [2]Medical Laboratory LOMA, Opole, Poland,*

Lipemia is a very well known interfering factor with many chemistry assays. Thus, dyslipidemias common in diabetes may affect glucose monitoring. The aim of this study was to evaluate the effect of increased plasma lipids concentrations on glucose assays performed using point-of-care analyzers utilizing different measurement techniques.

Results obtained using the Optium XidoTM glucose meter, the HemoCue® glucose analyzer, and the Modular P laboratory chemistry analyzer were compared. 162 blood samples were divided into four groups based on total cholesterol (TC) and triglycerides (TG) levels: A) TC<7.0 mmol/L and TG<4 mmol/L, B) TC≥7.0 mmol/L and/or TG≥4.0 mmol/L, C) TG≥4.0 mmol/L irrespective of TC concentrations, and D) TC≥7.0 mmol/L irrespective of TG concentrations.

The Optium Xido and HemoCue yielded higher glucose concentrations in group A and lower concentrations in groups B, C, and D as compared to the Modular P. The Optium Xido yielded lower glucose concentrations than the HemoCue in all groups, but significantly lower in group A only. Agreement between the results obtained using three devices was the strongest in group A (r, 0.980-0.997); in comparing the results from the Optium Xido and the Modular P in groups C and D, the correlation coefficients were 0.832 and 0.840 and the slopes were 0.69 and 0.65, respectively; the correlation coefficients and slopes for the HemoCue and the Modular P were 0.876 and 0.91, and 0.85 and 0.86, respectively. In multivariate analysis, the highest impact on the variability of inter-method differences between the Optium Xido and the Modular P, as well as between the HemoCue and the Modular P, was the TG concentration. In comparison the Opium Xido glucose meter with the laboratory method error grid analysis yielded 155 (95.7%) results in zone A, 5 (3.1%) results in zone B and 2 (1.2%) results in zone C and similarly for the HemoCue 155 (95.7%) results were located in zone A, 5 (3.1%) results in zone B and 2 (1.2%) results in zone C . In the case of both devices majority of results located in zones B and C were obtained in hyperlipidemic samples.

Significant inter-method differences exist between glucose measurements with point-of-care devices and the Modular P as a function of lipid concentrations, particularly triglycerides. Error grid analysis revealed minor clinical importance of inter-method differences found. However, distribution of results in error grid was significantly related to TC and TG concentrations. Dyslipidemia should be considered an interfering factor with point-of-care glucose assays.

D-26

The new POCT system "Banalyst" having incorporated micro fluidic chips has achieved rapid diagnosis of HbA1c and CRP

M. Ono[1], K. Kusaba[1], S. Matsumoto[2], M. Yamaichi[3], H. Okada[3], Y. Kageyama[3], A. Yokogawa[3], T. Mori[3], Y. Okumura[2], T. Hotta[1], Y. Kayamori[1], D. Kang[1]. *[1]Department of Clinical Chemistry and Laboratory Medicine, Kyushu University Hospital, Fukuoka, Japan, [2]USHIO INC., Hyogo, Japan, [3]ROHM Co., Ltd., Kyoto, Japan,*

[Background] Banalyst is a new POCT microblood analyzing system comprising chips (l50mm, w40mm, t4.5mm) developed based on the μTAS (Micro-Total Analysis System) technology and desktop compact measuring instrument. It is easy to operate, does not require any supplying/drainage of water and can perform centrifugal separation of the sample. Rohm Co., Ltd. and Ushio INC. have developed 2 types of quantitative chips to test the percentage of HbA1c and the concentration of CRP (C-reactive protein). In Japan, Banalyst Ace HbA1c and Banalyst Ace hsCRP have been sold from Sanwa Kagaku Kenkyusho Co., Ltd. This time, the performances of Banalyst were evaluated with these chips at Kyushu University Hospital.

[Methods] In the chip solution is moved by the centrifugal force produced by the rotation of the stage and the directions of the chip are changed-over on the stage, thereby making treatment of solution such as metering and mixing. In the HbA1c chip, the following 4 operational steps function Step1: A minimum of 4μL whole blood is centrifuged, Step2: Only 0.4μL blood cells are metered, Step3: It is sequentially mixed into the solution reagent (dilution, latex reagent and antibody solution) for automatic analyzer built in the chip, Step4: 635nm spectral-measurement is conducted, and the result is obtained in 7.5 minutes.

[Examinations and Results] HbA1c: The reproducibility was CV 1.0% (mean 5.1%, n=10) for the normal patient whole blood, and CV 1.1% (mean 7.7%, n=10) for the abnormal whole blood. The daily reproducibility was CV 1.0% (mean 5.5%) in the ordinary concentration region, and CV 1.0% (mean 8.1%) in the high concentration region at the 7 days'/5 times' continuous measurement of the domestic certified reference material JCCRM423-5 at each concentration. The linearity was favorable below 12.2%. The accuracy was 5.5 - 5.7% to 5.56%, 7.8 - 8.0% to 7.99% and 11.1 - 11.4% to 11.07% at the 5times' measurement of JCCRM423-5 at each concentration. It has only JDS% certificated values 5.16%, 7.55% and 10.55%. They correspond to 5.56%, 7.99% and 11.07% in NGSP% (NGSP% = 1.019JDS% + 0.30). hsCRP: The reproducibility was CV 0.0% (mean 0.05mg/dL, n=10) for the normal patient serum, CV 1.9% (mean 3.48 mg/dL, n=10) for the abnormal serum. The daily reproducibility was CV 8.1% (mean 0.05mg/dL) for the normal patient serum, and CV 2.5% (mean 3.52mg/dL) for the abnormal patient serum at the 10 days' continuous measurement. The linearity was favorable below 4mg/dL. The lower detection sensitivity was 0.01mg/dL in the 3SD method at the 5times'

measurement of the diluted system produced with the patient serum. The accuracy was 100 - 106% to 0.31mg/dL and 101 - 106% to 1.08mg/dL at the7 times' measurement of the in-company standard product adjusted in conformity with the international standard CRM470.
[Conclusion] The fundamental performance of Banalyst was favorable. It excels as a POCT system in that HbA1c is highly accurate, and CRP can be detected up to a low concentration. It is expected that changing the solution reagent in the chip will allow other items to be measured.

D-27

A Comparison of 3 units of POCT Analyzers applying CRP & HbA1c Reagent with the Medical Laboratory Methods

K. Kusaba[1], M. Ono[1], S. Matsumoto[2], M. Yamaichi[3], H. Okada[3], Y. Kageyama[3], A. Yokogawa[3], T. Mori[3], Y. Okumura[2], T. Hotta[1], Y. Kayamori[1], D. Kang[1]. *[1]Department of Clinical Chemistry and Laboratory Medicine, Kyushu University Hospital, Fukuoka, Japan, [2]USHIO INC., Hyogo, Japan, [3]ROHM Co., Ltd., Kyoto, Japan,*

Introduction: Banalyst (Collaborated by USHIO INC. & ROHM Co., Ltd.) is an immuno-assay analyzing system based on the Micro-TAS technology. It can analyze the conventional C-reactive protein (Range: 0.1-20.0 mg/dL), High-Sensitivity CRP (Range: 0.01-4.00 mg/dL) and Hemoglobin A1c (Range: 3.4-12.5 NGSP%, NGSP% = 1.019JDS% + 0.30) with a small amount of whole blood. The model AS100 Afinion (Axis-Shield PoC AS) is also a compact analyzer and it can analyze CRP (Range: 0.5-16.0mg/dL for serum and plasma, 0.8-20.0mg/dL for whole blood), HbA1c (Range: 4.0-15.0%). The DCA2000+ (Siemens Healthcare Diagnostics Inc.) is the most popular of the 3 units as a HbA1c analyzer (Range: 2.5-14.0%). These 3 units of Point-Of-Care-Testing analyzers are mainly used by practicing doctors. This time, we compared them to the medical laboratory methods for CRP/hsCRP and HbA1c in terms of the reproducibilities and the correlativities.
CRP/hsCRP result: The correlation formula between Banalyst Ace hsCRP(Y) and the biochemical analyzer in the medical laboratory(X: Hitachi 7700 applying CRP-Latex (II)X2 SEIKEN Assay Kit); Y=1.016X+0.012 (n=100, Sample: serum) and correlation factor r=0.997. Y=1.005X+0.005, r=0.998 (n=100, Sample: (Y)whole blood (X)plasma). Between Afinion(Y) and the medical laboratory method(X); Y=0.918X+0.029, r=0.995 (n=44, Sample: serum). Y=0.850X+0.097, r=0.993 (n=31, Sample: (Y)whole blood (X) plasma). The reproducibilities (serum) were CV0.0% (mean 0.1 mg/dL, n=5) for Banalyst Ace CRP, CV0.0% (mean 0.05 mg/dL, n=10) and CV1.9% (mean 3.48 mg/dL) for Banalyst Ace hsCRP CV8.6% (mean 1.04 mg/dL, n=5) for Afinion.
HbA1c result: The correlation formula between Banalyst Ace HbA1c(Y) and the HPLC analyzer in the medical laboratory(X: TOSOH HLC-723G7 calibrated by the standard which is traceable to IFCC standard); Y=0.992X+0.124, r=0.997 (n=100). Between Afinion and the medical laboratory method (X); Y=0.953X+0.623, r=0.989 (n=99). Between the DCA2000+ and the medical laboratory method (X); Y=0.991X+0.358, r=0.988 (n=99). The reproducibilities were CV1.0% (mean 5.1 NGSP%, n=10) and CV1.1% (mean 7.7 NGSP%, n=10) for Banalyst Ace, CV0.9% (mean 5.1 NGSP%, n=10) and CV1.1% (mean 7.8 NGSP%, n=10) for Afinion, CV1.3% (mean 5.5 NGSP%, n=10) and CV1.8% (mean 7.6 NGSP%, n=10) for the DCA2000+. And sampling errors were occurred in Afinion and The DCA2000 several times.
Turnaround time: Afinion was 3 min, 6 min for DCA2000+, and 7.5 min for Banalyst Ace.
Conclusion: The data shows all devices have enough performances for POCT Analyzer. Especially Banalyst Ace has a good reproducibility and a high correlativity with the medical laboratory methods.

D-28

Comparative Cost Analysis of Point Of Care and Central Laboratory Glucose Testing

S. Malhotra, P. J. Howanitz, J. H. Howanitz, S. Alfaro. *SUNY Downstate, Brooklyn, NY,*

We determined the cost of Point Of Care (POC) glucose testing, and compared it to the cost of glucose testing in our central clinical laboratory for 2009. Previous cost evaluations have not included rigorous credentialing costs of large numbers of nurses nor the use of data management software specific to glucose meters.
Our University Hospital has 383 beds, over 60,000 Emergency Department visits yearly, and five Intensive Care Units. The instruments used for POC glucose testing were the Precision Xceed Pro meters along with the corresponding Precision Web POC data management system (Abbott Laboratories, Chicago IL). The analyzers used in our central laboratory are two Olympus AU2700s and an AU640 (Beckman Instruments, Brea CA). Both the POC instruments and central laboratory glucose analyzers were acquired through reagent rentals from their respective vendors.
During 2009, 305,070 POC glucose tests were performed in our enterprise by 1051 personnel at 41 inpatient and 17 outpatient locations using 102 glucose meters. Of these, 258,119 tests required patient specimens, 42,691 tests were for daily quality control, 3060 tests were part of instrument validation, and 1200 tests were for our proficiency testing programs. Salaries used for the calculations of labor costs included benefits, and nurses and POC personnel agreed that testing for POC glucose took five minutes.
Total costs for POC glucose testing were $2,102,567. Of this cost, $1,300,920 (61.9%) was for patient tests, $215,163 was for daily QC, $6432 was for proficiency testing, $15,422 was for instrument validation, $72,929 was for credentialing, $258,119 was for LIS reporting, and $231,582 was the administrative costs for 3.4 Full Time Equivalents (FTEs) supporting glucose testing within our POC department. In addition, $2000 was the cost of replacing two lost meters.
The reagent/instrument cost for all tests performed was $119,361, whereas nursing labor costs for performing the testing was $1,418,575. The largest percentage of the cost per test was for labor (82.0%) followed by computer reporting (12.3%) and reagent/instruments (5.7%). Our cost per patient test for POC glucose testing was $8.14.
In comparison to POC testing, 151,134 glucose tests performed in central laboratory were on patient specimens and 60 for proficiency testing. The total cost for central laboratory glucose testing was $122,701. Of this,$50,700 was for phlebotomy and specimen centrifugation, $3305 for pneumatic tube transport, $1663 for phlebotomy supplies, $32,040 for measuring glucose in patient specimens, $13 for proficiency testing, $33,048 for LIS reporting costs, $1,932 for credentialing costs and there was no charge for QC tests and validation. The labor cost was $66,542 (54.2 % of the total cost), reagent/instrument cost was $18,143 (14.8% of total), pneumatic tube was $3305 (2.6% of total) phlebotomy supplies $1663 (1.4% of total) and the LIS reporting cost was $33,048 (27.0% of total cost). Our cost per patient test for central laboratory glucose testing was $ 1.23.
We conclude that POC glucose testing is much more costly than central laboratory glucose testing. The major proportion of cost in both POC glucose testing and central laboratory testing is attributed to the labor.

D-29

Performance Characteristics of Point-of-Care Test for the Determination of NT-proBNP in Whole Blood Based on Super-Paramagnetic Nanoparticle

M. Han[1], H. E. Chang[1], D. H. Ko[1], Y. Yoon[2], H. Park[3], J. Q. Kim[4], J. Song[1]. *[1]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Sungnam, Korea, Republic of, [2]Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Korea, Republic of, [3]Department of Laboratory Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of, [4]Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea, Republic of,*

Background: N-terminal proBNP (NT-proBNP) is known as a useful biomarker for congestive heart failure, of which early diagnosis and confirmation of treatment response are important. MICT (Magnetic Immuno-Chromatographic Test) NT-proBNP assay utilizes super-paramagnetic nanoparticle as the labeling agent, and measures in the entire chromatographic matrix, not limited to the surface. It is reported that the use of whole blood, higher sensitivity and quantitative measurements can be possible by MICT assay. In this study, we evaluated the performance characteristics of the whole blood based, point-of-care test, MICT NT-proBNP assay.
Methods: We evaluated the limit of blank (LOB), limit of detection (LOD), dilution linearity and imprecision of MICT assay system. Method comparison was also performed with E170 NT-proBNP (Roche Diagnostics) by Passing and Bablok fit. All the evaluations were performed according to the appropriate CLSI guidelines.
Results: The LOB and LOD of the MICT NT-proBNP assay were 23.8 and 42.2 ng/L, respectively. Total imprecision showed coefficients of variation from 17.7 to 18.2% at concentrations of low and high levels. Linearity was acceptable at the concentration range of 37.9 to 4538 ng/L ($r^2 = 0.93$). Passing and Bablok regression analysis with E170 NT-proBNP assay revealed a slope of 1.55 (95% CI 1.11 to 1.76) and an intercept of 28.6 (95% CI 3.95 to 81.36) (n = 47)
Conclusion: The MICT NT-proBNP assay shows acceptable performance for use in the point-of-care environment. It will be helpful in the early diagnosis and confirmation of treatment response of congestive heart failure.

D-30

Multi-parameter Performance Qualification of the New InnovaStar® POCT Clinical Chemistry Analyzer

A. Martins, D. Ehlers, A. Kawalek, E. Metzmann, T. Hektor. *DiaSys Diagnostic Systems GmbH, Holzheim, Germany,*

POCT is a fast growing market. Due to clear advantages like immediate available results, reduced potential errors and mobile laboratories point-of-care diagnostics is seminal for different countries and markets. The major drawback of point-of-care testing is that a multitude of established POCT systems have only limited functionality and therefore results the necessity to use different instruments for different clinical parameters. The new multifunctional InnovaStar® POCT system was designed to overcome these drawbacks and allows the adaption of a variety of clinical chemistry and immunoturbidimetric assays. The performance of the InnovaStar® POCT flow-through photometric system is comparable to laboratory based automated clinical photometric analyzers. Reagents are ready to use, pre-filled in barcoded unit dose cartridges and pre-calibrated. Previous sample preparation is not necessary. Within one run up to 3 parameters are determined from one specimen. The photometric unit has a wavelength range from 400 to 700 nm which is detected in total by a micro diode array at every measuring point. Linearity is given up to 2.2 absorbance units with a blank stability of ±0.5 mA. Correlation data of the InnovaStar® photometer compared to a commercially available photometer indicates a slope of m=1.002 and an axis intercept of b=-0.007.

The first adapted clinical parameters on the InnovaStar® are glucose and HbA1c to provide an advanced diabetes profile. A method comparison to commercially available tests delivered for InnovaStar® HbA1c the correlation y=1.00x+0.11, r=0.972, n=94 and for InnovaStar® Glucose y=0.969x-0.957, r=0.995, n=56, respectively. Precision data according to CLSI guideline EP 5-A2 show for a normal range sample (5.3 % HbA1c DCCT) a precision within run CV=2.5 %, between day CV=2.53 %, between run CV=1.86 %, and total precision CV=4.0 %. For a pathological sample (9.5 % HbA1c DCCT) precision data are within run CV=1.5 %, between day CV=1.8 %, between run CV=2.3 %, and total precision CV=3.3 %. Precision data for glucose at 88.8 mg/dL are within run CV=2.5 %, between day CV=0.4 %, between run CV=2.8 %, and total precision CV=3.8 %. For glucose at 285 mg/dL precision data are within run CV=1.8 %, between day CV=1.5 %, between run CV=1.0 %, and total precision CV=2.6 %.

Using the multifunctional opportunities of the instrument hemoglobin has been adapted to the system as a further example for clinical chemistry parameters. Correlation data to a commercial hematologic system were obtained: y=0.987-0.152; r=0.997, n=103. Intra-assay precision has a CV < 1.6%, inter-assay precision a CV of < 2.5%.

CRP as an important clinical inflammation parameter has been adapted to the system as well. Intra-assay precision for CRP has a CV of 5.8% at 4 mg/L and CV=1.6% at 21 mg/L. No interference was observed up to 2000 mg/dL triglycerides.

Summary: The InnovaStar® is a unique multifunctional POCT clinical chemistry analyzer. Almost every parameter which can be adapted to photometric systems can be applied onto the instrument. Further clinical chemistry and immunoturbidimetric assays for InnovaStar® will follow in near future.

D-31

Point of Care in a Health Screening Environment using Fractionated Cholesterol to identify Heart Disease Risk Factors

L. Springer[1], P. Sampson[2], M. Evans[1], J. Brock[1], F. M. LaDuca[1]. *[1]Polymer Technology Systems, Indianapolis, IN, [2]Stewart Memorial Community Hospital, Lake City, IA,*

Increasingly Point of Care testing (POCT) has been used in health screening applications to promote wellness. A POCT analysis of fractionated cholesterol is an integral part in the determination of relative risk of Heart Disease (HD). In a health screening setting we evaluated the comparative effectiveness of the CardioChek POCT device compared to a chemistry reference instrument (Siemens RXL Max) in order to determine if consistent and proper classification of HD risk based upon fractionated cholesterol factors could be made, and if deemed comparable, whether the POCT device was commutable or a diagnostic equivalent. Three separate evaluation studies were performed within a 4 month period using the CardioChek PA point-of-care device to screen a set population of staff at community hospital. Phases 1 and 2, employed multi-analyte POCT lipid panels (POCT-A, POCT-B) consisting of Cholesterol, HDL, and Triglyceride; Phase 3 employed single analyte tests (POCT-C). All samples were tested using venous blood in the CardioChek and plasma from a lithium heparin sample on the reference analyzer. Results were captured and categorized based on the traditional risk category for the three analytes. This categorization determined that across not only multiple devices but also reagent strip lots and lipid panels that the CardioChek consistently classified patients within the appropriate risk category based on the reference lab classification to initiate physician follow-up and further treatment.

Conclusion: This study establishes that the utilization of a POCT device for fractionated cholesterol can be an effective health screening tool in a large "healthy population" to identify patients requiring further medical follow-up for prevention of heart disease POCT testing provides immediate test results suitable for patient counseling.

Study		Population Risk Classification Assignment (%)								
		Cholesterol (mg/dL)			HDL (mg/dL)			Triglyceride (mg/dL)		
System	Phase	<200	200-240	>240	<40	40-60	>60	<150	150-200	>200
Reference (n=44)	1	66	22	12	15	53	32	38	28	34
POCT A (n=44)	1	67	26	7	18	57	25	40	33	27
POCT B (n=44)	1	62	26	12	13	53	34	40	35	25
Reference (n=32)	2	72	25	3	13	43	44	88	12	0
POCT A (n=32)	2	82	15	3	18	53	29	81	13	6
POCT B (n=32)	2	70	21	9	9	53	38	81	10	9
Reference (n=31)	3	77	19	4	10	57	33	74	16	10
POCT C (n=31)	3	81	16	3	10	56	34	71	16	13

D-32

Evaluation of clinical performance of Abbott Precision Xceed Pro ketone meter

H. E. Yu, M. Agus, M. Kellogg. *Children's Hospital Boston, Boston, MA,*

Background: Diabetic ketoacidosis (DKA) is a serious condition that presents most commonly in acutely ill type 1 diabetes mellitus patients. Traditionally, diagnosis and management of DKA rely on the measurement of urine ketone levels along with blood glucose, anion gap and pH. These values provide estimates of the beta-hydroxybutyrate (BOH) blood concentration, and an indication of the severity of DKA. However, these indirect measurements do not reliably reflect the severity of ketoacidosis. Direct measurement of blood BOH with point-of-care meter is, therefore, thought to better aid DKA diagnosis and management.

Methods: Abbott Precision Xceed Pro ketone meter is an FDA approved point-of-care meter that measures BOH from whole blood. We evaluated the clinical performance of Abbott ketone meter at the core laboratory. Heparinized venous whole blood BOH concentration from DKA patients were measured by the Abbott ketone meter, and compared with the plasma BOH concentration measured with StanBio BOH reagent on the Roche Cobas c501.

Results: 51 pairs of specimens with plasma BOH between 0-10 mmol/L were measured. When the BOH measurements by Abbott ketone meter and Roche Cobas were compared, 36 pairs of the specimens were within a TEa of 0.3 mmol/L or 20%. Of the remaining 15 pairs of specimens, 14 pairs displayed a difference of 25% - 43%, most of which had a plasma BOH concentration greater than 5 mmol/L. In those patients, plasma ketone concentrations (StanBio) trended downward during treatment, whereas whole blood ketone (Abbott) concentrations remained relatively unchanged.

Discussions: Measurement of BOH by the Abbott ketone meter up to ~ 5mmol/L correlates well with the StanBio method when measured in the core laboratory. While a linearity of 0-5 mmol/L may be sufficient to aid in the diagnosis of DKA, the Abbott ketone meter may not be accurate enough to allow physicians to follow the progress and response to therapy in hospitalized DKA patients with BOH values greater than 5 mmol/L.

D-33

Comparison of the AQT90 FLEX and Mini Vidas Assays in D-dimer Analysis for the Determination of Vascular Traumatic Events.

E. N. Shea, A. H. Wu, S. Tu, A. Wong. *UCSF, San Francisco, CA,*

Objective: D-dimer, a protein fragment remaining after the degradation of a blood clot, can be measured in the blood to determine the likelihood of a vascular traumatic event. Most commonly the test is used to rule out deep vein thrombosis and pulmonary embolisms. From a diagnostic standpoint these VTEs can share the same symptoms (shortness of breath, chest pain, and leg swelling) as a host of other medical problems so it is imperative to rule out a thrombosis as soon as possible. Point-of care devices shorten the turn around time for blood test results and therefore prove valuable in an emergent situation. Our study measured D-dimer levels on the AQT90 FLEX (Radiometer, Inc), a point-of-care device against the mini Vidas (bioMérieux, Inc), used in the clinical laboratory to determine the degree of correlation between the two assays.

Methods: Data was collected from 116 unique individuals enrolled at UCSF. Of the subjects, 12 were diagnosed with PEs and one with a DVT. The plasma samples were collected on sodium citrate and were obtained frozen from the clinical labs after being

used for clinical testing. The samples were defrosted and tested in batches on the AQT90 FLEX and mini Vidas within 2 hours of each other. Subjects who were not diagnosed at time of enrollment with a VTE were then contacted 30 days later to determine if any new thrombosis had occurred.
Results: The subjects consisted of men (n=49) and women (n=67) with a mean age of 51.1 years (SD=17.1). Their ethnicities were covered in five groups; White(n=40), Black(n=31), Asian(n=12), Hispanic(n=8) and Other(n=16). Well's scores captured for 51 subjects included Low(n=30), Moderate(n=19) and High(n=2), although none of the VTEs received a Well's score. Of the 103 patients who did not have a VTE, 89 were contacted and none had thromboses at the 30 day follow up point. One patient expired due to unrelated causes and another suffered a PE several months later. A regression analysis of the two assays shows a slope of 0.908 and intercept of 77.019 with results above 5000ng/mL removed as outliers as results above 500ng/mL are considered a positive test. Comparing the subjects, non-VTEs vs. VTEs have linear regressions of y= 0.950x +1.177and y=0.577x +2.409 respectively. There was concordance between the two assays with all but four results in respect to positive and negative tests. The AQT correctly identified a PE as positive and two subjects as negative that the Vidas reported to the contrary. The AQT also reported one false positive that the Vidas did not. The remaining 11 VTEs were identified as positive on both assays.
Conclusion: There is a strong concordance between the AQT90 Flex results and the predicate Vidas assay. As D-dimer is used as a rule out test, done before confirmatory imaging, the AQT provides accurate results from a point-of-care location to help health care providers make time sensitive diagnoses.

D-34

Accuracy Comparison Test of the Response Biomedical Corporation RAMP® NT-proBNP Assay Across Multiple Test Centers

S. Tu[1], A. H. B. Wu[1], F. S. Apple[2], M. M. Murakami[2], F. Koura[3], R. Silverman[4], J. A. Cottral[4], S. M. DeCicco[4], R. Christenson[5], S. Henderson[5], T. S. Sligh[6], S. P. Sligh[6]. *[1]UCSF, San Francisco, CA, [2]Hennepin County Medical Center, Minneapolis, MN, [3]Kentucky Lung Clinic, Hazard, KY, [4]North Shore Long Island Jewish Hospital, New Hyde Park, NY, [5]University of Maryland School of Medicine, Baltimore, MD, [6]Providence Clinical Research, Burbank, CA,*

Background: The RAMP NT-proBNP Assay is an in vitro point-of-care (POC) device that identifies NT-proBNP, a biological marker that assists in the diagnosis of congestive heart failure. Point-of-care devices such as this one can provide results immediately and in a timely manner.
Method: This is a multi-center study to test for accuracy of the RAMP NT-proBNP assay. Sites will enroll patients that are 18 years and older that correspond to the three groups exhibiting Heart Failure, non-Heart Failure and healthy patients (those that do not have heart failure, hypertension, diabetes, renal or pulmonary disease). EDTA whole blood is tested on the RAMP NT-proBNP POC device. EDTA plasma samples are taken from centrifuged whole blood, frozen and then sent to the Central Lab to be tested by Roche Elecsys.
Results: A total of 322 evaluable cases were the outcome from 6 sites and had NT-proBNP levels from 29 ng/L to 21598 ng/L on the RAMP assay and 8.42 ng/L to 25061 ng/L on the Roche assay. Out of the total subjects, 222 were from the Heart Failure group under NYHA (New York Heart Association) classifications; 48 subjects were from healthy individuals and 52 were non-healthy individuals with diabetes, hypertension etc. In a Linear Regression comparison between RAMP and Roche, the correlation was RAMP NT-proBNP = 284.7 + 0.8334 x Roche Elecsys with R = 0.922. The R-squared and adjusted R-squared is 0.85; the p-value in the slope resulted with <0.0001. When analyzing the Ramp and Roche assay results by Passing Bablok regression, the correlation was RAMP NT-proBNP = 27.57 + 0.98 x Roche Elecsys with the null hypothesis of constant bias and proportional bias. The 95% confidence interval of constant bias was 17.84 - 35.70 ng/L where as the proportional bias was 0.94 - 1.02 ng/L.
Conclusions: The point-of-care RAMP NT-proBNP Assay results show a strong positive correlation when compared to the Roche Elecsys assay results from a central laboratory.

D-35

Clinical Utility of Point-of-Care Prothrombin Times and International Normalized Ratios in Predicting Coagulation Deficiency in Patients Undergoing Cardiopulmonary Bypass

A. Chin, R. Molinaro, A. Duncan, C. Fantz, K. Tanaka. *Emory University, Atlanta, GA,*

Background: Coagulation factor deficiencies are common immediately after cardiopulmonary bypass (CPB) and their prompt identification is warranted to manage excessive bleeding episodes. The prothrombin time (PT) and international normalized ratio (INR) could be potentially useful in determining the indication for fresh frozen plasma (FFP) transfusion in the event of bleeding after CPB. Point-of-care (POC) PT/INR testing within the operating room while the patient is on CPB may provide immediate feedback on the patient's coagulation status and reduce delays in FFP transfusion if needed (Ho A, Anesth Analg 2003; 96:15-20).
Objective: To determine the clinical utility of POC PT/INR in predicting coagulopathy during CPB.
Methods: Venous whole blood obtained from seven patients before (baseline), during (on-pump), and after CPB (post-protamine) were used to measure PT/INR by the CoaguChek S meter and strips (Roche Diagnostics) and the Hemochron Signature Elite meter and cuvettes (ITC Medical). Citrated venous plasma was also obtained to measure PT/INR by reference methods (Siemens BCS Thromborel S and the Stago Neoplastine CI Plus). Assay precisions were determined using respective manufacturer controls. On-pump whole blood POC PT/INR was measured after pre-treatment with heparinase (Hepzyme, Siemens) to neutralize heparin.
Results: PT/INR intraassay precisions for the CoaguChek were 3.1/6.5% (4.5 sec./1.4) and 4.5/8.7% (23.3 sec./3.7), while the Hemochron were 4.4/8.0% (29.1 sec./2.0) and 4.7/8.1% (54.5 sec./5.8). Treatment with heparinase led to better normalization of on-pump CoaguChek PT/INR readings as compared to the Hemochron and the CoaguChek was in close agreement with the Thromborel method (See Table). **Conclusion:** The CoaguChek exhibits very good precision and better correlation with the Thromborel as compared to the Hemochron. Utilization of POC PT/INR may prove useful for predicting bleeds in patients undergoing CPB.

CPB Patient Specimen Comparisons

	1		2		3		4		5		6		7	
	PT (sec.)	INR	PT (sec.)	INR	PT (sec.)	INR	PT (sec.)	INR	PT (sec.)	INR	PT (sec.)	INR	PT (sec.)	INR
Baseline														
CoaguChek	12.2	1.0	13.0	1.1	12.5	1.0	12.8	1.1	14.2	1.3	13.1	1.1	12.6	1.0
Hemochron	22.0	0.8	21.0	1.2	21.0	1.2	21.0	1.2	24.0	1.5	23.0	1.4	21.0	1.2
Thromborel	11.7	1.0	13.1	1.1	12.5	1.1	11.8	1.0	14.8	1.3	17.6	1.5	12.4	1.1
On-pump Heparinase-Treated														
CoaguChek	16.4	1.8	17.6	2.0	20.2	2.8	18.4	2.3	21.5	3.2	17.0	1.9	17.0	1.9
Hemochron	58.0	6.4	57.0	6.3	37.0	3.0	33.0	2.5	41.0	3.6	42.0	3.7	39.0	3.3
Off-pump Post-Protamine														
CoaguChek	15.0	1.5	13.4	1.2	error*	error*	error*	error*	14.8	1.4	error*	error*	error*	error*
Hemochron	28.0	1.9	28.0	1.9	26.0	1.7	25.0	1.6	27.0	1.8	33.0	2.5	27.0	1.8
Thromborel	19.7	1.6	15.8	1.4	15.6	1.3	15.9	1.4	17.5	1.5	17.6	1.5	18.5	1.6

*Analytical sampling error

D-36

Point-of-care system for rapid HIV-1, syphilis, and hepatitis B serodiagnosis

M. Lochhead[1], K. Todorof[1], M. Delaney[1], J. Ives[1], C. Greef[1], C. Myatt[1], X. Zhang[2], S. Reed[2], C. Benson[2], R. T. Schooley[2]. *[1]mBio Diagnostics, Inc., Boulder, CO, [2]University of California, San Diego, CA,*

BACKGROUND: Opportunistic infection in HIV infected individuals leads to increased morbidity and mortality. Ideally, co-infections would be identified at the time of initial HIV diagnosis. Unfortunately, co-infection screening is often cost-prohibitive and impractical, particularly in resource-limited settings. To address this unmet need, an inexpensive, point-of-care system to simultaneously diagnose HIV and co-infection status is presented. It is comprised of single-use cartridges and a simple fluorescence reader. A combination HIV-1, syphilis, hepatitis B combination assay is demonstrated here.
METHODS: Single-use assay cartridges incorporating antigen/antibody microarrays were designed for simultaneous detection of HIV-1 and *T. pallidum* antibodies in serum, plasma, or whole blood. Antibodies against three different antigens per pathogen are detected. The same fluidic channel also includes capture antibodies for a hepatitis B surface antigen (HBsAg) sandwich assay (direct antigen detection). The system uses 5 microliters of sample diluted into carrier buffer. After incubation with patient sample, a cocktail of detect antibodies (dye labeled anti-human IgG and dye-labeled anti-HBsAg) are added to the channel. Cartridges are read on a fluorescence imaging unit powered by a USB port. Total test time is ~25 min per sample, and up to 12 samples can be run simultaneously by a single operator. Clinical samples were collected at the UCSD Medical Center and selected for likely HIV, hepatitis B, and syphilis infection. Additional samples were from collections at the UCSD Antiviral Research Center, as well as commercial controls. All had known primary infection status determined by clinical laboratory methods. A subset of HIV-positives had known HBV or syphilis co-infection. The system was used to generate quantitative signal data for all antigens on all samples. Known negative samples were used to establish empiric cutoff values. Signal/cutoff values were then calculated and these values were compared to known disease status.
RESULTS: 170 samples had known HIV status (55 pos; 115 neg). 54/55 HIV(+) samples were called correctly. The one false negative was also neg on a commercial HIV rapid test (OraQuick). 115/115 HIV(-) samples were called correctly. Results for two seroconversion panels demonstrate equivalent performance to leading EIAs. 45/46 Trep(+) samples were called correctly with one indeterminate. 111 samples were RPR-negative; 108 were Trep(-) on the multiplexed system, with three Trep(+) / RPR (-) samples likely resolved infection or latent syphilis. HBsAg titration curves (antigen spiked serum) demonstrate lower-limit of detection of 0.5 ng/mL in this simple assay.
CONCLUSIONS: The point-of-care system presented here is low cost, robust, and has been successfully demonstrated for a HIV-1/syphilis/HBV combination assay. The system has particularly utility for limited resource settings.

Wednesday PM, July 28

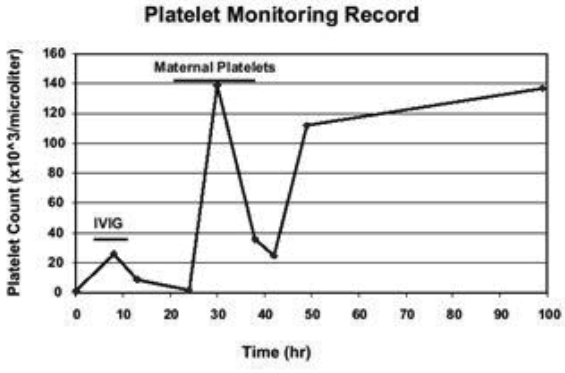
Poster Session: 2:00 pm – 4:30 pm
Hematology/Coagulation

D-37

A Rare Neonatal Thrombocytopenia Case Report-Platelet less than 1,000/µl

X. Liu, B. H. Durham, J. Grishaber, M. Creer, C. A. Vogler. *Saint Louis University, Saint Louis, MO,*

Background: Neonatal Alloimmune Thrombocytopenia (NAT) is the most common cause of severe fetal and neonatal thrombocytopenia. It is due to a transplacental maternal alloantibody, IgG, against paternally inherited platelet antigens on the fetal platelets but absent from the maternal platelets. It occurs in 1 in 2000 births. In the US Caucasian population, the most common antigen is HPA-1a. Clinical presentations vary from petechiae, hematomas, and gastrointestinal bleeding in the newborn to hydrocephalus in utero. The most severe complication is intracranial hemorrhage. Treatment is usually started when platelets are less than 30,000/µl. A transfusion of washed, irradiated, maternal platelets is the first choice; intravenous IgG and corticosteroids also help. Previously, the lowest platelet count reported in NAT was less than 10,000/µl.
Clinical case: A male infant was born to a 29 y/o G1P1 mother at 42+2/7 weeks' gestational age. APGAR scores were 9/9 at 1/5 minutes. Bruising of the right ear lobe was noted at delivery. Thirty five hours later, circumcision was performed. Five hours later, the infant started oozing blood from the left base of the penis. At that time, his platelets were 9,000/µl; the repeated platelet count was 11,000/µl. The patient was transferred to our hospital, and the platelet count was less than 1,000/µl. Appropriate management was instituted. On hospital day 5, the patient's platelet count was 107,000/µl, and the patient was discharged. The follow up platelet count was 205,000/µl four days later, and the infant was in good condition. The infant's blood drawn on the second day of life for antibody screening was read as reactive on the 10/13 wells that are HPA-1a antigen positive and not reactive on the 3/13 wells that are HPA-1a antigen negative.
Conclusion: Most likely, this infant's thrombocytopenia is due to NAT anti-HPA-1a antibodies. The platelet monitoring chart is attached.



D-38

Rapid detection of cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex 1 (VKORC1) polymorphisms associated with warfarin dose requirement by SMart Amplification Process

E. Miyajima, H. Hirose, S. Kida, H. Tanaka, Y. Kato, Y. Katayama, H. Yonezawa, S. Sumita. *Yokohama City University Medical Center, Yokohama, Japan,*

Background: The aim was to validate a rapid detection method for polymorphic alleles related to warfarin dose requirement using the Smart Amplification Process (SMAP), which is a newly developed method for single nucleotide polymorphism (SNP) detection by Mitani et al. Furthermore, clinical usefulness of this rapid detection method was also assessed.
Methods: 120 patients receiving anticoagulation therapy with warfarin and 30 untreated healthy volunteers were participated in this study. The cytochrome P-450 2C9 enzyme (CYP2C9) and vitamin K epoxide reductase complex 1 (VKORC1) polymorphisms were identified in all samples using a Warfarin Dosage Test kit (DANAFORM, Yokohama, Japan) based on the SMAP. In brief, blood samples were treated with an approximately twofold volume of pretreatment solution and heated at 98 ºC for 3 min. A 25 µl reaction mixture containing 1 x Reaction Mix, 1/100,000 diluted SYBR Green I (Molecular Probes), 1.0 µl Aac DNA polymerase, 2.0 µl of each allele-specific primer mix, and 1.0 µl pretreated blood sample was incubated at 60 ºC. Amplification was monitored with a real-time PCR instrument, the MX 3005P System (Stratagene), for 40 min, and determined according to fluorescence intensity. 37 samples were re-tested by PCR-RFLP analysis.
Results: The results of genotyping using the Warfarin Dosage Test kit were all consistent with those obtained by PCR-RFLP analysis. The time required for this assay by SMAP was only 60 min, and the procedure for sample preparation was very simple and easy. The frequencies of CYP2C9*1/*1, CYP2C9*1/*3, VKORC1-1639G>A and VKORC1-1639A were 0.96, 0.04, 0.17 and 0.83, respectively, and were consistent with the results obtained by other investigators in Japanese populations.
Conclusion: Genotyping with the Warfarin Dosage Test kit is accurate, rapid and has simple sample preparation, and is suitable for routine testing in clinical laboratories and contributes to making quick therapeutic decisions.

D-39

Hypotension during hemorrhagic SHOCK, oxidative burst and severity of lung injury

V. Villiotou[1], O. Livaditi[2], P. Prigouris[2], P. Paneris[2], E. Douzinas[2]. *[1]Anticancer Hospital of Piraeus, Piraeus, Greece, [2]3rd Dpt of Critical Care, University of Athens, Medical School, Athens, Greece,*

Background: Hemorrhagic shock is the major factor in post injury multiple-organ failure; prolong intensive care stay and late trauma mortality. Hemorrhagic shock- resuscitation can be viewed as a global hypoxia- reoxygenation injury insult. Although hypoxia alone can produce tissue injury, it is well known that exposure of hypoxic tissue to oxygen upon reperfusion is the major cause of tissue damage. The sudden reintroduction of oxygen results in a burst of reactive oxygen species (ROS) which may increase the tissue damage through oxidative stress. Thus, hemorrhagic control and optimal resuscitation are the main goals in the management of severely injured patients.
Methods: Twenty-one rabbits were equally allocated into three groups: sham-operated (n=7); Shock-40 bled within 30 minutes to mean arterial pressure (MAP) of 40 mmHg; and Shock-30, bled within 30 minutes to MAP of 30 mmHg. Shed blood was re-infused with two volumes of Ringer's lactate within 30 minutes and animals were monitored for another 120 min. At the end of the resuscitation period bronchoalveolar lavage (BAL) and lung histology were performed and parameters of oxidative stress were measured. In the BAL fluid malondialdehyde (MDA), total antioxidant capacity (TAC), and oxidative burst were determined, on lung tissue histopathologic score was evaluated and myeloperoxidase (MPO) levels were determined.
Results: The median values of MDA were significantly increased in BAL fluid from shock-40 to shock-30 groups ($p=0.001$), and from sham to shock-30 ($p=0.001$), but not between sham -group and shock-40. An inverse pattern was observed for TAC values. The median values of TAC were significantly decreased in BAL fluid from sham to both shock groups ($p<0.05$ for both comparisons), but not between shock groups. The median values of MPO were significantly increased in BAL fluid from sham to both shock groups ($p=0.001$ for both comparisons), but not between shock-30 and shock-40 group. The median values of fluorescence intensity GMFI quantifying oxidative burst as well as lung histopathology score were significantly increased from sham group to shock-40, to shock-30 group ($p=0.001$ between sham and shock groups, and $p<0.05$ between shock groups).
Conclusion: Parameters of BAL fluid oxidative stress are related with the degree of lung injury arising during post-ischemic hemorrhagic shock resuscitation and both parallel to the extent of shock. Whole blood reactive oxygen species, the parenchyma neutrophil content as it was estimated by myeloperoxidase activity and the histopathologic score were elevated with statistically significant difference ($p<0.05$) in the shock-30 vs to shock-40 showing that the degree of inflammation and lung injury were arising during post-ischemic hemorrhagic shock resuscitation period and were depended of the extent of the shock. BAL fluid oxidative stress as MDA index and TAC were related with the extent of hemorrhagic shock. The degree of hypotension during hemorrhagic shock has a significant impact on the oxidative burst and the severity of lung injury and the optimal resuscitation for example a hypoxemic resuscitation could contribute to the good management of severely injured patients.

D-40

Vkorc1, Cyp2c9 And Cyp4f2 Pharmacogenetics: Relevance In Warfarin Maintenance Dose Prediction Among Italian Patients.

C. Zambon[1], D. Basso[1], S. Moz[1], P. Fogar[1], S. Schiavon[1], M. Pelloso[1], E. Greco[1], F. Navaglia[1], A. Frigo[2], V. Pengo[3], R. Padrini[4], M. Plebani[1].
[1]Dept. Laboratory Medicine University of Padova, Padova, Italy, [2]Dept. of Environmental and Public Health University of Padova, Padova, Italy, [3]Dept. of Cardiothoracic and Vascular Sciences, University of Padova, Padova, Italy, [4]Dept. of Pharmacology and Anesthesiology, University of Padova, Padova, Italy,

Background. Warfarin is a widely used anticoagulant with a narrow therapeutic index and relevant inter-individual variability in dosing requirements. The present study was conducted to ascertain the influence of pharmacogenetics on the maintenance dose of warfarin.

Methods. We retrospectively selected 437 Italian patients (277 males and 160 females, mean age 71.9yrs, range 28.9-92.8 yrs) under stable warfarin therapy (target INR=2.5). The indications for oral anti-coagulation were: atrial fibrillation and venous thromboembolism. Clinico-demographic features as well as information on smoking habit, alcohol and coffee consumption were collected. The gene polymorphisms of VKORC1 (-1639G>A SNP), CYP2C9 (*1,*2 and *3 alleles)[§] and CYP4F2 (*1 and *3 alleles)[§] were analyzed by means of PCR with TaqMan fluorescent chemistry starting from whole blood extracted DNA. In plasma samples ALT, GGT and albumin levels were measured.

Results. The mean weekly warfarin dose (WWD) (range 3.75 - 80 mg), higher in males than females (t=3.47; p<0.001), was associated inversely with age (F=10.96; p<0.0001) and directly with Body Surface Area (BSA) (F=15.39; p<0.0001), coffee consumption (t=2.50; p<0.05) and ALT levels (r=0.199, p<0.0001). WWD was associated with VKORC1, CYP2C9 and CYP4F2 polymorphisms (F=127.68; p=<0.0001, F=28.80; p<0.0001 and F=6.52; p<0.005 respectively) being lower in VKORC1 and CYP2C9 mutated homozygotes, intermediate in heterozygotes and higher in wild type homozygotes (CYP2C9*1*1 or VKORC1 G/G). By contrast it was lower in CYP4F2*1*1 homozygotes compared to subjects bearing at least one CYP4F2*3 mutated allele.

Multiple linear regression analysis was performed and a pharmacogenetic algorithm for warfarin maintenance dose prediction was derived and validated: the R^2_{adj} was 65.4% and 55.5% while the mean Absolute Error (± SE) was 6,80 ± 0,38 and 7,03 ± 0,58 mg/week in the derivation and validation cohorts respectively. The algorithm included as significant predictors VKORC1, CYP2C9 and CYP4F2 polymorphisms, BSA and age.

The percentage of cases whose predicted WWD was within 20% of the actual maintenance dose was 51.8% considering patients overall; taking into account patients requiring low (43.75 mg/week) maintenance dose, the percentages were 36.2%, 66.2% and 55.4% respectively. Overall the pharmacogenetic algorithm could correctly assign 73.8% and 63.2% of patients to the low and high dose regimens respectively.

Conclusions. The pharmacogenetic algorithm developed in this study, new with respect to others in that it comprises CYP4F2 polymorphisms, allows the correct prediction of low or high warfarin dose regimens in the great majority of cases. Patients in these categories may therefore benefit greatly from a pre-treatment application of the maintenance dose prediction.

[§] according to the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (http://www.imm.ki.se/CYPalleles/).

D-41

Comparative performance of the UniCel DxH 800, Cell-Dyn Sapphire, and LH 780 hematology analyzers in a Tertiary Care Hospital

B. Tan, A. Nava, T. George. *Department of Pathology, Stanford Univeristy, Stanford, CA,*

INTRODUCTION: The new DxH 800 hematology analyzer (Beckman Coulter, Miami, FL) is a scalable, fully automated hematology analyzer system capable of analyzing up to 100 samples per hour and providing a CBC, WBC differential, NRBC and reticulocytes from 165uL of sample. This new design incorporates a new flow cell design, advanced algorithms, and significantly less tubing in a much smaller analyzer footprint than its predecessor, the Beckman Coulter LH 780 analyzer. We compared performance of the DxH 800, the LH 780 (Beckman Coulter) and the Cell-Dyn Sapphire hematology analyzer (Abbott Diagnostics Division, Santa Clara, CA). Our aims were to compare the CBC, automated differential, NRBC count and overall efficiency of each analyzer on samples collected in a busy tertiary care hospital.

METHODS: We ran a total of 430 adult samples on all instruments in parallel, according to NCCLS guidelines. We compared numerical test results from the DxH 800, the LH 780 and the Sapphire, as well as each instrument's WBC differential versus a reference 400-cell manual differential. We also compared flagging efficiencies by determining whether flagged specimens showed abnormalities on a peripheral blood smear as defined by criteria from the International Committee for Standardization in Haematology.

RESULTS: Using linear regression analysis, the CBC showed excellent correlation between the DxH 800 and LH 780, including WBC (r = 0.998), RBC (r = 0.997), HGB (r = 0.995), MCV (r = 0.985), and PLT (r = 0.997). The DxH 800 also correlated well to the Sapphire, including WBC (r = 0.999), RBC (r = 0.990), HGB (r = 0.995), MCV (r = 0.980), and PLT (r = 0.998). The automated differential from the DxH 800 compared well with the reference 400-cell manual differential (neutrophil r = 0.852, lymphocyte r = 0.939, monocyte r = 0.510, eosinophil r = 0.943, and basophil r = 0.522). Similar performance was observed with the Sapphire (neutrophil r = 0.842, lymphocyte r = 0.931, monocyte r = 0.355, eosinophil r = 0.850, and basophil r = 0.392) and LH 780 (neutrophil r = 0.850, lymphocyte r = 0.942, monocyte r = 0.580, eosinophil r = 0.940, and basophil r = 0.149). Correlation of the automated NRBC count was similar for the DxH 800 (r = 0.982), Sapphire (r = 0.990) and LH 780 (r = 0.958). The DxH 800 flagged specimens with an abnormal peripheral blood smear with a sensitivity of 86.7% and a specificity of 72.7%, yielding an overall efficiency of 76.5%. By comparison, the sensitivity, specificity, and efficiency of the Sapphire flags were 91.8%, 67.3%, and 74.0%, respectively, while those of the LH 780 were 93.9%, 56.2%, and 66.5%, respectively.

CONCLUSIONS: The DxH 800 provides CBC and NRBC results comparable to the LH 780 and the Cell-Dyn Sapphire. All three analyzers provide an automated differential that shows good correlation with a reference 400-cell manual differential. In our evaluation, which includes a high proportion of specimens from critically ill patients, both the DxH 800 and Sapphire show better flagging efficiency than the LH 780.

D-42

Hemoglobin Korle Bu has two different chromatograms

P. J. Howanitz[1], J. H. Howanitz[1], Y. S. Chauhan[2]. *[1]SUNY Downstate and Kings County Hospital Center, Brooklyn,, NY, [2]Kings County Hospital Center, Brooklyn,, NY,*

Hemoglobin identification and quantification is most commonly performed in U.S. clinical laboratories using High Performance Liquid Chromatography (HPLC) equipment, and of these systems, the Bio-Rad Variant II β-thalassemia Short Program instruments (Bio-Rad, Hercules CA) are the most frequent. Common hemoglobin variants elute during the 5.30 minute run time into specific timed windows, although other rare variants also elute into these specific windows. The A_2 window currently extends from 3. 30 to 3.90 minutes, and the D window from 3.90 minutes to 4.30 minutes, but there have been small changes in the boundaries of these windows over the years depending on the reagent and cartridge lots. The chromatograms are calibrated according to the amount of hemoglobin F and A_2 present, but if no A_2 is present, the ordinate changes from % hemoglobin to volts. Variants are identified on chromatograms by their retention times, the variant peak shapes, and variant percentages. Unknown variants are confirmed by hemoglobin electrophoresis, and if clinically significant, by further laboratory studies.

Hemoglobin Korle-Bu (G-Accra) is a hemoglobinopathy (β-73, Asp→Asn) that is the most frequent rare beta chain point mutation in populations from West Africa. Since 2003, we have evaluated approximately 1,500,000 hemoglobin chromatograms and have diagnosed Hemoglobin Korle-Bu in 61 cases. Fifty-eight of these cases were heterozygotes (AKorle-Bu), and three were double heterozygotes (SKorle-Bu).

We have found that hemoglobin Korle-Bu presents with two distinct chromatographic patterns making Korle-Bu identification difficult, as its elution straddles the juncture of the A_2 and D windows. In the majority of the heterozygotes (39/58), hemoglobin Korle-Bu eluted in the hemoglobin A_2 window with average elution time of 3.84 minutes (range 3.72-3.89 minutes) with an A_0 retention of 2.46 minutes (range 2.44-2.53 minutes) and the ordinate expressed as % hemoglobin. In 19 or the 58 heterozygotes, hemoglobin Korle-Bu eluted in the D window with an average retention time of 3.94 minutes (range 3.87-4.07 minutes), whereas A_0 eluted at 2.48 minutes (range 2.43-2.54 minutes). In one of the double heterozygotes, Korle-Bu eluted in the A_2 window at 3.85 minutes (A_0 2.28 minutes) and in the other two double heterozygotes, Korle-Bu eluted in the D-window at 3.90 and 3.94 minutes, (A_0 2.33 and 2.28 minutes respectively). In all cases where Korle-Bu eluted in the D window, the ordinate was expressed in volts, as A_2 was not detected. Lower column temperatures cause hemoglobin variants to elute earlier, and when we lowered the column temperature for a Korle-Bu variant eluting at 3.94 minutes in the D window, we found that this variant eluted in the A_2 window at 3.78 minutes. Consequently, when we elevated the elution time for a Korle-Bu variant eluting in the A_2 window, this variant eluted in the D window.

We conclude that interpretation of HPLC chromatograms for hemoglobin identification and quantification is far more difficult than electrophoresis, as for hemoglobin Korle-Bu, there are two different chromatographic patterns. We caution that the variability of elution times of various hemoglobins over a period of years is greater than what previously has been described.

D-43

Hemostatic Abnormalities During Complex Cardiac Surgery For Congenital Heart Disease: Shone´S Syndrome

J. Noval Padillo, C. Serra-Gomez, A. Leon Justel, F. Garcia Torrado, R. Hinojosa, A. Hosseinpour, J. Luis-Navarro, J. Guerrero Montavez. *Universitary Hospital Virgen Del Rocio, Sevilla, Spain,*

OBJECTIVE: To present a case that illustrates the usefulness of a Mobile Laboratory Unit within the operating area for the monitoring of coagulation defects in the context of complex cardiac surgery.RELEVANCE: Repeat cardiac surgery and the possible use of hypothermia during complex procedures augment the risk of coagulopathy.It is difficult to foresee who would be affected to what degree and in which aspect of the coagulation system. Therefore, the therapy offerred is often empirical with unpredictable results. For the therapy to be more specific and precise, more information regarding the coagulation profile of the patient is required rapidly.

METHODOLOGY: We present the case of an eighteen-year-old man with Shone syndrome who underwent repeat cardiac surgery for augmentation of the left ventricular outflow tract (the Konno procedure) and aortic and mitral valve replacement. Blood samples were taken at induction of anesthesia, on bypass, and after bypass once protamine had been given. These samples were analysed on site,to assess the coagulation profile, full hematological profile and gasometry of each patient in each of the three surgical phases.

VALIDATION: The coagulation defects are monitored using rotational thromboelastometry (ROTEM®) using different reagents. Therefore, the coagulation profile and both the intrinsic and extrinsic pathways (inTEM®, exTEM®) were assessed. The hematologic study is done with a hematologic counter (Sysmex® poch-100i). The gasometry is done with astandard gasometry machine (Cobas® b 221).

RESULTS: The duration of bypass was 240 minutes with a minimum temperature of 28 degrees. During bypass, the ROTEM indicated a platelet dysfunction with an MCF of 41 (Normal values > 50), and a functional deficit with respect to fibrinogen with an MCF of 4 (Normal vlaues > 9). After rewarming and the adjustment of the biochemical parameters, the relevant blood products were administered as indicated by the tests. Such thereapy improved the two MCFs to 49 and 8 respectively. Further administration of the relevant blood products resulted in values of 57 and 9 in the ICU.

CONCLUSION: This case shows that the Mobile laboratory Unit within the operating area is able to provide us rapidly with information on specific coagulation defects. needed.

D-44

Fresh and citrated whole blood speicmens can produce different thromboelastography results in patients on extracorporeal membrane oxygenation

E. A. Jaben, C. D. Koch, B. J. Krekelberg, B. S. Karon. *Mayo Clinic, Rochester, MN,*

Background: The use of citrated whole blood has been published as an alternative to fresh (not anticoagulated) whole blood for use in thromboelastography (TEG). We compared TEG tracings from fresh and citrated whole blood samples obtained from patients on extracorporeal membrane oxygenation (ECMO) and on cardiopulmonary bypass.

Methods: Samples of fresh and citrated whole blood, collected nearly simultaneously, were analyzed for 6 patients on ECMO and 19 patients immediately after cardiopulmonary bypass. TEG was run using kaolin as an activator in a plain plastic cup. TEG tracings were begun within four minutes of sample collection for fresh whole blood specimens and within 15 minutes for citrated samples. In ECMO patients, heparinase cups were also used with both fresh and citrated whole blood samples. Citrated samples were recalcified using calcium chloride per the manufacturer's recommendations prior to analysis. Parameters including R, angle, and maximum amplitude (MA) were evaluated and compared for differences between fresh and citrated whole blood samples.

Results: Samples were collected from a total of six ECMO patients. In three patients citrated whole blood samples showed near-normal coagulation status (R, angle and MA values only slightly outside the normal ranges), while fresh whole blood samples demonstrated profound coagulopathy attributed partially to heparin administration. For these patients the TEG tracing in citrated whole blood was nearly identical to the tracing produced after heparinase treatment of fresh whole blood. In two patients there was the appearance of partial reversal of heparin in the citrated sample. In one patient, little difference between fresh and citrated whole blood TEG results was noted. Following this observation, we collected fresh and citrated whole blood from 19 patients who had TEG ordered following cardiopulmonary bypass (after protamine reversal of heparin). The majority of TEG tracings from citrated samples had slightly shortened R values and mildly increased MA, while the angle was not different between sample types. This mildly pro-coagulant effect of citrate anticoagulant has been reported previously. We did not observe the apparent heparin reversal effect in any of the cardiopulmonary bypass patients.

Conclusion: There is some factor, which at present is poorly understood, causing a significant difference in the TEG tracings of some ECMO patients when citrated samples are used. In these patients, use of citrated samples for TEG appears to reverse the anticoagulation effects of heparin *in vitro* and could potentially result in inappropriate heparin administration or excessive anticoagulation. We were unable to reproduce this in patients following cardiopulmonary bypass, despite their inherent coagulation abnormalities. Until this effect is understood, patients on ECMO should have fresh whole blood samples collected for TEG testing.

D-45

Immunophenotyping In The Diagnosis And Classification Of Acute Leukemia: An Indonesian Cancer Hospital Experience

L. Setiawan, A. S. Kosasih, S. H. Harijanto, S. B. Kresno. *Dharmais Cancer Hospital, West Jakarta, Indonesia,*

Background Immunophenotyping is one of the methods recommended in the diagnosis and classification of leukemia. We present data on the role of immunophenotyping in the diagnosis of acute leukemia in the Clinical Pathology Laboratory, Dharmais Cancer Hospital from January 2005 until July 2007.

Methods In this retrospective study, subjects were divided into 2 groups: children (<18 years) and adult.

Cytomorphology, cytochemistry and immunophenotyping data were compared and analyzed descriptively. We used Wright stain for cytomorphology, and Sudan Black B for cytochemistry. Immunophenotyping was performed on FACSCalibur flowcytometer, with myeloid panel (CD33, CD13, CD14), lymphoid panel (CD3, CD5, CD7, CD10, CD19, CD20, CD22), CD34 and HLA-DR.

Result During the period, there were 451 cases of acute leukemia, including 279 new cases, 162 evaluation of therapy and 10 relapse cases. There were 77 children, 191 adults and 11 subjects whose age was not recorded.

Of 337 requests for immunophenotyping, 189 (41.6%) was leukemia cases. Specimens submitted included marrow aspirates (170 cases, 90.0%), peripheral blood (18 cases, 9.5%) and lymph node juice (1 case, 0.5%).

Classification by cytomorphology and cytochemistry according to FAB criteria were: in children group 59(76%) had ALL and 18 (24%) AML. ALL-L1 subtype was most frequently found (43; 73%), followed by ALL-L2 (15; 25%). AML-M5a was the most frequent AML subtype in children (4; 22%).

In adult, AML was more frequently found (149; 78.0%) than ALL (42; 22.0%). The most frequent subtype was AML-M2 (40; 27%), followed by M4 (28; 18.9%), M1 and M5a (each 23 cases, 12.2%), M3 (13; 8.8%), M5b (6; 4.1%) and M6 (3; 2.0%). We found 1 M0 case which was morphologically indistinguishable from ALL and SBB negative, and the diagnosis was based on immunophenotyping.

According to phenotype, we found 9 cases of T-ALL, 64 B-ALL and 92 myeloid lineage. Two (22%) of T-ALL cases showed co-expression of one of the B-lineage antigen, while 1 (11%) case showed co-expression of myeloid antigen (CD33). Co-expression of antigen was found in 18 cases (28%) of B-ALL: myeloid antigen(s) CD33 (8 cases), CD13 (4 cases), CD 33 and CD13 (4 cases), and T-lineage antigen CD5 (1 case). One case of B-ALL showed co-expression of CD7 and CD13.

Lymphoid antigen was more frequently co-expressed in AML (31 cases, 34%). The most frequent lymphoid antigen co-expressed was CD19 (18 cases) and CD7 (9 cases). One case of AML expressed CD10 and CD19, and 2 cases showed co-expression of CD7 and CD19.

There were several cases in which FAB classification did not match or the diagnosis cannot be established without immunophenotypic analysis, as in the case of AML-M0. There were 2 cases with negative SBB, but proven to be AML by immunophenotyping. Two other cases were classified as M5a according to their morphology, but showed B-lineage immunophenotype.

Conclusions This study confirmed the usefulness of immunophenotyping in determining diagnosis of lineage, distinguishing between ALL and AML, especially in cases with negative SBB as in M0 and M5a, differentiating between B-ALL and T-ALL, and to diagnose cases of biphenotypic/ mixed lineage leukemia.

D-46

Experimental study of proliferation and adherence of TF-silenced endothelial progenitor cells from human umbilical cord blood

s. xiong, Q. Wang, L. Zheng, J. Bao. *Laboratory Medicine Center, Guangzhou, China,*

Background Endothelial progenitor cells (EPC) participate not only the embryonic vascularization, but also the vascular neogenesis after born and repair process after endothelial injury. Tissue factor (TF) is the primary cellular initiator of blood coagulation and also functions in vascular development, angiogenesis and tumor cell metastasis. TF expression could be up-regulated in endothelial cells by inflammatory cytokines.

However, the induction and inhibition of TF expression in EPC remains unclear. In this study, TF expression in EPC stimulated by TNF in the addition of siRNA against TF and the proliferation and adherence of TF-silenced EPC were studied.

Methods EPC were isolated from healthy human umbilical blood, the biological functions of EPC were examined with a laser scanning confocal microscope, the expression of specific surface mark CD133, CD34 and KDR were assessed by fluorescence activated cell sorter (FACS) analysis. TF expression were measured by RT-PCR and ELISA. TF2 and TF6, constructed to decrease the TF expression, were lentivirus packaged and collected. EPC proliferation and adherence were assayed by MTT assay and colorimetry respectively.

Result Adherent EPC were double positive for DiL-acLDL uptaking and lectin binding, the expression profile of cultured EPC included CD133 (27.05±2.94)%,CD34(16.37±2 .69%), and KDR (56.67±7.29%). TF mRNA and antigen were remarkably increased in EPC stimulated with 1000u/ml TNF, both lentivirus TF2 and TF6 could suppress the TF expression in EPC stimulated by TNF, and the inhibitory effect were more obvious in TF2 than that of TF6. There is no difference in proliferation and adherence in EPC and TF-silenced EPC by TF2.

Conclusion TNF could stimulate TF expression in EPC and TF-silenced EPC did not change the proliferation and adherence in vitro, which provide basis for EPC's further study and clinical application.

D-47

The inter-relation pattern of coagulation and fibrinolytic factors in Bleeders of Unknown Cause differs from healthy subject's pattern.

M. Henríquez[1], T. Quiroga[1], M. Goycolea[1], O. Panes[2], E. Aranda[2], J. Pereira[2], C. Vallejos[3], G. Marshall[3], D. Mezzano[2]. *[1]Departamento de Laboratorios Clínicos. Pontificia Universidad Católica de Chile, Santiago, Chile, [2]Departamento de Hemato-oncología. Pontificia Universidad Católica de Chile, Santiago, Chile, [3]Facultad de Matemáticas. Pontificia Universidad Católica de Chile, Santiago, Chile,*

Background: A significant proportion of patients with primary non-thrombocytophenic mucocutaneous bleeding remain without etiological explanation even after exhaustive laboratory explorations. This group of patients, currently named as "Bleeders of unknown cause (BUC)" entails a substantial diagnostic and therapeutic challenge and is remarkably understudied (Quiroga et al, 2007).

Based on clinical presentation, the bleeding symptoms in BUC have been explained in terms of unidentified deficits in primary hemostasis, most probably related to platelet-vessel wall interaction. However, due to recent evidence suggesting close relationship between primary and secondary hemostasis in vivo, a more careful search for alterations in secondary hemostasis in BUC patients is mandatory.

Objective: To explore eventual alteration in secondary hemostasis in BUC, with special focus on the relative balance of coagulation and fibrinolytic factors.

Methodology: Fasting blood samples were collected from 96 subjects (from 5 to 15 years old) previously categorized as bleeders of unknown cause and 227 controls at the same range of age. Clotting factor levels were determined by one-stage modified APTT or PT assays, using plasmas depleted in the respective factors (Dade-Behring, USA). Fibrinogen levels were determined by the Claus method. Protein C and Antithrombin III levels were assessed by means of ELISA standard assays. Protein S activity was assessed by a chromogenic standard method.

In order to explore inter-relations among coagulation/fibrinolytic factor levels, we conducted a cluster analysis by the VARCLUS method (SAS Institute, Cary, NC) inside the studied groups. VARCLUS method allows to divide large multidimensional data sets into few non-overlapping unidimensional clusters, each one of them conformed by variables that are as much correlated as possible among themselves and as uncorrelated as possible with variables in another clusters.

Results: BUC and controls were clinically comparable in regard to the basic hemostatic parameters and coagulation factors activity. All BUC patients included in this study presented normal activity for every one of the coagulation and fibrinolytic factors.

In spite of this, the inter-relation pattern among coagulation/fibrinolytic factors identified by means of the cluster analysis was completely different in BUC and controls. Control subjects presented 3 well differentiated clusters, the first one enclosing factors II, V, VII, X and fibrinogen, the second one enclosing factors VIII, IX, XI and XII and the third one enclosing the antithrombotic factors AT III, proteins S and C. The abovementioned cluster pattern was lost in the BUC group, which presented 3 less-defined clusters, the first one comprising factors II, V, VII and X, the second one comprising antithrombotic factors PS, PC, ATIII, plus factor XII, and a third cluster comprising factors VIII, IX, XI and fibrinogen.

Conclusions: Our results suggest that alterations in the balance among coagulation/fibrinolytic factors may be relevant in the pathogenesis of bleeding in BUC, even when the activity levels of these factors are in the normal range when analyzed separately.

D-48

Performance Characteristics of Serotonin Release Assay Using Low Molecular Weight Heparin and Fondaparinux

L. Song[1], J. S. Dlott[1], C. L. D. Haley[1], N. N. Gould[1], P. Chou[1], M. A. Sahud[2]. *[1]Quest Diagnostics Nichols Institute, Chantilly, VA, [2]Quest Diagnostics Nichols Institute, San Juan Capistrano, CA,*

Introduction: Heparin-induced thrombocytopenia (HIT) and HIT-associated thrombosis (HITT) are caused by antibodies binding to the platelet factor 4 (PF4)/heparin complex, resulting in platelet activation with aggregation and release of coagulation factors. These antibodies are found in up to 40% of patients receiving unfractionated heparin (UFH). HIT has a lower incidence (0.6%) in patients exposed to low molecular weight heparin (LMWH) and is rare in patients receiving fondaparinux, a small synthetic pentasaccharide. HIT antibodies in patient sera can be detected by anti-PF4 ELISA and serotonin release assays (SRAs) using UFH, but these assays have not been evaluated for use with LMWH or fondaparinux in our laboratory.

Objective: To provide an investigational tool to clinicians, we developed and characterized two SRAs using LMWH and fondaparinux.

Method: For both assays, washed donor platelets containing [14C]serotonin are prepared before the test. Patient serum is incubated with platelets in the presence of low and high concentrations of LMWH (enoxaparin) or fondaparinux for 60 minutes at room temperature. After the reaction is stopped and platelets are spun down, the [14C]serotonin in the supernatant is measured and the degree of platelet activation is determined by the percentage of [14C]serotonin released from platelets. A positive result is defined as >or = 20% serotonin release at the low concentration of LMWH or fondaparinux, with a ≥50% reduction at the higher drug concentration. An indeterminate result means that high [14C]serotonin release is observed in the absence of LMWH or fondaparinux and cannot be reduced by ≥50% at high drug concentration. A negative result is defined as <20% serotonin release at both the low and high drug concentration. To evaluate the LMWH-SRA and fondaparinux-SRA assays we used 10 serum samples from clinically confirmed HIT/HITT patients with positive UFH-SRA and anti-PF4 ELISA results and 10 samples with negative UFH-SRA results.

Results: The LMWH-SRA yielded positive results in 9 of 10 (90%) samples from HIT/HITT patients; the remaining sample was indeterminate. The fondaparinux-SRA was negative in 7 HIT/HITT samples, positive in 1, and indeterminate in 2. Indeterminate fondaparinux-SRA results persisted when these 2 samples were tested using different donor platelets and when diluted 4-fold. All 10 UFH-SRA-negative samples had negative LMWH-SRA and fondaparinux-SRA results. Conclusions: The high rate of positive LMWH-SRA results in HIT/HITT patients (90%) shows that this assay can detect antibody activation caused by LMWH. Despite heat inactivation of complement and the addition of hirudin to neutralize any thrombin contamination, indeterminate SRA results are seen across the board of all 3 types of heparin used in our study. The finding that 2 of the UHF-SRA-positive HIT/HITT cases had indeterminate fondaparinux-SRA results, suggest that some HIT antibodies can recognize platelet-bound PF4 in the absence of an exogenous source of heparin. These findings suggest that the SRA-fondaparinux assay may be useful for in-vitro investigation of suspected fondaparinux-induced HIT/HITT; further studies with samples from patients with known fondaparinux-related HIT/HITT are needed to validate this use.

D-49

Modification of a Chromogenic Anti-Factor Xa Method for use as a Pharmacokinetic/Pharmacodynamic Marker in Clinical Trials

B. Litzenberger, C. Starks, M. Lordes Genato, J. Dharmadhikari. *Huntingdon Life Sciences, East Millstone, NJ,*

Background: The purpose of this project was to develop and validate a fit for purpose method for measuring Anti-Factor Xa to be used as a pharmacokinetic/pharmacodynamic marker in clinical trials. The method selected was the Diagnostica Stago Stachrom® Unfractionated Heparin Test Kit. The quantitiation specifications of the kit are 0.1 to 1.0 IU/mL. The fit for purpose limit of quantitation for the clinical trial was defined as 0.025 to 0.375 IU/mL. In order to achieve the target quantitation specifications, a modified calibration curve and appropriate quality control materials were developed. The method was then validated to meet the regulatory expectations summarized in the FDA Guidance for Industry on Bioanalytical Method Validation (FDA-BMV) issued May 2001.

Materials and Methods: The Stachrom® Unfractionated Heparin Test Kit (product # 00906) is based on a method by Teien, *et al*, which is designed to determine the plasma level of unfractionated heparins (UFH) or low molecular weight heparins (LMWH) by the measurement of their anti-Xa activity using the amidolytic method with chromogenic substrate..The assay was applied to the Diagnostica Stago Compact (Software version 106.7) following the vendor's assay parameter specifications. In order to extend the calibration range, unfractionated heparin supplied by NIBSC, was diluted in Tris EDTA

buffer to prepare standard stock solution which was used to prepare standards in sodium citrate plasma (depleted of heparin binding protein) at nominal concentrations of 0.025, 0.040, 0.060, 0.075, 0.100, 0.150, 0.225, and 0.375 IU/mL. Unfractionated heparin supplied by NIBSC, was diluted in Tris EDTA buffer to prepare a QC stock solution which was used to prepare QCs in sodium citrate plasma (depleted of heparin binding protein) at nominal concentrations of 0.025 (LLQC), 0.060 (LQC), 0.100 (MQC), 0.0200 (HQC) and 0.375 (ULQC)IU/mL.

Results: The inter-assay calibration curve precision was demonstrated by comparing the mean back-calculated concentration value of each standard with its theoretical (nominal) value (n=7). The values ranged from 95.6% to 140.0% recovery. The precision of the method was evaluated by determining the relative standard deviation (RSD) of mean back calculated concentrations of QC samples. The mean intra-assay precision measurements ranged from 2.1 to 8.5 %RSD. The mean inter-assay precision measurements ranged from 4.5 to 21.4 %RSD. The accuracy of the method was evaluated by determining the percent relative error (%RE) of the QC samples..The mean intra-assay accuracy ranged from -21.7 to 28.0 %RE. The mean inter-assay accuracy ranged from 1.0 to 12.0 %RE. Dilutional linearity was demonstrated by assaying serial dilutions of a high concentration of heparin anti-Xa solution (3.750 IU/mL) in sodium citrate plasma. Dilutional integrity was established at 1:128.

Conclusion: The results of the method validation demonstrate that the assay is considered robust and compliant with the regulatory standards defined in the FDA-BMV Guidance for Industry issued May 2001.

D-50

A semi-automated method for the measurement of hepcidin in plasma by on-line extraction coupled to liquid chromatography-tandem mass spectrometry

M. E. Franklin, S. Hall, P. J. Taylor, R. Saal, L. Fletcher, D. Mudge, R. Bird. *Princess Alexandra Hospital, Brisbane, QLD, Australia,*

Introduction: The iron regulatory peptide hormone, hepcidin, is a promising biomarker for the diagnosis and monitoring of iron metabolism disorders. The study of hepcidin has been hindered by the availability of reliable analytical methods. The aim of this investigation was to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the measurement of hepcidin that could be used to facilitate human investigations of this biomarker. **Methods:** Samples (100 µL) were pre-treated with an aqueous solution of $^{13}C_{18}$,$^{15}N_3$-hepcidin, labeled internal standard, (200 ng/mL, 100 µL) and submitted to on-line extraction coupled to LC-MS/MS. The on-line extraction involved the loading of sample (100 µL) onto pre-conditioned Hysphere C8 extraction cartridges (10 µm, Spark Holland). The cartridges were sequentially washed with water (1 mL), 25% acetonitrile in 0.1% ammonium hydroxide (1 mL), 25% acetonitrile in 0.1% formic acid (1 mL). Chromatography was performed on a Sunfire C18 analytical column (50 x 2.1 mm, 5 µm, Waters) under gradient conditions at a flow rate of 0.3 mL/min. The mobile phases consisted of 2 mmol/L ammonium acetate and 1 mL/L formic acid in water (solvent A), and2 mmol/L ammonium acetate and 1 mL/L formic acid in methanol (solvent B). The linear gradient started at 20% B up to 100% B over 5 minutes and followed by 2 minutes of re-equilibration. Mass spectrometric detection was by selected reaction monitoring (hepcidin *m/z* 930.4→1143.7; internal standard *m/z* 937.2→1149.0) using positive electrospray ionization conditions (3 kV). Linearity was tested over the range of 5 to 100 ng/mL. Accuracy and precision were determined using quality controls across the analytical range (5, 15, 40 and 80 ng/mL) and above the upper limit of quantification (250 ng/mL). A pilot study was undertaken to compare of hepcidin and ferritin concentrations using patient samples from routine iron investigations (n = 34).

Results: Under the mass spectrometric conditions the predominant precursor ion was the triply charged species, $[M+3H]^{3+}$. Total chromatographic run time was 7 min. The assay had an analytical range of 5 to 100 ng/mL ($r^2 > 0.998$, n = 12). Inter- and intra-day accuracy and imprecision for quality control samples (15, 40, 80 ng/mL) were 95.4 to 106.2% and <8.3% (n = 8), respectively. The lower limit of quantification was 5 ng/mL; with an inter-day accuracy and precision of 105% and 9.8%, respectively. A dilution protocol (1/5) using an out of range quality control sample (250 ng/mL) showed acceptable inter- and intra-day analytical performance. Comparison of hepcidin concentrations (range: <5 to 164 ng/mL) against ferritin concentrations (range: 7 to 1760 ng/mL) revealed the following equation: $y = 0.051x + 14.5$ ($r^2 = 0.43$, n = 34). **Conclusion:** We report a reliable, robust and precise mass-spectrometry based assay for hepcidin measurement. The approach to sample handling minimises such problems as sample stability and potential absorption. This methodology should assist in understanding disease states in which hepcidin are a key mediator.

D-51

Ex vivo megakaryocyte expansion from human peripheral blood stem cells

Ö. bingöl özakpınar, A. Maurer, C. Adiguzel, F. Uras, M. Bayık. *Marmara University, Istanbul, Turkey,*

Thrombocytopenia remains a serious problem in patients treated with intensive high-dose chemotherapy and bone marrow transplantation. Megakaryocytes (Mk) are the specialized precursors of platelets and are contained in hematopoietic progenitor cell products, but their number is variable and relatively low. The infusion of ex vivo expanded Mk precursors to patients has been proposed as a strategy for shortening the time to platelet engraftment. The development of in vitro culture methods to obtain sufficient numbers of Mks from haematopoietic stem cells is an important target for basic and clinical research. The aim of this study was to develop an ex vivo expansion method for Mk progenitors from peripheral blood stem cells (PBSC). PBSC were harvested from two healthy adult donors. Briefly, CD34+ cells were isolated and cultured in serum free media supplemented with thrombopoietin (TPO), Interleukin 3 (IL-3) and Interleukin 6 (IL-3) for 14 days followed by TPO and IL-6 for another 7 days. The differentiation of Mk was monitored by flow cytometry (CD34+/41+). The morphology of the cultured cells was studied by light microscopy.We observed that the percentage of CD41+ cells was more than 70 on the 21st day. Morphological analysis of cells obtained after 7 days of culture showed aspects typical of developing Mk. This study showed that CD34+ cells isolated from peripheral blood can be used to expand megakaryocytic cells using two step culture method. It is obvious that this promising method needs further development for clinical applications.

D-52

Ala-9Val Polymorphism Of Mn-Sod Gene Is Not Associated With Beta Thalassemia Major Pateints

S. Sogut[1], O. Ozturk[1], A. Yigit[2], N. Yilmaz[1], H. Yilmaz[2], E. Gali[3], E. Uz[2], H. Kaya[4]. *[1]Mustafa Kemal University, Medical Faculty Deparment of Biochemistry, Hatay, Turkey, [2]Suleyman University, Medical Faculty Department of Medical Biology and Genetics, Isparta, Turkey, [3]Antakya State Hospital, Pediatric Unit, Hatay, Turkey, [4]Mustafa Kemal University, Medical Faculty Deparment of Internal Medicine, Hatay, Turkey,*

Background: Beta -Thalassemia is an autosomal recessive disease characterized by impairment of hemoglobin production, in which there is complete or partial failure to synthesize beta-globin chains and a subsequent imbalance in alpha / beta -globin chain ratio that results in chronic haemolytic anaemia. In beta thalassaemia, beta-globin mutations cause excess alpha-globin subunits. Any imbalance between the alpha and beta globin chains plays a crucial role in producing oxidative stres. Superoxide dismutases (SODs), including Cu,Zn-SOD, Mn-SOD, and extracellular (EC) SOD, are protective enzymes against oxidative stress. The present study was aimed to assess whether there is a genetic association between a functional polymorphism (Ala-9Val) in the human Mn-SOD gene and beta thalassemia major (BTM).

Methods: The data in the present study was obtained from human Mn-SOD gene polymorphism of BTM patients (n=111) and healthy controls (n=141) using a PCR/RFLP method. Alanine or valine polymorphism in the signal peptide of Mn-SOD gene was evaluated using a primer pair to amplify a 107-bp fragment followed by digestion with NgoM IV.

Results: The results obtained are given in Table 1.

Table 1. The genotypes of Ala-9Val polymorphism and allele frequencies in controls and thlassemia patients.

	Genotypes[a]			Allele frequencies[b]	
	Val/Val, n (%)	Val/Ala, n (%)	Ala/Al n (%)	-9 Val	-9 Ala
Thalassemia (n=111)	51 (% 46.0)	52 (% 46.8)	8 (% 7.2)	0.69	0.31
Control (n=141)	70 (% 49.6)	63 (% 44.7)	8 (% 5.7)	0.72	0.28

[a] No significant difference in genotype frequencies between patients and controls ($x^2 = 0.471$, df = 2, P = 0.79). The genotypic distributions in both patients and controls were within the Hardy-Weinberg equilibrium.

[b] No significant difference in allele frequencies between patients and controls.

Conclusion: Our results suggest that Mn-SOD is not associated with the physiopathology of thalassemia major. However other antioxidant and oxidant enzymes could be potential candidates responsible for oxidant/antioxidant imbalance in thalassemia major. Therefore they should be taken into account in future studies.

D-53

Non-Transferrin Bound Iron, Iron Burden And Hypoxia Induced Low-Grade Inflammation In Patients With Thalassemia Intermedia

I. Papassotiriou[1], V. Ladis[2], E. Kanavakis[3], C. Kattamis[2], A. Kattamis[2].
[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]First Department of Pediatrics, Athens University Medical School, Athens, Greece, [3]Department of Medical Genetics, Athens University Medical School, Athens, Greece,

Background: Inflammation is known to play an important role in the pathogenesis of thalassemia. Serum levels of pro-inflammatory cytokines such as IL-6 and TNF-α are known to be elevated in thalassemic patients, suggestive a low-grade inflammatory status. These levels are comparable to the ones observed in patients with diabetes, obesity and atherosclerosis. Altered redox status has also been shown in thalassemic erythrocytes. Reactive oxygen species (ROS) are generated in increased amounts after the precipitation of excess unmatched globin chains, the deposition of non-heme iron and hemichromes and the induced inflammation. In this study we assessed the level and possible causes of inflammatory status in patients with thalassemia intermedia (TI).

Patients and Methods: Thirty-five patients with TI, 13 men and 22 women, aged 8-63 years were included in the study. None of the patients had received any transfusion therapy at least 6 months prior of sampling, while 25/35 patients had been splenectomized. We measured the hematologic and biochemical parameters, including Hb, HbF, ferritin and soluble transferrin receptors (sTfR) with standard methodology. Serum concentrations of non-transferrin bound iron (NTBI) were estimated using graphite furnace atomic absorption spectrometry. Determination of high-sensitivity CRP (hs-CRP or cardiophase-CRP) concentration was performed using the Siemens Advia 1800 Clinical Chemistry System. Furthermore, we obtained P_{50} values from oxygen equilibrium curves (OEC) drawn in fresh whole blood. Oxygen delivery and release parameters were calculated using the "Siggaard-Andersen's Oxygen Status Algorithm".

Results: hs-CRP levels were elevated 2.12±0.55 mg/L compared to lean control values 1.2±0.19 mg/L ($p<0.008$). Most of the patients (60 %) showed evidence of low-grade inflammation based on the hs-CRP levels. NTBI levels were significantly elevated (2.4±2.2micromol/L), with only 10/30 patients having levels <0.5micromol/L, which is proposed as normal limit. As accepted P_{50} values were indicative of relative tissue hypoxia (almost all patients had increase oxygen affinity). All of the patients showed evidence of increased eryhtropoeitic activity, as indicate by the elevated sTfR levels ranged from 4.0 to 22.9mg/L (3- to 19-fold increase of erythroid marrow activity). The main results of the evaluated correlations showed that: a) hs-CRP levels correlated positively with NTBI concentrations ($r=0.741$, $p<0.0001$), b) hs-CRP levels correlated positively with ferritin levels ($r=0.522$, $p=0.004$) and c) hs-CRP levels correlated negatively with P_{50} values ($r=-0.409$, $p=0.03$), while no correlation was found between hs-CRP levels and the degree of ineffective erythropoiesis expressed as sTfR concentrations ($p> 0.223$).

Conclusions: These findings demonstrate that patients with TI have a chronic low-grade inflammation. Similar inflammatory status has been also shown in patients with atherosclerosis, diabetes and obesity. The level of inflammation correlated with indexes of iron homeostasis, alteration of which is commonly observed in patients with TI. Thus, it seems plausible that the oxidative effects of NTBI and increased iron burden result in chronic inflammation in these patients. The observed negative correlation of inflammation and P_{50} is of interest, as it indicates possible involvement of tissue hypoxia in inflammation processes.

D-54

Coagulation testing in the context of the automated hospital lab and optimizing for the effects of common interferences

N. Marcus. *Huntsville Hospital, Huntsville, AL,*

Coagulation testing is central to diagnosis of patients with hemostasis disorders and is critical for monitoring of antithrombotic therapies. Despite this, there is a lack of clarity in the published literature on the impact of hemolysis on the measurement of PT/aPTT using automated coagulation measurement instrumentation.

Objective: To evaluate the impact of hemolysis on the accuracy of the standard coagulation tests PT and aPTT in patients not undergoing anticoagulation therapy and in patients undergoing heparin therapy.

Methodology: Na-citrate serums were obtained from an emergency room setting and processed within 2 hours of collection. Two patient populations were collected 1) 60 patients excluding those undergoing anticoagulation therapy; 2) 20 patients actively undergoing heparin therapy. For both populations, samples were divided and one aliquot was lysed mechanically by drawing through a 25 gauge needle. Both aliquots were then separated on a Hettich centrifuge and for each paired sample the degree of hemolysis was measured as a serum index on the Roche Modular DPP and the coagulation measurements PT/aPTT were determined on the Diagnostica Stago STA-R Evolution®.

Results: Mechanical shearing of serum samples by passage through a 25 gauge syringe produced significant hemolysis, as 87% of samples had serum indices <10 prior to hemolysis, compared with 87% having serum indices greater than 20 following hemolysis. In comparing mean PT and aPTT values in normal vs hemolysed samples we observed no significant difference for either value ($p = 0.67$ PT; $p=0.65$ aPTT) for the patients not undergoing anti-coagulation treatment. While not statistically significant, we observed a trend to decreasing values for the aPTT in the hemolyzed samples. To establish performance parameters, we applied acceptable biological variation values (PT=5.3%, aPTT=4.5%) and compared the the frequency of ΔPT and aPTT results pre and post hemolysis. By this analysis, 100% of the PT values fell within the acceptable range regardless of the hemolysis Index, whereas for aPTT, 21% of samples exceeded the acceptable variation cutoff. Employing a hemolysis Index cutoff of < 313, 75% of samples were tested and of these 95% fell within the acceptable variation.

In preliminary analysis of the data, we observed an apparent interference in the aPTT test with heparin therapy and the degree of hemolysis. We examined this in further detail in a collection of 20 patients on heparin therapy. In comparing the aPTT data between the heparin treated and the non-coagulation results we observed the expected increased aPTT time. However, within the heparin therapy group, 19 of 20 samples (95%) had a ΔaPTT value outside the acceptable variation range (4.5%), compared with only 21% in the non coagulation group.

Conclusions: The data in this study demonstrates that in establishing an automated coagulation analysis workflow, it is essential to include characterization of the potential interference of hemolysis with the aPTT assay, whereas the PT assay appears to be more robust with respect to hemolysis. Furthermore, where samples are likely to include heparin treated patients, characterization of the effect of hemolysis is essential since 95% of samples exceeded the acceptable biological variation.

D-55

Quantification of unfractionated heparin in human plasma and whole blood by means of novel fluorogenic anti-FXa assays

V. Castro-Lopez[1], L. Harris[1], J. O'Donnell[2], T. Killard[1]. *[1]Biomedical Diagnostics Institute, National Centre for Sensor Research, Dublin City University, Dublin, Ireland, [2]Haemostasis Research Group, Trinity College Dublin, and National Centre for Hereditary Coagulation Disorders, St. James's Hospital, Dublin, Ireland,*

Objective: Unfractionated heparin (UFH) is a drug commonly used in the prevention and treatment of thrombosis and cardiovascular diseases, but which has to be monitored due to its pharmacokinetic limitations. UFH can be quantified in human plasma using both traditional clot-based and chromogenic assays. Among several limitations, clotting assays suffer from inter-laboratory variability in responsiveness to therapeutic UFH concentrations while chromogenic assays can only be applied to platelet-poor plasma. Thus, novel and sensitive plate-based fluorogenic anti-FXa assays were investigated to quantify therapeutic UFH concentrations in human plasma and whole blood, respectively.

Methods: The assay is based on the complex formation of heparin with endogenous antithrombin and exogenous FXa added in excess. Any FXa not inactivated is free to react and cleave the fluorescently labelled peptide substrate, Mes-D-LGR-ANSN(C_2H_5)$_2$ containing the fluorescent reporter group 6-amino-1-naphthalene-sulfonamide (ANSN). The relative change in fluorescence was monitored with excitation at 352 nm and emission at 470 nm. Human blood samples were drawn through antecubital venapuncture from six healthy volunteers (30-40 years of age) who had not ingested any drugs that might affect coagulation. All samples were spiked with pharmacologically relevant concentrations (0-1.6 U/ml) of therapeutic UFH and performed in triplicate. Reaction rates were analyzed and statistical analysis by means of one-way ANOVA, and when necessary, post-hoc analysis (Scheffe test), was performed. The mean difference was significant at the 0.05 level.

Results: Two fluorogenic anti-FXa assay methods were defined for low and high pharmacologically relevant UFH concentration ranges in pooled human plasma. 100 nM FXa in the presence of 25 μM Mes-D-LGR-ANSN(C_2H_5)$_2$ was found to be sensitive in the low therapeutic UFH range of 0-0.6 U/ml with significant differences at intervals of 0.2 U/ml ($P<0.05$). The enzymatic reaction of 200 nM FXa with 75 μM ANSN-based fluorogenic substrate showed significant differences in the high therapeutic UFH range of 0.6-1.2 U/ml every 0.2 U/ml ($P<0.05$). The response profile was not linear in both assays and the data was fitted to a third order polynomial with a correlation coefficient of 1. Assay percentage coefficients of variation were below 5%. The assay was also optimized for whole blood with 350 nM FXa in the presence of 87.5 μM ANSN-based fluorogenic substrate. This was capable of differentiating UFH concentrations at intervals of 0.2 U/ml ($P<0.05$) in the range of 0-0.4 U/ml. The data was normalised and the averaged results were fitted to a second order polynomial with a correlation coefficient of 1.

Conclusions: Fluorogenic anti-FXa assays to measure therapeutic UFH concentrations in pooled human plasma and whole blood have been investigated. The results obtained in this study will assist diagnostic laboratories towards improved monitoring of UFH therapy.

D-56

Serum ferritin concentration in various lymphoproliferative diseases

D. Begovic, S. Kapetanovic, M. Radojkovic, S. Ristic, M. Plecas. *Clinical Health Center Dr Dragisa Misovic, Belgrade, Serbia,*

Background: Ferritin is major storage protein for iron. The high serum ferritin concentration has been attributed to reticuloendothelial cells disturbances, release of ferritin from damaged cells, or increase of ferritin synthesis by neoplastic cells. Elevated serum ferritin were detected in patients with lymphoproliferative diseases. Various other conditions, however, could increase serum ferritin (increased metabolism and inflammation). Recent hypothesis suggested that in hematologic malignancies, high concentration of serum ferritin is result of its increase synthesis by neoplastic cells. The aim of our study was to determinate level of serum ferritin concentration in various lymphoproliferative diseases. Serum CRP was measured as an indicator of inflammation.
Methods: Twenty-seven patients (age 63±12years) in advanced stages of lymphoproliferative diseases (lymphoma n=14, plasmacytoma n=8 and chronic lymphoid leukemia-CLL n=5) were observed in study. Following tests were performed: ferritin , CRP, fibrinogen, Fe, UIBC and other biochemical and haematology parametars. Ferritin was examined using a chemiluminescent assay (reagents from DPC company, on analyzer Immulite 1000), and reference ranges are 6-195 ng/ml. Serum CRP was assessed by an immuno-turbidimetric method. Fe, UIBC and fibrinogen concentration were determinated by colorimetric methods.
Results: An increase of ferritin concentration was found in all patients with lymphoproliferative diseases (mean value 358 ng/mL). High levels of serum ferritin were found in plasmacytomas and lymphomas, but there was slightly increased ferritin level in CLL. Although 48% participains had high levels of CRP (above 10mg/mL) and 37% increased fibrinogen (>3.9g/L), even 71% had ferritin higher than 200ng/mL. Results show no significant correlation between levels of ferritin and CRP level (Spearman coefficient q=0.34, p>0.05), ferritin and fibrinogen (Spearman coefficient q=0.1079, p>0.05), and ferritin and Fe (Spearman coefficient q = 0.07131, p>0.05).
Conclusions: Our result suggest the serum ferritin measurements may be clinical usefful in the initial evaluation in patients with lymphoproliferative diseases. Also, our results suports the hypothesis that in hematologic malignancies, high concentration of serum ferritin is a result of synthesis by neoplastic cells.

D-57

Hemoglobin A_2 Values, quantified by High Performance Liquid Chromatography (HPLC), in samples from patients with sickle cell disease in the presence or absence of alpha thalassemia.

S. F. Fonseca[1], L. Assunção[2], N. Boa Sorte[2], A. Purificacao[2], R. Ribeiro[2], T. Amorim[2], S. S. S. Costa[3]. *[1]Unb, Brasilia, Brazil, [2]Apae, Bahia, Brazil, [3]Sabin Laboratory, Brasilia, Brazil,*

In the diagnosis of sickle cell disease, the quantification of Hb A_2 helps in the differential diagnosis between sickle cell anemia (SS) and $S\beta^0$-thalassemia. In 1996, Suh reported that values of Hb A_2 obtained by HPLC were significantly elevated in samples containing Hb S, and raised the possibility of SS individuals having been wrongly diagnosed with $S\beta^0$-thalassemia. In 2000, Shokrani proposed that in samples of SS patients analyzed by HPLC, values of Hb A_2 of up to 5.9% may be considered normal. Mass spectrometry studies suggested that these elevated values of Hb A_2 are a consequence of the co-elution of smaller components of Hb formed by a β^S chain associated with an alpha-globin chain, which was modified by carbamylation, after translation. Additionally, Head concluded that the percentage of Hb A_2 is higher in individuals with sickle cell trait and alpha thalassemia than in those without alpha thalassemia, and suggested that this occurs because the δ chains have a greater affinity for the alpha chains than the β^S chains. Objective: Considering the broad utilization of HPLC in daily laboratory practice, and the importance of a correct diagnosis for a better planning of the clinical and laboratory follow-up of patients, the authors propose to analyze the range of variation normally found in the Hb A_2 values quantified by HPLC, in SS and hemoglobinopathy SC (SC) patients, in the presence or absence of alpha thalassemia (AT), and establish reference values for the Brazilian population. Material and Methods: Study of a transverse cut, with casuistic composed of 143 SS children and 136 SC children, diagnosed and followed by the Reference Service on Neonatal Screening, APAE, Bahia, Brazil. The children's age ranged from 2 to 6 years old. The hemoglobin study was done by automated HPLC, using the *Variant II* and the *Beta-thal Short Program* (Bio-Rad, USA). The 3.7 kb α_2 thalassemia deletion was investigated by allele-specific PCR. The values for Hb A2 were described using measures of central trend and dispersion, and compared according to type of hemoglobinopathy utilising ANOVA and the Tukey test. Results: Interestingly, the values of Hb A_2 were elevated in the 279 samples containing Hb S, principally in the presence of alpha thalassemia. The Hb A_2 average in the SS samples (n=193) was 3.62% (SD +/- 0.63); in the SS samples heterozygous for AT (n=29) was 4.00% (SD +/- 0.64); in the SS samples homozygous for AT (n=3) was 4.73% (SD +/- 0.25). The Hb A_2 average in the SC samples (n=136) was 3.95% (SD +/- 0.50); in the SC samples heterozygous for AT (n=26) was 4.23% (SD +/- 0.43); in the SC samples homozygous for AT (n=3) was 4.86% (SD +/- 0.25). Our results demonstrate that the hemoglobinopathy laboratory should be informed of this apparent elevation of the Hb A_2 values as determined by HPLC, in samples containing HbS, and that other factors, such as the family history and clinical and laboratory data, must be considered before the diagnosis of sickle cell anemia and $S\beta^0$-thalassemia are confirmed.

D-58

Development And Evaluation Of An eSensor® Test For Thrombophilia Risk On The Xt-8 Instrument

A. R. Jacobs, Z. Wang, S. Panuganti, N. Babbar, A. Zuniga, V. Headley, Y. Liu, C. Argueta, S. Marsh, M. Wood. *Osmetech Molecular Diagnostics, Pasadena, CA,*

Background and Purpose: Thrombosis is one of the most common types of blood coagulation disorders, affecting 1 in 1000 individuals with a fatality rate of 1-2%. Inherited Thrombosis is associated with congenital predisposing risk factors such as Factor II (Prothrombin), Factor V (Leiden) proteins involved in a blood coagulation enzyme activity cascade and Methylenetetrahydrofolate Reductase (MTHFR). MTHFR that converts homocysteine to methionine as part of the pathway that coverts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. FII, FV and MTHFR mutations are present in ~2%,5% and ~40-50% in individuals with N. European ancestry respectively. Inherited Thrombosis is characterized by increased risk of deep vein thrombosis, ectopic pregnancy, pulmonary embolism, myocardial infarction, cardiovascular disease and other complications related to abnormal blood. The American College of Medical Genetics (ACMG) recommended in 2007 that FV DNA testing should be performed in patients with any type of venous thrombosis. Additional recommendations also include molecular genetic testing for the other most common risk factors such as FII and MTHFR for patients tested positive for FV. We have developed a genotyping test for this panel based on the eSensor® DNA detection technology and here report the evaluation of its performance.
Methods: Genomic DNA (gDNA) was subjected to multiplex PCR amplification, exonuclease digestion and a hybridization-based genotyping step that was performed in a single eSensor® cartridge for each sample, using the eSensor® XT-8 instrument. The system reports the genotyping results for all mutations or a subset based upon user selection from the panel. A reproducibility study was performed by testing 5 blood gDNA samples in duplicate in each of 5 runs with at two external sites and one internal site by 6 operators (a total of 300 tests). A method comparison study was performed by testing 219 gDNA samples by the eSensor® method and by DNA sequencing. The assay range was established by testing 20 replicates each of 2 Blood extracted gDNA samples) at 0.01, 0.1, 1, 10, 100 and 500 ng of total input DNA per PCR. The effect of potential interfering substances was evaluated for, Heparin, Cholesterol, Bilirubin, Hemoglobin and excess EDTA
Results: The reproducibility and method comparison studies gave 100% final call rates and 100% agreement with DNA sequencing after a single retest of no-call results. The assay range study determined the Limit of Detection (LOD) for gDNA sample input to be 1 ng. No effect of interfering substances on DNA extractions, PCR yields and genotyping call rates was observed. **C**
onclusions: Under the conditions of this study, the eSensor® Thrombosis Risk Test and the eSensor® XT-8 instrument provided accurate and reproducible results for the detection and genotyping of Factor II G20210A, Factor V G1691A and MTHFR C677T and A1298C mutations in patients with suspected thrombophilia from isolated genomic DNA obtained from whole blood samples.
Use of the eSensor® Thrombophilia Risk Test is for investigational use only; the performance characteristics of this product have not been established. The eSensor ® Thrombophilia Risk Test is 510(k) pending with the US FDA and not yet available for commercial sale.

D-59

Biclonal gammopathies: review of 19 cases.

S. Guastafierro, U. Falcone, M. G. Ferrara, R. R. Parascandola, P. Fiorillo, C. Cuomo, S. Santangelo, A. Sica. *Division of Hematology, Second University of Naples, Naples, Italy,*

Background: The simultaneous presence of two serum monoclonal components (MCs) is a rare occurrence. MCs can be detected or suspected by high-resolution serum protein electrophoresis (SPE), but serum immunofixation (IF) is necessary for a definitive diagnosis. We report 19 patients with double MC and we also describe the eventual presence of comorbidity.
Patients and Methods: Between 1995 and 2009, 604 patients with MC were observed

at our Division of Hematology. Thirty-one of them presented a double MC, but 12 were not included in this study because of insufficient data or transience of one of the two MCs. The age of the remaining 19 patients (12 M, 7 F) ranged from 55 to 91 years (mean age: 73 years). All the selected patients underwent clinical and laboratory work-up, including high resolution SPE on agarose gel, bone marrow aspiration and/or biopsy. Monoclonal characterization was performed by immunofixation using agarose film and antisera monospecific for the light and heavy chains of human immunoglobulins. Antisera were tested for specificity against known cases of multiple myeloma.

Results: The observed incidence of double MC was 3.1%. Serum IF detected 2 MCs in all cases, even in the 10 patients in which a single peak had been showed by SPE. Thirty-five of 38 MCs were intact immunoglobulins. In 3 cases, one of the two MCs was γ-, α-, or λ-chain, respectively. The most frequent combinations of MCs were IgG+IgA (4 cases) and IgG+IgG (4 cases). The most frequent combination of light chains was κ+λ (12 cases). Only in 4 patients (21%) the biclonal gammopathy was not associated with another disease. In 13/15 remaining cases, a hematologic disease was present (lymphoprolipherative diseases -including NHL, Waldenstrom macroglobulinaemia, CLL, and crioglobulinaemia- 6 cases; myeloprolipherative diseases: 4 cases; myeloma: 2 cases; ITP: 1 case).

Conclusions: Biclonal gammopathies represent 1-6% of all monoclonal gammopathies. IF or capillary zone electrophoresis/immunosubtraction can detect an additional little MC that could be not suspected by SPE. A higher incidence of malignancies in patients with monoclonal gammopathy has been reported by some authors but not subsequently confirmed in other studies. Our data, furthermore, seem to support the hypothesis of an ulterior increase in neoplasm incidence when 2 or more MCs occur. In conclusion, when 2 MCs are present, a prompt evaluation should be performed, in order to rule out a possible occult malignancy, especially a hematologic one.

D-60

Automation Of Hepascore As A Biochemical Index Of Liver Fibrosis In Patients With Thalassemia: The Importance Of Hyaluronic Acid

I. Papassotiriou[1], M. Papastamataki[1], P. Delaporta[2], E. Premetis[3], A. Kattamis[2], V. Ladis[2]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]First Department of Pediatrics, Athens University Medical School, Athens, Greece, [3]Hematology Laboratory, "Aghia Sophia" Children's Hospital, Athens, Greece,*

Background: Patients with transfusion depended thalassemia major often develop liver fibrosis due to liver iron overload and/or hepatitis virus C (HCV) infection. Hyaluronic acid (HA) plays a prominent role in the pathogenesis of liver fibrosis, and the elevation of serum HA concentration is due to either increased synthesis by inflammatory cells and hepatic stellate cells or impaired degradation by sinusoidal endothelial cells (SECs) and thus is proposed as a non-invasive biomarker of liver fibrosis either by itself and/or included in the HEPASCORE formula[1].

Patients and Methods: In this study we evaluated prospectively a screening of liver fibrosis in 201 adult patients aged 19-54 years with transfusion depended thalassemia major, based on HA measurements. 46/201 patients were HCV-mRNA(+). HA was measured with a turbidimetric assay (Wako Chemicals GmbH) applied on Siemens ADVIA 1800 Clinical Chemistry Analyser. This assay contains hyaluronic acid binding protein (HABP), which binds the HA in serum forming a complex that combines with latex particles coated by anti-HABP antibody. The above insoluble aggregate increases turbidity in the solution. The Hepascore was computed from the results by using the model previously published: Hepascore=y/(1+y) with y=exp [–4.185818–(0.0249 age (years))+(0.7464 sex (M=1, F=0)+(1.0039 α2-macroglobulin (g/l))+(0.0302 HA (μg/l))+(0.0691 bilirubin (μmol/l))–(0.0012 GGT (U/l))].

Results: The main results of the study showed that: a) HA levels were increased in 110/201 (55%) thalassemia patients 85.0 ± 10.3 ng/ml, ranged from 15.0-1495.0 ng/ml, compared to 20.8 ± 7.4 ng/ml reference laboratory values b) HA levels were significantly higher in HCV-mRNA(+) compared to HCV-mRNA(-) patients, 192.9 ± 40.5 vs 53.0 ± 2.7 ng/ml, p<0.0001 c) no significant correlations were found between HA levels and/or Hepascore with ferritin and liver iron content (LIC) assessed with MRI (p>0.324 and p>0.270, respectively).

Conclusions: In recent years, there has been considerable interest in the use of noninvasive markers of hepatic fibrosis and cirrhosis in patients with thalassemia, because liver biopsy is an invasive and expensive procedure with potential complications. Our findings indicate that hyaluronic acid measurements contribute to the assessment of liver fibrosis in patients with thalassemia and might be helpful for further evaluation of patients with liver biopsy if this is truly needed. Furthermore, these results are in accordance with the results published recently with the other non-invasive method Fibroscan (transient elastography), indicating that liver fibrosis in thalassemia is independent from liver siderosis2.

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D-61

Comparison of the Detection of P2Y12-receptor Blockade in Pre-angiocath Subjects with cardiovascular Disease by Light-transmittance and Whole-blood Aggregometry, Verify Now® P2Y12 and INNOVANCE® PFA P2Y*

D. L. McGlasson, A. D. Shah. *Wilford Hall Medical Center, Lackland AFB, TX,*

The purpose of this study was to compare the results of the INNOVANCE® PFA P2Y*, a new test cartridge for the PFA-100 ® system to those obtained by light transmittance (LTA) with 20 μM ADP and whole blood aggregometry (WBA) using 5 and 10 μM of ADP, and the Verify Now ® P2Y12 cartridge by Accumetrics. Blood was collected with 3.2% and 3.8% sodium citrate from 102 subjects with cardiovascular disease after receiving clopidogrel (6-24 hours post loading with 300 or 600 mg) or after 7 days of 75 mg daily P2Y12 receptor blockade was detected with INNOVANCE ® PFA-P2Y* using a cut-off of >106 sec. Only the INNOVANCE system was tested with 3.2 and 3.8% sodium citrate. All others were tested with 3.2% sodium citrate only. Cut offs for other systems were VerifyNow >20%; WBA 5 <5 ohms; WBA 10 <8 ohms; LTA <50% amplitude.

The following results indicate the comparison of methods for detection of the influence of clopidogrel: Sensitivity (%): P2Y 3.2%=59%; P2Y 3.8%=95%; VerifyNow=60%; WBA 5μM=88%; WBA 10 μM=89%; LTA 20 μM=72%.

The total concordance (%) for this set of post drug patients was computed and the results are as follows: P2Y 3.2% to VerifyNow®=71%; to WBA 5 and 10μM=64 and 65% respectively; to LTA 20μM=69%. P2Y 3.8% to VerifyNow®=71%; to WBA 5 and 10μM=90% for both; LTA 20μM=76%. VerifyNow® to WBA 5 and 10μM=68 and 67% respectively; LTA 20μM=72%

The INNOVANCE® 3.8% result of 95% compares favorably with the results obtained in both WBA ADP concentrations. The INNOVANCE® 3.2% data compares closely with the VerifyNow® cartridge system. Concordance with the Verify Now® cartridge system was favorable at 71% for both INNOVANCE® sodium citrate concentrations. However, when comparing with WBA the 3.8% citrate results with the INNOVANCE® cartridge was 90%. The INNVOANCE® PFA-P2Y agrees favorably with other methods for detection for P2Y12-receptor blockade agents.

D-62

Evaluation of the accuratic platelet counting by electrical impedance, fluorescent optical and manual methods in microcytic blood samples

L. Pan. *Chang Gung Medical Foundation, Chang Gung Memorial Hospital, Kaohsiung, Kaohsiung, Taiwan,*

Automated hematology analyzers provide high precision and accuracy for platelet counting. The machines distinguish red blood cells (RBCs) from platelets (PLTs) according to size. Generally, the size difference between the RBCs and PLTs is large enough to discriminate the two cell types of particles. However, spurious overestimation occurs when there are small particles similar to the PLTs, such as red cell fragments and leukemic cell debris. Microcytic anemia as well, it is characterized by small red cell size with mean corpuscular volume (MCV) less than 80 fL and most commonly caused by iron deficiency, thalassemia, infection, chronic disorder and malignancy. Particularly in patients with hematological malignancies receiving chemotherapy, either therapeutic or prophylactic platelet transfusions for thrombocytopenia are frequently prescribed. Among these patients, a spurious overestimation of platelet counts is not uncommon resulted from false recognition of the microcytic RBCs and tumor cell debris by the automated hematology analyzers. The delayed platelet transfusion may result in severe bleeding and even death of the patients. In this study, 203 K_2 EDTA anticoagulant blood samples were obtained from department of laboratory medicine to evaluate the accuracy of PLTs count. Two different modes, the routine method of electro-impedance (PLT-I) and advance method of fluorescent optical (PLT-O) of the Sysmex XE-2100, and reference method (PLT-M) were compared in platelet counting. PLT-M used Unopette diluting system, a microscope to count PLTs on improved Neubauer hemacytometer by senior medical technologists. 203 samples were divided into 4 groups. Group 1, 45 samples with normal MCV (>80fL) and PLTs (>140*10^3/μL) were used as control. 41 samples with normal MCV but abnormal low PLTs (<140*10^3/μL) were recruited in group 2. Group 3, 68 samples with microcytic RBCs (MCV<80fL) and normal PLTs were recruited. Group 4, 49 samples with microcytic RBCs and abnormal low PLTs were recruited. Our results show that the agreement gave a correlation of 0.954 ($P < 0.001$) between PLT-I and PLT-O, 0.932 ($P < 0.001$) between PLT-I and PLT-M and 0.969 ($P < 0.001$), between PLT-O and PLT-M. The electro-impedance method overestimates PLTs compared with fluorescent optical method ($P < 0.001$) and reference method in microcytic blood samples ($P < 0.005$). An accurate platelet count is essential in many diseases for platelet transfusion. The results with limitation of PLT-I in microcytic blood samples of disease states, an alternative confirmative method, such as PLT-O and manual Unopette microscopy platelet counting are recommended for patients with microcytic anemia and low platelet count.

Our study highlights the potential inaccuracy of routine clinical use modes (PLT-I) and re-emphasizes the need for alternative method to improve accuracy of PLTs counting. PLT-O is easy and rapid to perform which requires no specialized equipment and skilled technology. For the accurate practice in routine use, we propose that PLT-O could be an alternative method of platelet counting in microcytic blood samples, and each technology employed on the XE-2100 analyser for patients with microcytic thrombocytopenia, the advance method of fluorescent optical combined with reference method double checked platelet counting were suggested.

D-63

Elevated levels of the fibrin monomer complex and D-dimer indicate high risk of venous thromboembolism after spinal surgery

I. Kitajima[1], M. Tani[1], Y. Uji[1], K. Yoshioka[2], T. Kabata[2]. *[1]University of Toyama, Toyama, Japan, [2]University of Kanazawa, Kanazawa, Japan,*

Background: The fibrin monomer complex (FMC) has attracted attention as a quick and useful factor to detect thrombophilia. FMC is produced when thrombin sequentially cleaves fibrinopepptide A and B from the aminoterminals of A- and B-chains of fibrinogen. To determine the incidence of deep venous thrombosis (DVT) and pulmonary embolism (PE) after spinal surgery. To clarify the rapid changes of FMC and D-dimer in the perioperative period of spinal surgery for early diagnosis of venous thromboembolism (VTE).

Materials and Methods: The subjects were 72 patients who underwent spinal surgery. Before surgery, all patients had a standard laboratory screening that included a complete blood count and lupus anticoagulant. FMC and D-dimer were measured six times: at induction of general anesthesia, just after the implantation or in the middle of surgery, immediately following surgery (one, three and seven days after surgery). To measure the FMC, we used Auto LIA FM (Roche Diagnotics, Tokyo, Japan) which is an F405 antibody raised with des AA-fibrin as an immunogen. This is rapid, sensitive latex immunoturbidimetric assay using latex-immunobilized anti-FMC monoclonal antibody. To measure D-dimer, we used the latex aggutination method using NANOPIA D-dimer, respectively (Daiichi Pure Chemicals, Tokyo, Japan). All patients were examined with duplex ultrasonography assessments of both lower extremities and lung perfusion scintigraphy 7-10 days after surgery. If DVT or PE was suspected, the patient underwent multidetector computed tomography (CT) venography. All statiscical analysis were performed using the SPSSII software package (SPSS, Tokyo, Japan). This study was approved by Ethics Comittee of University of Toyama and Kanazawa University Hospital, and informed consent was obtained from all patients.

Results: Six patients (8.3%) showed VTE, of whom four patients had DVT, one patient had PET and one patinent had PE with DVT. There were no significant difference in age (p=0.49), gender (p=0.41), body mass index (p=0.59), operative time (p=0.19), blood loss during operation (p=0.06). Patients with VTE had significantly higher FMC levels one day after surgery compared with those without VTE (55.9±17.2 μg/ml versus 11.1±2.89 μg/ml, P<0.01). Patients with VTE had significantly higher D-dimer levels seven days after surgery compared with those without VTE (12.5±2.95 μg/ml versus 4.3±0.39 μg/ml, P<0.01). The receiver operating characteristic (ROC) analysis showed that FMC was more useful than D-dimer for the diagnosis of VTE. When the cutoff value were set up at >12.8 μg/ml for FMC, sensitivity was 100%, and specificity was 78.8%.

Conclusions: In our study the prevalence of VTE after spinal surgery was 8.3%. FMC measured one day after spinal surgery is considered to be useful as an indicator of VTE.

D-64

Comparative performance of the UniCel DxH 800 hematology analyzer and the Cell-Dyn Sapphire hematology analyzer in a Tertiary Care Children's Hospital

B. Tan, A. Nava, T. George. *Department of Pathology, Stanford Univeristy, Stanford, CA,*

INTRODUCTION: Analysis of pediatric samples on hematology analyzers is often problematic due to small blood volumes and because instrument flags are typically optimized to detect abnormalities in adult specimens. These unique aspects result in more manual interventions and a much higher slide review rate for pediatric specimens. We evaluated the new DxH 800 hematology analyzer (Beckman Coulter, Miami, FL) versus the Cell-Dyn Sapphire hematology analyzer (Abbott Diagnostics Division, Santa Clara, CA). Our aims were to compare the CBC, automated differential, NRBC count and overall efficiency of each analyzer on pediatric samples collected in a busy tertiary care children's hospital.

METHODS: We ran a total of 156 pediatric microtainer samples on both instruments in parallel, according to NCCLS guidelines. We compared numerical test results from the DxH 800 versus the Sapphire, as well as each instrument's differential versus a reference 400-cell manual differential. We also compared flagging efficiencies by determining whether flagged specimens showed abnormalities on a peripheral blood smear as defined by criteria from the International Committee for Standardization in Haematology.

RESULTS: Using linear regression analysis, the CBC showed excellent correlation between the DxH 800 and Sapphire, including WBC (r = 0.994), RBC (r = 0.992), HGB (r = 0.998), MCV (r = 0.988), and PLT (r = 0.995). The exception was MPV (r = 0.817), a parameter not reported at our institution. The automated differential from the DxH 800 compared well with the reference 400-cell manual differential (neutrophil r = 0.959, lymphocyte r = 0.948, monocyte r = 0.793, eosinophil r = 0.907, and basophil r = 0.271). Similar performance was observed with the Sapphire (neutrophil r = 0.952, lymphocyte r = 0.955, monocyte r = 0.781, eosinophil r = 0.710, and basophil r = 0.190). The NRBC count on the DxH 800 correlated better with the manual count (r = 0.987) than the Sapphire (r = 0.901). The sensitivity and specificity of the DxH 800 flags for detecting specimens with an abnormal peripheral blood smear were 95.7% and 67.7%, respectively, to yield an overall efficiency of 76.6%. In comparison, the sensitivity and specificity of the Sapphire flags were 91.3% and 57.6%, respectively, with an overall efficiency of 68.3%. The DxH 800 showed both a lower false negative rate (1.4% vs 2.8%) and lower false positive rate (22.1% vs 29.0%) versus the Sapphire.

CONCLUSIONS: Both the DxH 800 and Sapphire provide comparable CBC results and an automated differential that shows a high degree of correlation with a reference 400-cell manual differential. In our evaluation at a busy, tertiary pediatric laboratory service, the DxH 800 was more accurate at detecting NRBCs, and the instrument flags were more sensitive and specific at detecting abnormal peripheral blood specimens.

D-65

Comparison of New Access Soluble Transfer Receptor Assay to Roche Diagnostics Modular P Test

P. J. Nelson, M. G. Rush, J. Bockman, C. L. Wiley. *PSHMC and PAML, Spokane, WA,*

Background: Anemia of chronic disease (ACD) and iron deficiency anemia (IDA), the most common forms of anemia, are difficult to differentiate by standard tests which are affected by inflammatory processes. Soluble Transferrin Receptor (sTfR) is unaffected by inflammation providing an advantage over standard measures.

Objective and Methods: The study was performed at Providence Sacred Heart Medical Center to determine assay performance of the sTfR assay on the Beckman Coulter DxI600 (Access® Enzymatic Immunoassay System) when compared to Roche Diagnostic's Modular P analysis (Particle-enhanced immunoturbidimetric assay). The Beckman Coulter (BCI) sTfR assay is a two-site immunoenzymatic ("sandwich") assay. A serum or heparinized plasma sample is added to a reaction vessel along with paramagnetic particles coated with anti-sTfR antibody and an anti-sTfR alkaline phosphatase conjugate. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. The chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of sTfR antigen in the sample. The amount of sTfR in the sample is determined from a stored, multi-point calibration curve. The time to first result is 35 minutes.

Results: Using a Deming Regression analysis, 78 patient samples ranging from 1.2 to 36.2 mg/L were compared using the BCI sTfR assay and Roche method. The slope was 0.413 (0.384 to 0.442[95% CI]), the intercept was 0.03 (-0.24 to 0.30[95% CI]) and the correlation coefficient was 0.95. The concordance study showed 89% agreement in the diagnostic accuracy of both assays in discriminating between patients with IDA and ACD. The intra-assay imprecision for three controls at levels using 30 data points each are as follows: 0.7 mg/L (3.8%), 1.9 mg/L (4.2%) and 6.8 mg/L (3.0%). Inter-assay imprecision studies for three levels over 10 days yielded 7.2% (0.7 mg/L), 5.2% (1.8 mg/L) and 5.6% (6.6 mg/L). The Accuracy, Reportable Range, and Linearity of sTfR were analyzed on BCI DxI600 over a range of 0.0 to 10.8 mg/L. This analysis assumes accurate assigned values. Allowable systematic error (SEa) was 1.2 mg/L or 12.0%. The maximum deviation for a mean recovery from 100% was 5.5%. Six of 6 mean recoveries were accurate with the SEa. Eighteen of 18 results were accurate within the allowable total error (TEa) of 3.6 mg/L or 36.0%. The results are linear. The analytical measurement range of the sTfR assay (0.2 - 11.1 mg/L) was verified by the linearity experiment. A multiplication factor of 0.0738 was used to convert sTfR values from nmol/L to mg/L.

Conclusions: The sTfR values by the BCI assay were 2.4 times lower when compared to Roche method. This study demonstrated that there is good correlation between methods with a concordance study showing that the diagnostic accuracy of both assays in discriminating between patients with IDA and ACD is strong.

D-66

Hemostasis Test Compatibility with Capped Tubes

K. Finnegan[1], G. Cutsforth[2]. [1]*SUNY Stony Brook, Stony Brook, NY,* [2]*Diagnostica Stago, Inc., Parsippany, NJ,*

Several manufacturers have introduced cap-piercing technology on their automated blood coagulation analyzers. The study was initiated to determine the substantial equivalence of routine hemostasis testing (PT, INR, APTT and fibrinogen) on capped versus uncapped evacuated blood collection tubes from two manufacturers. The use of cap-piercing technology reduces technologist exposure to blood borne pathogens. However, cap-piercing technology must be validated to ensure that a bias or additional variability is not introduced. Seventy one volunteers donated two hemostasis collection tubes (one each of two manufacturers) and the PT, PTT and fibrinogen assays performed first on the capped tube, then on the same tube, uncapped. Demographic data (age and sex) and coumadin/coumarin/warfarin therapy were collected.

Samples were drawn by routine venipuncture and processed for platelet poor plasma within 4 hours of collection adhering to CLSI H21 A5 Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline. The PT, INR, APTT and fibrinogen were performed on a Stago STA®-Compact® with STA® System Controls N&P, STA® Neoplastine CI+, STA® PTTA and STA® -Fibrinogen reagents. Assays were performed in singlicate and the values were compared between capped and uncapped by linear regression and Student's T-test analysis. Linear regression analysis demonstrated a strong correlation between capped and uncapped values ($R^2 > 0.90$) for all assays. Student's t-test was not peformed on the data sets because the differences in values is dependent on the level of the value.

Linear Regression & Student''s T-test Values

Assay	Linear Regression	Linear Regression	Linear Regression	Student's T-test
	Slope	Intercept	R/R2	
PT	1.009	-0.34	1.00 / 0.99	
INR	1.012	-0.03	0.99 / 0.99	
PTT	0.955	1.36	0.99 / 0.98	
Fibrinogen	0.968	32.03	0.95 / 0.90	

Our conclusion is that there is no significant difference between PT, PTT and fibrinogen analysis nor on the INR calculated from the PT, on the capped or uncapped blood collection tubes tested in this study.

D-67

False-positive Results on ELISA-based anti-FVIII antibody Assay May Occur with Lupus Anticoagulant and Phospholipid Antibodies

M. A. Sahud[1], J. Popov[1], O. Zhukov[1], K. Mo[1], S. Ruden[1], M. Belter[2], J. Dlott[2]. [1]*Quest Diagnostics Nichols Institute, San Juan Capistrano, CA,* [2]*Quest Diagnostics Nichols Institute, Chantilly, VA,*

Background: The standard laboratory method for detection of factor VIII (FVIII) inhibitors is a clot-based Bethesda assay modified by the Nijmegen method, a relatively labor-intensive process. An ELISA-based assay was recently introduced to screen for FVIII inhibitors; only samples with positive results require confirmation with the neutralizing Bethesda/Nijmegen assay. When monitoring the performance of the FVIII IgG ELISA-based assay, we often detect LA in samples that test positive for FVIII antibodies with the ELISA screen but negative with the Nijmegen/Bethesda neutralizing assay. Here we examine whether clinical samples positive for LA or anti-phospholipid antibodies (APAs; ie, cardiolipin IgG and anti-β-2 glycoprotein IgG) exhibit cross-reactivity in the FVIII IgG ELISA.

Methods: The FVIII IgG ELISA (GTI Diagnostics, Waukesha) was performed on a total of 289 plasma samples from patients with suspected antiphospholipid syndrome (APS) that were positive for LA (hexagonal phase confirmation assay; Staclot LA, Diagnostica Stago; n=143), cardiolipin IgG (Varelisa; Phadia; n=84), or anti-β-2 glycoprotein IgG (The Binding Site; n=62). Control plasma samples positive for antibodies to ASCA, parvovirus B19, or *T pallidum*, as well as healthy adults, were also tested with the FVIII IgG ELISA. All specimens were also tested for antibodies to factor IX (FIX) using an ELISA-based in-house assay. Selected samples with positive results on the FVIII IgG ELISA screen were tested with the Nijmegen/Bethesda assay to confirm the presence of neutralizing antibodies to FVIII.

Results: Positive results on the FVIII IgG ELISA were significantly more common in samples positive for LA, cardiolipin IgG, and β-2 glycoprotein IgG than in samples from healthy donors. None of the samples positive for LA or APA tested positive for FIX IgG antibody by ELISA.

Conclusions: Lupus anticoagulant and APAs may cross-react with this ELISA-based FVIII antibody assay, yielding false-positive results. This could lead to labor-intensive additional testing.

Specimen reactivity (n)	**Anti-FVIII IgG- positive, No. (%)**	***P****
Lupus anticoagulant (143)	18 (13)	<0.001
Anti-Cardiolipin IgG (84)	15 (18)	<0.001
Anti β-2 glycoprotein IgG (62)	6 (10)	0.01
Anti ASCA IgG (22)	0 (0)	NA
Anti *T pallidum* (6)	2 (33)	NA
Anti parvovirus B19 IgG (13)	0 (0)	NA
Healthy donors (101)	1 (1)	NA
Note: No samples tested were positive for anti-F IX IgG.		
*Vs Healthy donor; chi-square test		

Wednesday PM, July 28

Poster Session: 2:00 pm – 4:30 pm
Lipids/Lipoproteins

D-68

Association of high-sensitive C-reactive protein and anti-oxidized LDL antibody in Nepalese hypertensive individuals

R. Shrestha, S. C. Jha, M. Khanal, P. Gyawali, M. Shrestha, B. Jha. *Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal,*

Background: Identifying the individuals with high risk for developing cardiovascular disease (CVD) is an important aspect in primary prevention. Necessary intervention at right time could prevent the future cardiovascular events. Traditional risk factors including age, total cholesterol (TC) concentration or LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), blood pressure (BP), diabetes, obesity and smoking status are being assessed to predict the future cardiovascular events from decades. However, established traditional risk factors alone is not sensitive enough to predict the risk. Though, numbers of emerging risk factors, including high-sensitive C-reactive protein (hsCRP) and antibody to oxidized LDL (anti-oxLDL antibody), have been proposed as significant predictors of CVD, these are rarely used in our country. Very few laboratories provide hsCRP testing and no laboratory provides service of oxLDL antibodies assay. To this date the study on hsCRP and anti-oxLDL antibodies concentration have not been done in Nepalese hypertensive subjects.
Objective: This study was designed to determine the association of hsCRP and anti-oxLDL antibody levels in Nepalese hypertensive individuals and to determine the correlation of these two emerging risk factors with other risk factors.
Methods: This study comprises 150 hypertensive individuals (93 under anti-hypertensive medication and 57 newly diagnosed) and 150 healthy controls (80 with normal BP and 70 prehypertensive individuals). Traditional risk factors like body mass index (BMI), presence of diabetes, metabolic syndrome, dyslipidemia, microalbuminuria and chronic kidney disease (CKD), and smoking status were assessed. Blood samples were also analyzed for hsCRP and anti-oxLDL antibodies. Data were analyzed using SPSS for window.
Results: Apart from clustering of traditional risk factors like dyslipidemia, metabolic syndrome, CKD, microalbuminuria, increase in BMI and decreased estimated glomerular filtration rate (eGFR) in hypertensive individuals, increased concentration of hsCRP ($p<0.001$) and anti-oxLDL antibodies (IgM, IgG) ($p<0.001$) were also associated with hypertension. No significant difference in blood level of hsCRP and oxLDL antibodies were seen between treated and newly diagnosed cases of hypertension. hsCRP concentration was significantly higher in prehypertensive individuals compared to normotensive individuals ($p=0.012$). Increase in age, BP, BMI, uric acid, TC concentration, LDL-C, triglyceride, urine albumin, decrease in eGFR and HDL-C, and the presence of metabolic syndrome were significantly associated in individuals with hsCRP concentration more than 3.0 mg/L. The level of hsCRP does not appeared to depend on smoking status. Increase in age, TC, triglyceride, LDL-C, and decrease in eGFR and HDL-C were significantly associated with individuals with oxLDL-antibody concentration more than 30 U/L. Concentration of oxLDL antibodies was also significantly higher in smokers and individuals with metabolic syndrome. Significant positive correlation was seen between level of hsCRP and oxLDL antibodies with 10-years predicted risk (Framingham risk score). Further, the individuals with multiple risk factors count were associated with increase level of hsCRP ($p=0.049$) and oxLDL antibodies ($p=0.011$). A positive correlation was also observed between hsCRP and oxLDL antibodies concentration ($r=0.539$, $p<0.001$).
Conclusion: Concentration of hsCRP and oxLDL-antibodies were significantly increased in Nepalese hypertensive individuals. The increase levels also correlates with other traditional risk factors. Increase levels of these biomarkers add risk for CVD.

D-69

Relationship between Apoliporotein AII Gene -256T/C Polymorphisms and Type 2 Diabetes Mellitus with Coronary Heart Disease in Han Nationality

L. Zhang[1], S. Yan[2]. *[1]Peking Union Medical College Hospital, Beijing, China, [2]China-Japan Friendship Hospital, Beijing, China*

Background:Apolipoprotein AII (apolipoprotein AII, apoAII) is a major apolipoprotein in human HDL particle. The physiological and biochemical role of apoAII is still in dispute. Lacking of large population epidemiological study,apoAII has long been regarded as a less important component of apolipoprotein. Recently, with the exploration of gene expression regulation and new polymorphisms of apoAII encoding gene (*APOAII*), as well as the results of clinical trials and studies of transgenic mice, the role of *APOAII* in development and prevention and treatment of atherosclerosis and hyperlipidemia has absorbed more concern from the researchers. However, many contradictions existed in different study of *APOAII* polymorphisms and risk of cardiovascular diseases. Numerous genetic studies in different populations revealed the relationship between *APOAII* polymorphism and type 2 diabetes. Now, *APOAII* is a hot genetic target for type 2 diabetes and its complication all over the world.Our research is to explore the effect of the interatction between *APOAII* -256T/C on the presence of polymorphism and type 2 diabetes mellitus with coronary heart disease in Han nationality from northern China.
Methods: Genotypes of *APOAII* -256T/C polymorphisms of *APOAII* gene were analyzed by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 155 unrelated healthy individuals, 101 patients dignosed of type 2 diabetes mellitus(DM), and 78 patients of type 2 DM combined with coronary heart disease (CHD). The relationship of gene polymorphisms of APOAII -256T/C and levels of HbA1c, serum lipids, and glucose was also analysed.
Results: Neither the frequencies of genotypes nor frequencies of alleles of APOAII gene -256T/C polymorphisms were statistically different among DM patients, DM+CHD patients and controls ($P>0.05$). lipid levels, HbA1c and glucose was presented differently in the genotype subgroups.Regardless of C allele gene carriers or TT genotype, in the DM+CHD group, TC was lower than that of control group and DM group, and TG, GLU and HbA1c were higher than the presence in the control group, while LDL-C, GLU and HbA1c were lower than that in DM group. No relationship between gender, family history of hypertesion, BMI and *APOAII* gene -256T/C polymorphisms was found. Logistic regression analysis showed that age and HbA1c was the risk factor, but HDL-C was a protective factor(β=-0.649,Exp(β)=0.785,95%CI=0.287~2.148,P=0.02)for type 2 diabetes mellitus with coronary heart disease.
Conclusion: The -256T/C polymorphisms of *APOAII* gene may not the major genetic risk factors of type 2 diabetes mellitus with coronary heart disease in Han nationality.

D-70

Effect of use of Depo Medroxy progesteron acetate contraceptive on serum lipid profile in average Nepalsese women.

B. K. Yadav, R. K. Gupta, B. Jha. *IOM,TUTH, Kathmandu, Nepal,*

Background: A substance or device capable of preventing pregnancy is called contraceptive .Depo-medroxy progesterone acetate (DMPA, locally called Depo-Provera) is used in more than 80 countries as a long acting contraceptive administered as single intramuscular (IM) injection of 150mg/3 months. It is more commonly used contraceptive by Nepalese women. Therefore, this study was designed to investigate the effect of DMPA on serum lipid profile in average Nepalese women.
Method: 220 subjects with age range 20-42 years selected in this study .Out of them 140 were experimental subjects and 80 were control. Among 140 subjects .Group I (n=40) was formed by new acceptors (who were taken DMPA for less than 2 years) and Group II (n=140) was formed by long term DMPA users (more than 3 continuous years) .Women without hormonal contraceptive were selected as the control subjects. Fasting Blood sample (5.0ml) were collected after a 12 hour fasting and serum were separated and stored at -20^{0}C until analyzed. Lipid profile (TC, TG, HDL-C, LDL-C) were estimated by sphectrophotometric enzymatic method (in BT2000 chemistry analyzer) using commercial kit manufactured by Human.
Result: - The Mean serum total cholesterol and triglyceride, LDL-cholesterol level of long term DMPA users were significantly ($p<0.01$, $p<0.05$ and $p<0.05$ respectively) elevated in comparison to that of non users. The mean serum HDL-cholesterol in the long term DMPA users were increased in comparison to control but it is not statistically significant ($p>0.05$).In case of new acceptors group , the lipid parameters remain almost unchanged in comparison to that of control group.
Conclusion: The present study shows that long term DMPA administration induces significant adverse effect on lipid profile and may favor the development of atherogenicity and other cardiovascular disease.

D-71

A new simple, accurate formula for LDL cholesterol estimation

R. B. Carvalho[1], C. M. M. Cordova[2], S. J. Guyenet[3], P. Carrera-Bastos[4], S. Lindeberg[5]. *[1]Independent researcher, Lisbon, Portugal, [2]FURB - Universidade Regional de Blumenau, Blumenau, SC, Brazil, [3]University of Washington, Division of Metabolism, Endocrinology and Nutrition, Seattle, WA, [4]NutriScience – Education & Consulting, Lisbon, Portugal, [5]University of Lund, Center for Primary Health Care Research, Lund, Sweden,*

Background: Low-density lipoprotein cholesterol (LDL-c) is considered a major parameter for cardiovascular risk assessment. The generally accepted Friedewald's

formula (LDL-F), developed in 1972 with data from 448 individuals, was established to replace costly direct LDL-c measurements requiring ultracentrifugation. More recently, less costly direct methods for LDL-c determination have been developed. However, these are still expensive for routine clinical application. Therefore, we compared the accuracy of various formulas for LDL-c estimation using directly measured LDL-c as reference.
Methods: We used a database of serum samples from 10,664 fasting individuals in Brazil, with direct measurements of TG, TC, HDL-c and LDL-c . LDL-c was measured by the LDL-C Select FS (Diasys) system, a homogeneous method without centrifugation steps. We applied the Least Squares Percentage Regression method to obtain several formulas in the format LDL-c = a * TC + b* HDL-c + c* TG. We also derived formulas using non-linear numerical approaches, but none provided significantly higher accuracy than the linear method. We then selected the formula with the highest accuracy and simplicity for general clinical use.
Results: The simple formula LDL-c = 3/4 (TC - HDL-c) provided an accurate estimate of LDL-c. Estimated LDL-c from this formula showed not only a higher correlation (r = 0.93) than that from LDL-F (r = 0.87), but also higher accuracy, judged by the 50% smaller Mean Squared Relative Error (rMSE). Furthermore, our formula outperformed several other LDL-c formulas over a wide range of TC, HDL-c and TG values. We found a strong linear association between LDL-c and non-HDL (r = 0.93). We also found that LDL-c is nearly independent of TG levels, with approximately 3/4 of individuals with LDL/non-HDL ratio in the range 0.65-0.85, and a modest correlation of LDL-c with TG (r = 0.31).
Discussion: The simple formula LDL-c = 3/4 (TC - HDL-c) was found to be more accurate than the LDL-F for LDL-c estimation, in a very large Brazilian population, across a broad range of TC, HDL and TG values. Our analysis has the limitation of being based on measurements determined using a non-gold standard method for LDL-c determination. However, this approach employed a large population sample, with a broad range of TC, HDL and TG values, increasing its statistical significance and performance at extreme values. Our formula appears to provide a more accurate estimation of LDL-c for almost every possible TG value, from low to extremely high. As it depends solely on the non-HDL cholesterol value (TC-HDL), it does not require a fasting blood draw. The application of our formula in other populations is warranted.

D-72

Apolipoprotein E gene polymorphism in the Turkish healthy population: allele frequencies and relation to lipoprotein and lipid concentrations
Y. Ozarda[1], D. Sucu[1], E. Eroz[1], D. Aslan[2]. *[1]Uludag University, Bursa, Turkey, [2]Pamukkale University, Denizli, Turkey,*

Background: Apolipoprotein E (ApoE) plays a major role in lipoprotein metabolism and lipid transport. Three common alleles called ε2, ε3, ε4 have been described, which code for three protein isoforms (E2, E3 and E4).The aims of this study were to determine ApoE genotypes and allele frequency distributions in the Turkish healthy population and to investigate the effects of ApoE polymorphisms to lipid metabolism.
Methods: The study population consisted of 402 healthy individuals (179 male and 223 female). Blood samples were collected in EDTA and evacuated blood collection gel tubes (Vacutainer, Becton Dickinson, U.K.) from each participant. ApoE genotype was determined using the CVD StripAssay which is based on a Polymerase Chain Reaction-Reverse Hybridization technique. Allellic frequencies were estimated by gene counting metod. The Hardy-Weinberg expectation of genotype distribution was calculated from the estimated allele frequencies and a χ^2 -test was used to test for equilibrium. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were determined by automated enzymatic methods using Aeroset analyzer (Abbott, Wiesbaden, Germany. Low density lipoprotein cholesterol (LDL-C) was calculated by Friedewald formula.
Results: The frequencies of Apo E genotypes (%) were E2/2: 0.75, E2/3: 14.18, E2/4: 1.24, E3/3: 70.40, E3/4: 12.94 and E4/4: 0.50. Allellic frequencies were ε2=0.085, ε3=0.840, ε4=0.076. The Hardy-Weinberg equilibrium was χ^2 =0.07, df=5, p>0.05. Subjects with ApoE*4 alleles showed significantly (p <0.05) higher TC and LDL-C values as compared to ApoE*2 and ApoE*3 alleles carriers.
Conclusion: It is established that Turks have high prevalence of coronary heart disease. Lipis and lipoproteins are in focus. Studies of the Turkish population have showed that Turks have high TC, LDL-C and low HDL-C levels. The ε4 allele of the ApoE gene is known to be related with coronary artery disease through lipoprotein metabolism and atherogenesis. The present study provides the high TC and LDL-C levels in ApoE*4 carriers which can be contribute to high prevalence of coronary heart disease in Turks.

D-74

Plasma adiponectin levels are related to obesity, inflammation, blood lipids and insulin in type 2 diabetic and non-diabetic Trinidadians
S. Nayak, D. Ramsingh, D. Ramdath, P. Teelucksingh. *Faculty of Medical Sciences, University of West Indies, Mount Hope, Uriah Butler High way, Trinidad and Tobago,*

Objective: To examine the extent to which plasma adiponectin levels are related to obesity, inflammation, blood lipids and insulin resistance in type 2 diabetic (T2DM) and non-diabetic Trinidadians.
Methods: This was a cross-sectional study of persons with type 2 diabetes attending primary and tertiary healthcare settings in central Trinidad who were matched with non-diabetic controls for age, gender and ethnicity. Along with clinical history and anthropometry, adiponectin, TNF-α, IL-6, CRP, lipid profile, glucose, and insulin were measured in fasting blood samples and insulin resistance (HOMA-IR) was calculated.
Results: Compared to controls (n=140), diabetics (n=126) had higher (p<0.05) glucose, insulin, HOMA-IR, triglycerides (TAG), VLDL and systolic blood pressure, but lower (p<0.05) HDL and adiponectin levels. Obese individuals had lower (p<0.05) adiponectin regardless of diabetic status. There were significant gender differences in HDL, LDL and TAG. In controls adiponectin correlated (p<0.05) with triglycerides (r = -0.280), IL-6 (r = -0.216), HOMA-IR (r = -0.373) and HDL (r = 0.355). When adjusted for confounders the relationship between adiponectin and HDL (r = 0.288), and TNF-α (r = -0.355) remained significant (p<0.05). Among T2DM, adiponectin was inversely related to BMI (r= -0.294; p<0.05). When adjusted for age, blood pressure, BMI and lipids, adiponectin was inversely related to HOMA-IR (r = -0.330; p<0.05).
Conclusion: Adiponectin decreases with increasing adiposity and insulin resistance. Adiponectin and TNF-α appear to be related to differences in the insulin mediated glucose disposal.

D-75

Analysis of the plasma fatty acid composition in T2DM by high performance liquid chromatography
L. NING, X. Zhou. *Wuhan University Zhongnan Hospital, Wuhan, China,*

[**Abstract**] **Objective** To analyze the plasma fatty acid composition in patients with type 2 diabetes mellitus (T2DM).
Methods The subjects are divided into three groups: normal control, T2DM and T2DM with hyperlipidemia.High-performance liquid chromatography (HPLC) is used to detect the plasma free fatty acids(FFAs)composition. The FFAs are extracted with modified Doles method, and the bromoacetophenone is used for the pre-column-derived agent. The plasma FFA composition are assayed on Dionex Summit HPLC system and quantified by internal standard calibration curve. The detail conditions are as follow: C18 reversed-phase column; isocratic elution; column temperature at 37 °C; wavelength at 242 nm; mobile phase is methanol: acetonitrile : water = 82:9:9, flow rate is 1.0 ml / min.
Results Compare to normal control, the total concentration of plasma FFA in T2DM is increased (p<0.01), mainly the concentration of saturated FFA (C16: 0, p <0.01). There is no significant difference in unsaturated FFA (C16:1, C18:2, C18:1) between the two groups. The proportion of saturated FFA concentrations of T2DM is raised (C16:0, p<0.01), while unsaturated FFA is reduced (C18:2, p<0.01). Compare to T2DM , total FFA concentration is increased in T2DM with hyperlipidemia, almost all of the FFA are elevated except for C18:1,C18:2. And the proportion of unsaturated FFA decreases significantly (C18:1, p<0.01; C18:2, p<0.05).
Conclusion The plasma total FFA concentration of T2DM is increased, mainly of which is saturated FFA, and unsaturated FFA is not significant increased. The C16:0 (saturated FFA),C18:1(mono-unsaturated FFA),C18:2(poly-unsaturated FFA)are the most representative ones. The level of the three FFAs is related to the development of T2DM, they might be the new items for clinical monitoring the lipid disorder of T2DM in the future.

D-76

Correlation of Lipid profile in Coronary Heart Disease patients in Libya
R. Kondreddy[1], A. Chenak[1], S. Shakila[1], B. Meka[2], A. M. Jarari[3], N. Naseb[3], J. R. Peela[3]. *[1]Department of Biochemistry,Faculty of Medicine,Al-Tahady University., Sirt, Libyan Arab Jamahiriya, [2]Department of Medicine,Faculty of Medicine,Al-Tahady University., Sirt, Libyan Arab Jamahiriya, [3]Department of Biochemistry,Faculty of Medicine,Al-Arab Medical University., Benghazi, Libyan Arab Jamahiriya,*

Introduction Coronary heart disease (CHD) is widely prevalent across the globe. Cholesterol is a major risk factor implicated in the causation of CHD. Our study reiterated

this finding. There have been studies on the incidence and prevalence of CHD in most countries of the world where this disease imposes high personal, social and economic costs, and the role played by other risk factors. However, studies concerning Libya and other Arab countries are generally not available or are not published.
Objective The current study was carried out to estimate the predictive value and the correlation of lipid profile with CHD in the population of Sirt city, one of Jamahiriya cities located in North Central Libya, bordering the Mediterranean sea, where there have been no studies on CHD incidence.
Methods The current study was a case-control study; and was carried out on a total of 167 clinically healthy subjects aged 26 to 80 years who served as population-based controls, and 98 CHD patients (history of angina or surviving myocardial infarction) with or without DM and HTN, admitted and diagnosed in coronary care unit of Ibn Sina Teaching Hospital and Clinic of Cardiology in Sirt Central Polyclinic where clinical signs and symptoms, ECG criteria Echocardiography, cardiac enzymes (CPK, CK-MB & LDH) and Troponin-I are applied for the diagnosis of each case. All CHD cases with liver impairment, renal disease or thyroid disease were excluded. The blood collection was done early morning after an overnight fast of 12-14 hours in controls. In CHD patients, samples were collected the next morning after admission of AMI cases or like controls for MI survivors or angina cases.
Results Total cholesterol and LDL-C were significantly increased while HDL-C was significantly decreased among the CHD group. Total cholesterol in the CHD group was 195.44±21.58 mg/dL (Mean±SD) vs. 189.42±10.45 mg/dL in the control group ($p<0.05$). LDL-C level in the CHD group was 127.42±13.10 mg/dL vs. 117.67±7.10 mg/dL in the control group ($p<0.05$). HDL-C level in the CHD group was 34.84±1.66 mg/dL compared to 39.28±2.94 mg/dL in the control group ($p<0.05$). Triglycerides did not show a significant increase in the CHD group (164.78±28.76 mg/dL), compared to the control group (165.41±15.12 mg/dL). The TC/HDL-C and LDL/HDL-C were significantly increased in the CHD group; while there was no significant elevation in TG/HDL-C ratio.
Conclusion The findings of our study indicate that cholesterol is an important risk factor for CHD, especially an increase in total cholesterol and LDL-C and decrease in HDL-C. There is need for appropriate pharmacological and non-pharmacological methods to maintain cholesterol levels within normal limits.

D-77

Estimation of small dense LDL cholesterol from calculated and direct LDL-cholesterol

P. Srisawasdi[1], S. Chaloeysup[1], Y. Teerajetgul[2], A. Pocathikorn[3], S. Vanavanan[1], M. Kroll[4]. *[1]Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [2]Faculty of Associated Medical Science, Khonkaen University, Khonkaen, Thailand, [3]Faculty of Medicine, Prince of Songkla University, Songkla, Thailand, [4]Department of Laboratory Medicine, Boston Medical Center, Boston, MA,*

Background: Small, dense low density lipoprotein (sdLDL) particles are a powerful predictor of atherogenesis. However, most sdLDL methodologies are expensive, time consuming and technically demanding, making them too laborious for routine clinical practice. Several studies indicated that sdLDL concentration directly correlates with serum triglyceride (TG) and inversely correlates with high density lipoprotein cholesterol (HDL-C). The calculated LDL-cholesterol (cLDL-C) may differ from the direct measurement of LDL-C (dLDL-C), and this difference may depend on the presence of sdLDL particles in addition to the variation in TG and HDL-C concentrations. The presence of such a dependence would offer a simple means to estimate sdLDL. We studied the dependence of sdLDL on cLDL-C, dLDL-C and other variables.
Methods: We measured glucose, creatinine, total cholesterol (TC), TG, HDL-C and dLDL-C using standardized methods, in 178 sera from the outpatient clinics of Ramathibodi Hospital. For sdLDL-cholesterol, a novel homogenous enzymatic assay (Denka Seiken, Japan) was used. The cLDL-C (in millimole per liter) was calculated using the Friedewald formula: cLDL-C = TC - HDL-C - TG/2.2. We used stepwise regression to analyze the significance of these variables.
Results: The median age of the patients was 59 years. Stepwise regression analysis identified TC, HDL-C, dLDL-C and cLDL-C as significant variables ($P < .001$), $r = 0.9$ and the standard error of the estimate of 0.27. The median sdLDL cholesterol was 1.24 mmol/L. The regression equation was sdLDL (mmol/L) = 0.33 TC - 0.30 HDL-C + 0.52 dLDL-C - 0.58 cLDL-C - 0.23.
Conclusion: The sdLDL- cholesterol concentration can be estimated from the TC, HDL-C, dLDL-C and cLDL-C, providing a cost-effective method for screening patients for the risk of cardiovascular disease. Moreover, the identification of a simple inexpensive marker for sdLDL particles may pre-select patients who would most benefit from a more definitive subfraction workup.

D-78

Lipid Profile of Mongolians

T. Enkhjargal, D. Khishigbuyan, P. Gantuya, B. Sodnomtseren. *Public Health Institute, Ulaanbaatar, Mongolia,*

Background: Diseases of circulatory system are the leading cause of death in Mongolia (Health indicators 2007). Serum total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) are implicated as risk factors for coronary artery disease. Limited information is available on the lipid profile of Mongolians.
Methods: Fasting morning blood samples were collected from 436 adults (177 males and 259 females) 25-64 years of age from Ulaanbaatar, the capital city of Mongolia and four provinces located in the south, north, east and west of the country. The serum levels of total cholesterol, triglycerides, HDL-C and LDL-C were determined using photometric systems.
Results: The mean serum total cholesterol concentration was 5.03 mmol/L and 11.0% of the surveyed had raised blood cholesterol (>6.20 mmol/L). The overall mean serum level of triglycerides was 1.63 mmol/L (14.7% had raised triglycerides, i.e. >2.3 mmol/L), of LDL-C was 3.53 mmol/L (22.7% had elevated LDL-C, i.e. >4.15 mmol/L) and of HDL-C was 1.59 mmol/L (4.4% had low level of HDL-C, i.e. <1.0 mmol/L). There was no significant difference in the level of total cholesterol, triglycerides and HDL-C between males and females ($p>0.05$) as well as between smokers and non-smokers ($p>0.05$), heavy episodic alcohol drinkers and those who do not engage in heavy drinking ($p>0.05$) and adults with high level of physical activity and those who do not engage in vigorous activity ($p>0.05$). The mean LDL-C concentration was lower in females than in males (3.42 mmol/L vs. 3.79 mmol/L; $p=0.002$) and in adults who engage in high level of physical activity than in those with low level of activity (3.36 mmol/L vs. 3.59 mmol/L; $p=0.019$), but the differences in the LDL-C level between drinkers and non-drinkers and smokers and non-smokers were not significant ($p>0.05$). Adults living in the capital city had slightly lower level of cholesterol than those in the rural provinces (4.92 mmol/L vs. 5.10 mmol/L; $p=0.048$), but there were not significant differences by setting in concentrations of triglycerides, HDL-C and LDL-C ($p>0.05$). The mean total cholesterol level was highest in the age group from 45 to 54 compared to adults from 25 to34 ($p=0.006$), from 35 to 44 ($p=0.001$) and from 55 to 64 ($p=0.029$). Age-related differences were not significant in concentrations of triglycerides, HDL-C and LDL-C ($p>0.05$). No correlations were found between the levels of the blood lipoproteins of the surveyed and their Body Mass Index (BMI) values as well as their blood pressure levels. The survey results demonstrate that 8.5% of the Mongolian adults had two blood lipoproteins at the increased risk level and 2.3% had three lipoproteins at the high risk level.

D-79

Qualtative analyses for PC hydroperoxides in human plasma by LC/MS

S. P. Hui[1], H. Chiba[2], S. Jin[2], T. Kurosawa[1]. *[1]Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido, Japan, [2]Faculty of Health Sciences, Graduate School of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Sapporo 060-0812, Japan,*

Lipid peroxidation has been suggested to play a significant role in the pathophysiology of atherosclerosis, diabetes, aging and other conditions. In particular, PC hydroperoxides (PCOOH) have been the foci of lipid peroxidation studies because their precursor, PC, is a major component of cellular membranes and an important constituent of plasma lipoproteins in *vivo*. HPLC with chemiluminescence detection has been reported for the quantitative analyses of PCOOH (1), but is unable to provide structurally characteristic data for individual PCOOH components. In recent years, Mass Spectrometry (MS) has become a powerful tool for structural identification of phospholipids. Several groups have studied oxidized PC using liquid chromatography mass spectrometry (LC/MS), which allowed structures to be analyzed in detail. Here we report a specific LC/MS method for the qualitative analysis of PCOOH in human plasma. The standards of PCOOH used were synthesized chemically, and their structural characterization of the identified products were carried out by electrospray ionization mass spectrometry (ESI-MS), multistage tandem mass spectrometry (MS2, MS3), and time-of-flight mass spectrometry (TOF-MS). Reverse-phased LC separation was achieved using a column (Hypersil Gold, 2.1 × 100 mm, 5 µm, Thermo Fisher Scientific Inc. Waltham, MA, USA) at 60ºC. The gradient elution was performed on the mobile phase consisted of two solvents; solvent A was 5 mM ammonium acetate aqueous, B was methanol, and C was 2-propanol. The gradient was programmed as follows: 0.0-4.0 min 90% A and 10% B , 4.1-6.0 min 20%A and 80% C, 6.1-8.0 min 5% A and 95% C, 10.0 -12.0 min 90% A and 10% C. The flow-rate was 0.2 mL/min. The qualitative ESI-MS analyses were carried out by using a Finnigan LXQ linear ion trap mass spectrometer (Thermo Fisher Scientific Inc. Waltham, MA, USA) equipped with an ESI source. The mass range of the instrument was set at m/z 180-1000 and scan duration of MS at 0.5 s in positive and negative ion mode. We identified

1-palmitoyl-2-linoleoyl-PCOOH (PC 16:0/18:2-OOH) as Adachi et al (2), but for the first time, we identified 1-stearoyl-2-linoleoyl-PCOOH (PC18:0/18:2-OOH) in human plasma by LC/MS.

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D-80

The association of LDL-cholesterol and metabolic syndrome with chronic kidney disease based on estimated glomerular filtration rate in Japanese males

M. Orisaka[1], K. Kotani[2], M. Nakamura[3], A. Suwabe[4]. *[1]Iwate Health Service Association, Iwate, Japan, [2]Department of Clinical Laboratory Medicine, Jichi Medical University, Tochigi, Japan, [3]Osaka Medical Center for Health Science and Promotion, Osaka, Japan, [4]Department of Clinical Laboratory Medicine, Iwate Medical University, Iwate, Japan,*

Background: Metabolic syndrome (MetS) is a risk factor for chronic kidney disease (CKD), leading to cardiovascular disease. Low-density lipoproteins (LDL)-cholesterol can be a risk factor for CKD, while this association remains to be incompletely established. The diagnostic criteria of MetS do not need circulating LDL-cholesterol levels. Unexpectedly, there are no studies to observe simultaneously the association of LDL-cholesterol and MetS with CKD; therefore, the present study investigated the association in general males.
Methods: The study enrolled asymptomatic 6329 males, aged 35-80 years, who had no known history of cardiovascular, cerebrovascular, kidney and liver disease and who were not taking any medicine. The MetS was defined according to the NCEP-ATPIII criteria (more than 25 kg/m^2 of body mass index was substituted for waist), and the CKD was defined as less than 60 mL/min/1.73 m^2 of the estimated glomerular filtration rate (eGFR: the MDRD study equation). Serum LDL-C was measured using a homogeneous method(Kyowa Medex Co. Ltd., Tokyo, Japan). Analyses were performed by age-stratum of 35-50 (group 1), 51-65 (group 2) and 66-80 years (group 3).
Results: The MetS prevalence (9.5, 11.0 and 10.0% in the group 1, 2 and 3, respectively) and the LDL-C levels (3.3±0.8, 3.3±0.8 and 3.2±0.8 mmol/L, similarly) did not differ between the three groups, while the eGFR levels significantly decreased with an increase in the group number (85.3±12.7, 81.3±13.4 and 77.7±14.1 mL/min/1.73 m^2 in order). Multivariate logistic regression analyses on CKD adjusted for age, smoking, LDL-C and MetS revealed that LDL-C, rather than MetS, was significantly associated with CKD in the group 2 (LDL-C: odds ratio=1.01, 95%CI=1.00-1.02, p=0.005; MetS: 1.67 [0.96-2.91], p=0.072) and the group 3 (LDL-C: 1.01 [1.00-1.02], p=0.027; MetS: 0.89 [0.36-2.16], p=0.792), and that by contrast, MetS, rather than LDL-C, was significantly associated with CKD in the group 1 (LDL-C: 1.01 [0.99-1.02], p=0.068; MetS: 2.25 [1.04-4.84], p=0.039).
Conclusions: These results suggest that the impacts of LDL-C and MetS with CKD may differ by generation, and the examination and management of LDL-C, in addition to MetS, is important to prevent CKD in the Japanese older males particularly.

D-81

Calculation of cholesterol levels in major serum lipoproteins separated by qualitative agarose gel electrophoresis

S. Vanavanan, S. Chaloeysup, R. Kasetpibal, P. Srisawasdi. *Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand,*

Background: Major lipoproteins including, high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) are well accepted and widely used for the assessment of atherosclerosis, cardiovascular disease and its equivalents. Lipoprotein electrophoresis is important for investigation of patients with dyslipidemia to determine whether they have a type II, III, and IV patterns proposed by Fredrickson and Lees. Electrophoresis in an agarose gel by HYDRAGEL LIPO + Lp(a) (Sebia Inc., USA) is designed for the qualitative determination of lipid profile and also for screening lipoprotein (a), which is an important risk factor for coronary artery disease. Although, this method provides a relative lipid of individual fraction, it does not give the level of cholesterol carried on each major lipoprotein. This study developed the formulas for calculating a major lipoprotein cholesterol level from the qualitative electrophoresis.
Methods: Seventy-seven patient sera without chyromicrons and lipoprotein (a) were analyzed by two electrophoretic methods, the qualitative HYDRAGEL LIPO + Lp(a) and the quantitative HYDRAGEL LDL/HDL CHOL Direct with enzymatic staining of cholesterol (Sebia Inc.). Using the relationship between two methods by a linear regression model, we developed the formulas for calculating relative cholesterol (%) of individual fraction separated by the qualitative assay. The estimated lipoprotein cholesterol levels were calculated by multiply the percentage of relative cholesterol with total cholesterol concentration and dividing by 100. To apply the developing formulas for clinical use, the calculated lipoprotein cholesterol values obtained from 47 and 227 samples with and without lipoprotein (a), respectively was compared separately with the standardized lipoprotein cholesterol methods.
Results: Linear regression statistics obtained from the percentage of HDL, LDL and VLDL fractions between the quantitative (y) and the qualitative assays (x) were y = 1.014x - 8.3; r = 0.836, y = 1.017x + 21.0; r = 0.806, and y = 0.750x - 6.50; r = 0.849, respectively. To transform % relative lipid (x) to % relative cholesterol (y) for qualitative assay, we used the equation of y = x - 8, y = x + 21 and y = 0.75x - 6.5 for HDL, LDL and VLDL, respectively. The regression lines obtained between the calculated lipoprotein cholesterol levels (y) and the homogenous assays (x) in sample with and without lipoprotein (a) were close to the identity line with the regression coefficients of y = 1.07x - 0.18; bias = -0.079 mmol/L and y = 1.06x - 0.06; bias = 0.014 mmol/L, respectively for HDL-cholesterol, y = 0.90x + 0.32; bias = -0.097 mmol/L and y = 0.92x + 0.29; bias = -0.013 mmol/L, respectively for LDL-cholesterol, and y = 0.85x - 0.03; bias = -0.122 mmol/L and y = 0.90x + 0.32; bias = -0.019 mmol/L, respectively for VLDL-cholesterol.
Conclusion: The proposed formulas provide a reliable estimation of cholesterol concentration carried on HDL, LDL and VLDL fractions by qualitative Sebia Hydragel LIPO + Lp(a) lipoprotein electrophoresis.

D-82

Two apoE-containing HDLs, lipid-rich and lipid-poor: Need to avoid confusion

Y. Takahashi[1], H. Furumaki[1], T. Sakurai[1], A. Nagasaka[2], M. Fujikawa[3], S. Jin[1], S. Hui[4], Y. Min[5], H. Chiba[1]. *[1]Faculty of Health Sciences, Hokkaido University, Sapporo, Japan, [2]Department of Gastroenterology, Sapporo City General Hospital, Sapporo, Japan, [3]Department of Clinical Laboratory, Sapporo City General Hospital, Sapporo, Japan, [4]Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Japan, [5]Department of Advanced Medicine, Graduate School of Medicine, Hokkaido University, Sapporo, Japan,*

Background: HDL cholesterol is the strongest negative risk factor for coronary heart disease. Currently, two kinds of cholesterylester transfer protein (CETP) inhibitors, Anacetrapib (Merck) and Dalcetrapib (Roche/Japan Tobacco), are in Phase III clinical trials as new HDL cholesterol-raising drugs for cardiovascular disease, and preliminary results appear encouraging. HDL is classified into apoE-containing HDL (or apoE-rich HDL) and apoE-deficient HDL (or apoE-poor HDL). ApoE-containing HDL has been reported to increase in plasma in patients with hepatobiliary diseases and genetic CETP deficiencies, and also in humans and animals administered with CETP inhibitors. ApoE-containing HDL has potent in vitro ability to inhibit agonist-induced platelet activation and foam cell formation from macrophages, in comparison with apoE-deficient HDL. Hence, the increase of apoE-containing HDL due to administration of CETP inhibitor is expected to have anti-atherogenic effect. However, apoE-containing HDL is sometimes talked as proatherogenic in previous literatures including JAMA 297:1537, 2007. Possible heterogeneity of apoE-containing HDL might be a cause of this controversy. In order to make this point clear, here we isolated apoE-containing HDL and studied its cholesterol content.
Methods: Pooled normal human sera were used for this study. Total lipoprotein fraction was isolated by ultracentrifugation (d=1.225), and subjected to polyethylene glycol precipitation to remove contaminating LDL. Thus obtained total HDL fraction (apoE-containing HDL and apoE-deficient HDL) was separated by heparin-Sepharose affinity chromatography. After elution with sodium chloride gradient, each eluted fraction was analyzed for total cholesterol, apoA-I and apoE.
Results: ApoA-I eluted first from the heparin-Sepharose column, and then apoE secondarily. ApoE eluted first with cholesterol, and then with little amount of cholesterol.
Conclusion: We established an isolation method for apoE-containing HDL with different content of cholesterol. This method enabled us to carry out analytical experiments. The obtained results demonstrated the presence of two kinds of apoE-containing HDL: one was the cholesterol-rich one and the other was cholesterol-poor one. The previous results demonstrating the anti-atherogenicity of apoE-containing HDL were obtained for the cholesterol-rich one or apoE-containing HDL2. Additionally, CETP inhibitors are known to increase apoE-containing HDL2. On the other hand, possible relation to positive risk of atherosclerosis has been reported in a proteomics study on HDL3, but not HDL2 (J Clin Invest 117:746-56, 2007). We need to avoid confounding two apoE-containing HDLs, when we discuss about the role of apoE-containing HDL in coronary heart disease and also about new methods for HDL-cholesterol measurement.

D-83

The Relationship between serum Lipids and Breast cancer in Libya

J. R. Peela[1], A. M. Jarari[1], S. O. El Saiety[2], S. El Busaifi[1], H. El Awamy[1].
[1]Department of Biochemistry, Faculty of Medicine,Al-Arab Medical University,, Benghazi, Libyan Arab Jamahiriya, [2]Department of Surgery,7th October Hospital, Faculty of Medicine,Al-Arab Medical University,, Benghazi, Libyan Arab Jamahiriya,

Background: Carcinoma breast is one of the major surgical problems in Libya particularly in younger subjects. Since it is a common health risk it is important to know the etiology of breast cancer in Libya. There are conflicting reports regarding the association between dietary lipids and serum lipid profiles and breast cancer. It is a fact that local dietary habits, environmental factors along with genetic predisposition play a role in the evolution of breast cancer. The present study was undertaken to identify whether there is such an association between alterations in lipid profile in carcinoma breast in a local Libyan subjects..

Material and methods: 40 patients with various stages of breast cancer have been takenf or the present study. Their age varied from16 to 65 years with mean age of 37 years retrieved from department of surgery, 7th October hospital, Benghazi, Libya during 2009 and 2010. There were 21 healthy controls with age group of 20 to 55 years with mean age of 35 years. Out of 40 cases of carcinoma of breast 25 cases are premenopausal and 15 are post menopausal based on clinical history.It was observed that the premenopausal group fall in the age group<47 years and post menopausal group's average was> 47 years old. Serum Lipid profile was done in these paients' fasting blood samples.Cholesterol ,HDL-cholesterol and Triglycerides were measured by authentic methods and LDL cholesterol was calculated using Friedwald's formula.

Results: Serum cholesterol and HDL cholesterol were significantly elevated in patients with carcinoma breast when compared with controls (p= 0.0046 and 0.004 respectively). Whereas the levels of LDL cholesterol and triglycerides did not show any significant changes between the cases studied and the controls. (p= 0.42 and 0.092 respectively). In the case of premenopausal woman there is significant rise of total cholesterol(p= 0.0186) and HDL cholesterol(p= 0.0031) whereas triglycerides(p= 0.335) and LDL cholesterol(p = 0.2617) had not shown any significance. In the postmenopausal group there is significant elevation of serum triglycerides (p = 0.0094), total cholesterol (p = 0.0238) and HDL cholesterol (p = 0.0457) but LDL cholesterol had not shown any significant difference. There is a striking difference in triglyceride levels in postmenopausal woman from premenopausal woman (p = 0.0298).

Conclusion: The preliminary study shows a significant alteration in serum lipid profile in breast cancer patients in the local Libyan population. The surprise finding of high levels of HDL cholesterol in the patients studied needs further evaluation. In postmenopausal woman there was an increase in serum triglycerides levels along with an increase observed in serum total cholesterol and HDL-cholesterol levels compared to premenopausal women.

D-84

Circadian change of serum concentration of small dense LDL-cholesterol in type 2 diabetic patients

Y. Ito[1], S. Hirayama[2], S. Soda[3], T. Ueno[2], Y. Fukushima[2], H. Ohmura[2], O. Hanyu[4], Y. Aizawa[4], M. Higuchi[1], T. Miida[2]. *[1]Denka Seiken Co.,Ltd., Tokyo, Japan, [2]Juntendo University, Tokyo, Japan, [3]Niigata City General Hpspital, Niigata, Japan, [4]Niigata University, Niigata, Japan,*

Background: Type 2 diabetic patients (DM) have a higher risk of atherosclerosis than non-diabetic subjects with the same low-density lipoprotein-cholesterol (LDL-C). This difference may be attributable to increased levels of small dense low-density lipoprotein-cholesterol (sdLDL-C) in DM. As the sdLDL-C concentration is elevated in hypertriglyceridemia, which is exaggerated postprandially, we examined whether the sLDL-C level increases postprandially in type 2 diabetes.

Methods: The study subjects comprised DM (9 men and 6 women) and normolipidemic healthy subjects (control group; 8 men and 2 women). To elucidate the circadian rhythm of sdLDL-C, we obtained 7 blood samples (30min before and 2h after each meal, and at midnight) from 15 patients with DM and ten normal controls. We used the albumin concentration to correct for the effects of postural changes on the lipoprotein concentration. The total cholesterol and triglyceride concentrations were measured by enzymatic methods, and HDL-C and LDL-C by the homogeneous assay, the albumin by the bromocresol green method. sd-LDL-C was measured by the precipitation method and lbLDL-C was determined by the subtraction of sdLDL-C from LDL-C.

Results: The fasting sdLDL-C concentration was 60.3% higher in the DM than in the controls (1.01+/-0.21 vs. 0.63+/-0.21mmol/l, p<0.001), while there was no significant difference in lbLDL-C between two groups. The sdLDL-C concentrations in both the DM and control groups were highest in the fasting state, decreased after breakfast, and remained low until midnight. The maximal reduction in the absolute sdLDL-C concentration was 56.5% greater in the DM patients than in the controls (0.36+/-0.13 vs. 0.23+/-0.16mmol/l, p<0.05). Thus, the sdLDL-C/lbLDL-cholesterol (lbLDL-C) ratio was reduced with increases in lbLDL-C.

Conclusions: The sdLDL-C concentration decreases postprandially in diabetes. This absolute reduction in sdLDL-C may contribute to an acceleration of atherosclerosis in DM.

D-85

Biochemical characterization of LDL subfractions and verification of specificity of a new homogeneous assay for small, dense LDL-cholesterol

M. Fujimura, M. Ohta, Y. Ito. *Denka Seiken Co.,Ltd., Tokyo, Japan,*

Objective: We developed and characterized a novel homogeneous assay for quantification of small dense LDL cholesterol (sd LDL-C) along with biochemical features of LDL subfractions. For the development of the homogeneous assay for sd LDL-C, we focused on a difference in ratios of sphingomyelin to lecithin in various LDL subfractions, and we incorporated a sphingomyelinase in an assay reagent to decompose Large LDL particles which are believed rich in sphingomyelin. However, there is no publication describing profile of phospholipids on various LDL particles in detail. In addition, there is no clear evidence on the reactivity of our homogeneous assay to LDL particles with different ratios of sphingomyelin to lecithin.

Methods: We first obtained a series of 21 fractions from d=1.019 to d=1.063 by ultracentrifugation according to a modified procedure originally developed by Havel et al. These fractions were verified as LDL fractions by comparing recoveries by a total cholesterol measurement with those by a LDL-cholesterol measurement. We then tested such fractions by our new homogeneous assay for sd LDL-C as well as by a phospholipid, sphingomyelin, triglyceride and apoB measuring reagents and also by Transmission Electron Microscopy (TEM).

Results: Among the obtained 21 fractions, the largest LDL particle size was 20.9 +/- 1.5nm, the medium was 18.8 +/- 1.1nm and the smallest was 17.0 +/- 1.4nm. There is a clear relationship between the size of LDL particles and the ratio of sphingomyelin to lecithin. As the LDL particle size gets smaller, the ratio of sphingomyelin to lecithin gets also smaller (the ratio was 25.1% in the fraction with the largest LDL particle and it was 8.9% in the fraction with the smallest LDL particle). The reactivity of our new homogeneous assay for sd LDL-C was found proportional to the ratio of sphingomyelin to lecithin and the strongest to the LDL fraction with the smallest ratio.

Conclusions: These findings support the validity of our new homogeneous assay for sd LDL-C.

D-86

Comparison of reference ranges for small, dense LDL-cholesterol between the US population and Japanese population

M. Fujimura[1], M. Higuchi[1], Y. Sato[1], A. Minagawa[1], K. Kamata[1], K. L. Stanhope[2], P. J. Havel[2], K. Horvath[3], B. F. Asztalos[3], E. J. Schaefer[3], Y. Ito[1]. *[1]Denka Seiken Co.,Ltd., Tokyo, Japan, [2]UC Davis, Sacramento, CA, [3]Tufts University, Boston, MA,*

Objective: Small, dense LDL (sd LDL) is one of such subfractions of low-density lipoprotein (LDL) with a smaller particle size and higher density. It is known as an atherogenic lipoprotein, and many epidemiological and pathological studies have recently suggested the relationship between sd LDL-cholesterol (sd LDL-C) level and CHD occurrence. Several different methods have been employed for the determination of LDL particle size such as based on ultracentrifugation, electrophoresis, and nuclear magnetic resonance (NMR). All these methods require special equipment and very long assay time, making them too laborious for general clinical use. We recently developed a novel homogeneous assay for quantification of sd LDL-C which utilizes phospholipase and unique detergents. The new assay enables us to test numerous samples in a very short period of time. We report reference ranges on the Japanese population and US population and discuss racial difference in the sd LDL-C level.

Methods: We recruited 651 volunteers in two US regions (Sacramento, CA and Boston, MA). Fasting serum samples were collected and tested for sd LDL-C, LDL-C, HDL-C, triglyceride and glucose levels. Subjects with either LDL-C ≥ 160 mg/dL, HDL-C ≤ 40 mg/dL, triglycerides ≥ 200 mg/dL or glucose ≥ 126 mg/dL were excluded from the analysis (n=192). Data from 459 subjects were finally analyzed to calculate a reference range. The reference range was calculated as the central 95% confidence interval. We also recruited 620 volunteers in Japan and tested sd LDL-C, LDL-C, HDL-C, triglyceride and glucose levels in fasting serum samples. Subjects with either LDL-C ≥ 140 mg/dL, HDL-C ≤ 40 mg/dL or triglycerides ≥ 150 mg/dL were excluded from the analysis (n=160) according to the guidelines from Japan Atherosclerosis Society for hyperlipidemia. Data from 460 subjects were finally analyzed to calculate a reference range.

Results: Reference Ranges were 10.2 - 44.8 mg/dL (mean 24.1 ± 9.1 mg/dL) with the

US subjects and 9.4 - 34.0 mg/dL (mean 18.9 ± 6.2 mg/dL) with the Japanese subjects. It was 10 mg/dL higher with the US subjects compared to the Japanese subjects. Even if the reference range was re-calculated for the US population using the same exclusion criteria as applied to the Japanese population, there was no drastic change in the reference range and it remains higher than the Japanese population (6.1 - 45.9 mg/dL, n=372).
Conclusions: Racial difference in sd LDL-C was suggested by our study. Further investigations may be needed to clarify whether this difference is coming from different eating habit and/or genetic factors is involved.

D-87

Association of Apolipoprotein A5,triglycerides and Gestational Diabetes Mellitus in Chinese women
k. zhang, H. Wei. *Drum Tower Hospital Affiliated to Nanjing University Medical School, Nan Jing, China,*

Objective: Apolipoprotein A5 (ApoA5) was the newest member of apolipoprotein family, the association between ApoA5 gene polymorphisms and triglycerides (TG) levels is well established, but the role of ApoA5 in modulating triglyceride levels in humans is incompletely understood. Some researchers have reported positive correlations of ApoA5 with triglycerides while others have reported negative correlations. This study investigated the relations between maternal ApoA5 concentration and triglycerides and gestational diabetes mellitus (GDM).
Methods: 232 pregnant women who had normal glucose tolerance (NGT) and 182 women who had GDM at 20 to 36 wk of gestation were investigated. Maternal blood samples were drawn to determine plasma concentrations of ApoA5, insulin and blood lipids. Blood samples at 1 and 2 h after an oral glucose load were obtained to measure plasma glucose, and insulin concentrations.
Results: In comparison with the NGT group, women with GDM showed no significantly advanced age (P>0.05), and greater body mass index (BMI),ApoA5 and TG at the time of blood collection (P<0.01). As expected, TC and LDL-C were significantly higher, whereas HDL cholesterol level (1.1±0.2 vs 1.6±0.5 mg/dL, p<0.01) was markedly lower in the GDM group than in the NGT group. Plasma ApoA5 concentration was modestly increased in the third trimester of GDM group (169.3 ± 27.3 ng/mL) than in the same period of NGT group (132.2 ± 33.3 ng/mL). Significantly increased plasma fasting,1h,2h insulin, and TC concentrations were found in subjects at different trimester who had GDM compared with those who had NGT. Correlation analysis between plasma ApoA5 and cholesterol had been accomplished: A significant inverse correlation were observed between ApoA5 and total cholesterol(r= -0.412, p<0.001),LDL cholesterol (r= -0.202, p<0.05) in both NGT and GDM groups. Surprisingly, ApoA5 was positive associated with TG (r=0.324, p<0.001) in GDM. There was virtually no correlation between serum insulin levels and ApoA5 levels in patients with GDM. After adjustment for confounding factors including age, blood glucose, triglyceride, LDL cholesterol and HDL cholesterol, a multiple logistic regression analysis was performed To conform plasma ApoA5 level is an independent predictor of GDM(OR=1.02, 95%CI 0.8-1.95, p<0.05).
Conclusions: Our data indicate that in patients with GDM ApoA5 levels are positively correlated with triglycerides but are not correlated with insulin levels. ApoA5 is an independent predictor of GDM .
Keywords:Gestational diabetes mellitus;ApolipoproteinA5; Triglycerides

D-88

Absence of ApoB R3500Q Mutation in Lebanese Hypercholesterolemic Subjects
R. T. Daher, D. S. Sarieddine, R. Abdel Khalek, R. Tanios, N. K. Cortas, R. A. Mahfouz. *American University of Beirut, Beirut, Lebanon,*

Mutation in Apolipoprotein B 100 (apoB-100) has been associated with severe hypercholesterolemia and early onset atherosclerosis in many European populations. In this study we investigated the presence of the most common mutation in ApoB (R3500Q) in Lebanese Hypercholesterolemic patients. Analyses of cholesterol, triglyceride, LDL-C, and HDL-C were done using Roche Modular system (Mannheim, Germany). ApoA and ApoB-100 were analyzed using BN Prospec analyzer from Dade Behring (Marburg, Germany).
ApoB R3500Q mutation analysis was done using a sensitive PCR-reverse hybridization technique (CVD Strip Assay from Vienna Lab, Austria). Subjects were selected if their cholesterol was >240 mg/dL and ApoB-100 was above 1.25 g/L.
None of the 40 participants (20 males and 20 females) were positive for this mutation. The Lipid parameters for the study subjects are summarized below:

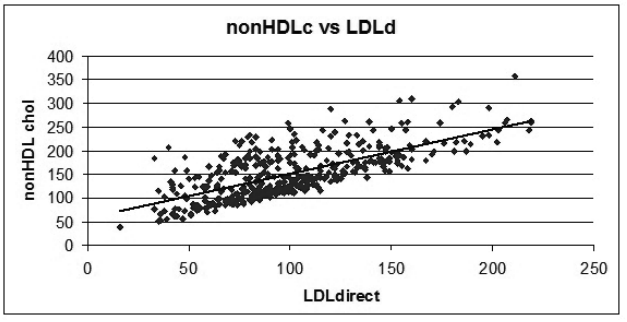
Analyte (Desirable cutoff)	Range	Mean	Std. Deviation
Age	25-73	51	12
Cholesterol total (120-200 mg/dl)	241-399	278	33
LDL (60-130 mg/dl)	147-238	186	18
HDL (M >45;F >55 mg/dl)	29-86	53	17
Chol/HDL (M <4.97; F <4.44)	3.43-11.00	5.80	1.91
TG (30-200 mg/dl)	45-1795	296	393
Apo A1 (1.1-2.15 g/l)	1.17-2.24	1.65	0.30
Apo B g/l (>1.25 g/l)	1.24-3.14	1.58	0.33
ApoA/ApoB (0.30-0.90)	0.46-1.59	1.08	0.25

Although this mutation has a wide European distribution, its trend showed a progressive geographic disappearance and was not reported in Turkey, Spain, and Israel. We have also confirmed in this study that this mutation is not prevalent among Lebanese Hypercholesterolemic patients.

D-89

Calculated nonHDL cholesterol - an alternative to LDL?
E. C. Dinovo[1], T. Howard[1], E. Dinovo[2]. *[1]VA Greater Los Angeles HCS, Los Angeles, CA, [2]Consultant, SRC, CA,*

LDL cholesterol has become the parameter and target of choice for evaluation and treatment of lipid disorders. Direct testing of the LDL cholesterol is not subject to the limitations of the Friedewald LDL cholesterol calculation and does not require the 14 hour fast. Increasingly, LDLdirect is being included in lipid testing profiles. The motivating premise is that examining the relationship between total nonHDL cholesterol (total cholesterol - HDL cholesterol) and the LDL test results would enlighten and perhaps lead to a simplified and cost effective lipid testing protocol.
Data for 425 consecutive Lipid Panels (Cholesterol, HDL, LDLdirect and Triglycerides) were extracted from the database. All reference to patient identifiers and demographics were removed and the raw laboratory data analyzed using the JMP statistics program. nonHDL cholesterol values were calculated and plotted against the LDL direct values. The linear regression equations was found to be:
nonHDLchol = 58.06 + 0.96*LDLdirect (p<.0001, r^2= 0.47)
Partitioning the data by triglyceride values, when triglycerides are below 400 mg/dL the linear fit to LDL direct is found to be 'nonHDLchol = 22.85 + 1.05*LDLdirect' (p<.0001, r^2= 0.90) and to the Friedewald LDL calculation is nonHDLchol = 27.47 + 1.01*LDLcalc (<.0001, r^2= 0.86). For triglycerides above 400 mg/dL, the linear fit equation is 'nonHDLchol = 88.66 + 1.09*LDLdirect' (p<.0001, r^2= 0.52) and to the LDLcalculated it is
nonHDLchol = 137.18 + 0.72*LDLcalc (<.0001, r^2= 0.53).
The coefficient of determination r^2 clearly indicates a tighter relationship between non-HDLchol and LDL direct or calculated when triglyceride values are below 400 mg/dL. Data and analysis support the contention that nonHDL cholesterol calculation may replace the LDL direct test or the Friedewald LDL calculation particularly when triglycerides are below 400mg/dL thus avoiding performing a triglyceride test and subjecting the patient to a 14 hour fast. Further studies are in progress.



D-90

Evaluation of oxidized LDL in patients with obstructive sleep apnea associated with dyslipidemia and hypertension.

M. C. Feres[1], F. D. Cintra[2], M. C. De Martino[3], S. C. Silva[4], P. G. Campana[4], D. Poyares[5], S. Tufik[6]. *[1]Universidade Federal de São Paulo- UNIFESP, São Paulo, Brazil, [2]Universidade Federal de São Paulo- UNIFESP and Instituo do Sono de São Paulo, São Paulo, Brazil, [3]Associação Fundo de Pesquisa a Psicofarmacologia- AFIP/ML, São Paulo, Brazil, [4]AFIP/ML, São Paulo, Brazil, [5]Universidade Federal de São Paulo- UNIFESP and Instituto do Sono de São Paulo, São Paulo, Brazil, [6]Universidade Federal de São Paulo- UNIFESPand Associação Fundo de Incentivo a Psicofarmacologia - AFIP/ML, São Paulo, Brazil,*

BACKGROUND: Obstructive sleep apnea OSA is a respiratory disorder involving multicomponent, with intermittent hypoxia (IH) as the main reason that is associated with metabolic and cardiovascular diseases. Pharyngeal obstruction during sleep leads to repetitive sequences of hypoxia and reoxygenation. IH induces many consequences as hemodynamic, hormonemetabolic and oxidative changes and immuno-inflammatory that can promote the formation of reactive oxygen species that oxidizes macromolecules and alter the system and activates redox signaling pathways in arterial changes favoring atherosclerosis. Atherosclerosis was found in patients with OSA free of other cardiovascular risk factors and is related to the severity of nocturnal hypoxia. Another consequence of OSA is systemic hypertension (SH) has a high prevalence in patients with OSA and that could lead to the conduct of arterial inflammation favoring oxidative stress exacerbating the atherosclerotic effects. No less important in the activation of the inflammatory cascade, dyslipidemia (DLP) which is another factor, along with OSA reinforce evidence of the existence of endothelial dysfunction and early clinical signs of atherosclerosis in these patients. Lipoprotein oxidative and anti-oxidized LDL (anti-oxLDL) has been detected in human plasma and in atherosclerotic lesions. However the role of these antibodies in the pathogenesis of vascular still isn't totality established in these conditions. In this study the authors assayed the oxidized LDL in patients with OSA and / or associated with DPL and hypertension compared with controls.

METHODS: The study selected 102 patients from the Sleep Institute of Sao Paulo with a mean age of 51.17 ± 7.98 years and BMI 28.16 ± 3.62 Kg/m^2 (55 women and 47 men). They were divided into 6 groups: G1 (**OSA** + **SH** + **DLP**), G2 (**OSA** + **DLP** - SH), G3 (**DLP** - OSA-SH), G4 (**contro**l), G5 (**OSA**-DLP-SH) and G6 (**SH**-OSA-DLP). Clinical evaluations, including laboratory polysomnography and measurement of oxidized LDL by chemiluminescent enzyme immunoassay (EIA-Mercordia-Uppsala, Sweden) were performed in all patients. *Statistics*: One-way ANOVA followed by the test of Tukey.

RESULTS: There were no significant differences in BMI among 6 groups, with G1 older than G2, G3 and G4 (56.23 ± 5.54; 45.83 ± 6.04; 46.44 ± 4.41; 43.92± 6.90 years) respectively and similar to the G5 and G6 with averages (51.87±7.46 e 53.77 ± 7.50 years, respectively.

There were significant difference in oxidized LDL between groups (F (5.94) interaction = 3.91, p = 0.003). **OSA** groups with associated co-morbidities G1, G2 and G3 with average 92.83± 41.32 U/L, 99.66 ± 34.87U/L e 98.33 ± 27.13U/L respectively showed p = 0.036, when compared with the G4 (**control**) with an average 67.45 ± 24.46 U/L while that G5 group (**OSA**) and G6 (**SH**) with average values of oxidized LDL = 65.12 ± 25.19 U/ L and 72.15 ± 25.19U/L respectively showed no significant differences in levels of oxidized LDL compared to the G4 (control).

CONCLUSION: The oxidized LDL has higher levels in individuals with OSA associated cardiovascular co-morbidities such as dyslipidemia and hypertension.

D-91

The effects of lifestyle modification treatment on the serum oxidized lipoprotein(a) levels in obese women: a comparison with native lipoprotein(a)

K. Kotani[1], S. Yamada[2], T. Yamada[1], M. Orisaka[3], N. Taniguchi[1], I. Sakurabayashi[4]. *[1]Jichi Medical University, Shimotsuke-City, Tochigi, Japan, [2]Central Institute, Shino-Test Corporation, Kanagawa, Japan, [3]Iwate Health Service Association, Iwate, Japan, [4]Saitama Memorial Hospital, Saitama, Japan,*

Background: Obesity and its related metabolic disorders are increasing and are continually worsening as a worldwide health problem. Oxidized lipoproteins play important roles in the atherosclerotic process in association with obesity. The biochemical measurements of oxidized lipoproteins have therefore gained a larger amount of attention in order to assess the pathophysiology of obesity related disorders. Oxidized lipoprotein(a) (oxLp(a)) may be a more potent indicator of atherogenesis than native Lp(a), a cardiovascular disease-relevant lipoprotein. Lifestyle strategies to modify diet and/or exercise factors are considered to be effective treatment approaches for obesity. The aim of this study was to investigate the effects of an anti-obesity lifestyle modification program on the serum oxLp(a) levels among obese subjects in comparison to the serum Lp(a) levels.

Methods: A total of 50 obese women (mean age of 59.7 years), who completed a 6-month lifestyle modification program on diet and/or exercise, were analyzed. In the pre- and post-treatment analyses, the atheroscrerosis-related variables including Lp(a) and oxLp(a) were measured. The serum oxLp(a) level was quantified using a sandwich ELISA system which contained the oxLp (a)-specific monoclonal antibody.

Results: The treatment program significantly reduced the levels of body mass index (BMI; 27.3 to 26.0 kg/m^2), mean blood pressure, serum total cholesterol, triglyceride and oxLp(a) (0.31 to 0.22 nmol/L), while the serum Lp(a) levels (0.10 to 0.14 mmol/L) did not significantly change. A simple linear regression analysis revealed the changes in the (log-)oxLp(a) levels to be significantly and positively correlated with those of the BMI (r = 0.281, p = 0.048). In a multiple linear regression analysis, adjusted for blood pressure, lipid panels and glucose, the changes in the (log-)oxLp(a) levels continued to show a significantly positive and independent correlation with those of the BMI (β = 0.466, p = 0.013). On the other hand, a similar simple analysis did not show any relatively significant correlation between the changes in the (log-)Lp(a) levels and those of other variables, while a similar multiple analysis showed a significantly inverse correlation between the changes in the (log-)Lp(a) levels and those of triglyceride (β = -0.474, p = 0.025).

Conclusions: These results suggest that weight loss by lifestyle modification may induce a reduction in the serum oxLp(a) levels among obese subjects even though no changes in the Lp(a) levels occur. This finding may be important for obtaining a better understanding of the different roles played by oxLp(a) in comparison to Lp(a), and also in turn demonstrate the significance of measuring the oxidized lipoprotein markers in obese individuals.

D-92

Kidney function as a determinant of high density lipoprotein and triglyceride concentrations; implications for cardiovascular health

U. Ray, N. Jordan, S. Cook, M. Smillie, S. Cook. *Royal Hobart Hospital University of Tasmania, Hobart, Australia,*

Aim: To investigate the effect of kidney transplantation on plasma lipoprotein concentrations in patients with chronic kidney disease on hemodialysis.

Background: The prevalence of chronic kidney disease (CKD) is increasing in Australia and worldwide. CKD is a potent risk factor for cardiovascular disease (CVD), which increases in a step-wise fashion to be greatest in end-stage kidney disease. CVD risk is relatively unaffected by conventional renal replacement therapy e.g. dialysis, yet significantly reduced by kidney transplantation, indicating a high degree of reversibility. Dyslipidemia, a recognised CVD risk factor, is frequently associated with CKD and may be the single largest contributor to CVD in CKD. CKD may also induce a state of insulin resistance, which then contributes to CVD risk and dyslipidemia. Whether dyslipidemia arises antecedent to, or as a result of CKD is uncertain. Dyslipidemia is a risk factor for CKD and may accelerate its progression; however dyslipidemia may also arise as a result of kidney impairment. If the latter finding is correct, then restoration of kidney function by kidney transplantation would be predicted to improve CKD-induced dyslipidemia, which may inturn contribute to the reduction in long-term CVD mortality observed following kidney transplantation.

Methods We conducted a retrospective before-after cohort analysis of sixty patients (Age = 27-68; M = 36, F = 24) cared for at the Royal Hobart Hospital Renal Unit who received a transplanted kidney in the last ten years. All patients were maintained on hemodialysis prior to transplantation. Outcome measures were high density lipoprotein (HDL) concentration, total cholesterol/HDL ratio, triglyceride concentration and triglyceride/HDL ratio. Triglyceride/HDL ratio was used as a marker of insulin sensitivity.

Results HDL was inversely associated with kidney impairment, a relationship that was apparent even in the earliest stages of kidney impairment. Triglycerides, low density lipoprotein (LDL), total cholesterol, total cholesterol/HDL ratio and triglyceride/HDL ratio were positively correlated with kidney impairment. HDL increased significantly in the weeks immediately following kidney transplantation ($p<0.001$), a time period that corresponds to the half-life for HDL biosynthesis. Insulin resistance may also act independently of kidney function to decrease HDL concentration in CKD patients. Total cholesterol/HDL ratio, triglycerides and insulin resistance (TG/HDL) decreased significantly following kidney transplantation ($p<0.001$, $p=0.002$ & $p<0.001$, respectively). Reductions in LDL and total cholesterol were not significant. Alterations in plasma lipoprotein profile where dependent on maintenance of graft function; if graft function was not maintained e.g. chronic rejection, then lipoprotein concentrations and ratios returned to their pre-transplantation values without exception.

Conclusion Kidney impairment was associated with pro-atherogenic changes in plasma lipoprotein profile, particularly triglycerides and HDL, which were reversed following 'rescue' of kidney function by kidney transplantation. The relationship between kidney function and plasma lipoproteins may contribute to the increase in CVD risk in patients with CKD and its' subsequent reduction following kidney transplantation.

D-93

Gas Chromatography-Isotope Dilution Mass Spectrometry Method for LDL Cholesterol Measurement Compared to the Betaquantification Reference Measurement Procedure

S. H. Edwards, S. D. Pyatt, M. M. Kimberly, G. L. Myers. *Centers for Disease Control and Prevention, Atlanta, GA,*

Background The CDC has historically used a beta-quantification (BQ) reference measurement procedure (RMP) to quantitate low density lipoprotein cholesterol (LDL-C). BQ is a multi-step procedure involving ultracentrifugation, removal of the VLDL layer, chemical precipitation of apolipoprotein B (apo B) containing lipoproteins with heparin/$MnCl_2$, and measurement of cholesterol in the bottom and HDL fractions using the Abell-Kendall (AK) RMP. LDL-C is determined indirectly by subtracting cholesterol in the HDL supernatant from the cholesterol in the ultracentrifuge subfraction (d > 1.006 kg/L).

In recent years, there has been a move towards establishing traceability to higher order RMPs. Mass spectrometry is generally accepted as the highest order analytical method. We previously developed a gas chromatography isotope dilution mass spectrometry (GC-IDMS) RMP for serum total cholesterol measurement[1] that will be used in the Lipid Standardization Program. Here, we have further evaluated this RMP for measurement of cholesterol in fractions separated by BQ.

Methods We performed BQ on 6 pools used in the LSP and compared the results to reference values previously assigned using CDC's traditional RMP. GC-IDMS analysis of these samples was performed as follows. After ultracentrifugation and precipitation of apo B-containing lipoproteins, we diluted the aliquots of bottom fractions (d > 1.006 kg/L) and HDL supernatants with tris-HCl buffer (50 mM, pH 7.4, 0.5% Triton-X 100) and spiked them $^{13}C_2$-cholesterol. We hydrolyzed the cholesterol esters in serum with a mixture of ethanol and potassium hydroxide (0.33 mol/L) and extracted free cholesterol with hexanes and water. The organic layer was evaporated down to dryness under nitrogen in a Turbovap evaporator. Subsequently, we converted the dried material containing cholesterol to the trimethylsilyl ether (TMS) derivative and then injected 1 µL on the GC-MS. We monitored mass ions 368.4 and 370.4 corresponding to unlabeled and labeled cholesterol, respectively, and used the ratio of the abundance of m/z 368.4 and 370.4 to quantify cholesterol in unknown samples with a multi-level calibration curve.

Results The percent difference of the AK RMP from the GC-IDMS measurement ranged from 1.3 % to 2.8% for LDL-C. Imprecision, calculated as the % CV ranged from 1.2% at 109 mg/dL to 1.58% at 152 mg/dL cholesterol. The average % CV was 1.4% for the sample set. The largest contribution to the bias resulted from the difference between the measurements of the bottom fraction cholesterol. Percent bias ranged from 1.44 % to 3.81 %.

Conclusion We have demonstrated that the GC-IDMS RMP combined with BQ can be used to quantify LDL-C in serum pools.

[1] Edwards SH, Pyatt SD, Stribling SL, Washburn R, Kimberly MM, Myers GL. Gas chromatography-isotope dilution mass spectrometry method for multi-level serum cholesterol analysis. Clin Chem 2007;53(6S), A43 [Abstract].

D-94

A Multicenter Comparison of Lipid Panel Assays to CDC Reference Methods

P. L. Platteborze, D. D. Bankson, R. H. Knopp, A. N. Hoofnagle. *University of Washington, Seattle, WA,*

Background. A lipid panel consisting of triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) is the most common biochemical approach to evaluate cardiovascular disease risk. Clinical guidelines are defined by the National Cholesterol Education Program Adult Treatment Panel III (ATP-III), and as a result, accurate laboratory results are essential.

Objective. The purpose of this study was to directly compare the accuracy of two commercial chemistry analyzer lipid panels to the CDC established reference methods (CDC-REF).

Methods. Specimens from 50 consenting lipid clinic patients were analyzed by three different Seattle laboratories. Fresh plasma samples were analyzed using CDC-REF at the Northwest Lipid Research Laboratory (NWLRL). Frozen serum aliquots were also analyzed on the Beckman Synchron DxC and Roche Cobas 6000 c 501 systems. In addition to TG, TC and HDL-C, LDL-C was directly measured (dLDL-C) and calculated using the Friedewald equation (LDL-CALC).

Results. The commercial assays correlated well to the reference methods. Pearson correlation coefficients (r^2) for the clinical analyzers vs. CDC-REF ranged from 0.9523 - 0.9974. Deming regression of the clinical analyzer results onto those from NWLRL demonstrated reasonable agreement for many of the assays (where x is CDC-REF): 1.01x - 4.89 and 1.04x + 4.44 (TG); 1.08x - 8.14 and 1.07x -2.00 (TC); 0.92x -7.02 and 0.90x - 1.40 (HDL-C); and 1.04x + 4.18 and 1.05x + 5.65 (dLDL-C) for the Beckman and Roche assays, respectively. Importantly, LDL-CALC had a significantly positive bias vs. CDC-REF with Deming equations of 1.13x + 5.58 (Beckman) and 1.08x + 6.94 (Roche) and HDL-C had a significantly negative bias. The average deviations were -3% and 8% (TG), 3% and 6% (TC), -23% and -13% (HDL-C), 11% and 13% (dLDL-C) and 20% and 17% (LDL-CALC) for the Beckman and Roche assays, respectively. All of these differences were statistically significant based upon paired Student's t-test ($p<0.0001$). To determine if the differences were clinically significant, we evaluated how many patients would be miscategorized when using the Beckman or Roche assays. Based upon the LDL-C goals for the ATP-III highest risk group, 11% and 9% of patients with the Beckman and Roche LDL-CALC, respectively, would have been considered for drug therapy that would not have been when using the CDC-REF method. The dLDL-C assay would have miscategorized 4% and 9% for the Beckman and Roche assays, respectively. Using the non-HDL-C ATP-III goals, 10% of patients analyzed on either platform would have been miscategorized as high. Using the ATP-III cutoffs, 35% and 18% of patients with the Beckman and Roche assays, respectively, would have been misclassified as having low HDL-C. A freeze-thaw cycle of 20 sera had no impact upon the Beckman HDL-C results.

Conclusions. In summary, there was good correlation between lipid panels from two manufacturers' and CDC-established reference methods. However, the HDL-Cs were significantly lower than the CDC-REF; this was especially problematic for the Beckman platform. The dLDL-C assay performed significantly better clinically than LDL-CALC on the Beckman platform.

D-95

Quantification of Apolipoproteins in Human Serum Using Liquid Chromatography Mass Spectrometry.

S. A. Agger, L. Marney, A. N. Hoofnagle. *University of Washington, Seattle, WA,*

Introduction. Liquid chromatography/tandem mass spectrometry (LC-MS/MS) is a powerful technique for the quantification of small molecules. However, it is not currently used to quantify proteins in clinical laboratories. Our objective was to develop the first multiplexed LC-MS/MS assay for apolipoproteins using human serum. Apolipoproteins are becoming important screening biomarkers for predicting cardiovascular risk in the general population. Some studies suggest that they may be stronger predictors than traditional serum lipid measurements. Apolipoprotein B (ApoB) is of interest clinically due to its association with atherogenic particles. In contrast, ApoA-I, the most abundant protein in high density lipoprotein is associated with atheroprotection via its reverse cholesterol transport and important anti-inflammatory properties. The ratio of the two may be more sensitive and specific than either protein alone.

Methods. Peptides from ApoA-I and ApoB were selected using shotgun proteomic analysis of density gradient ultracentrifugation purified HDL and LDL. The most abundant peptides for ApoA-I and ApoB were identified for use in selected reaction monitoring experiment. Isotopically labeled internal standards were synthesized for a tryptic peptide in ApoA-I (VQPYLDDFQK) and Apo B (VSALLTPAEQTGTWK). External calibrators were developed from commercially available serum based standards (Dade Behring, Deerfield, IL) which were matrix matched with fetal bovine serum by adjusting total protein to 60 g/L, levels expected in human serum. Tryptic digests of calibrators and patient samples were analyzed by LC-MS/MS at a flow rate of 0.4 mL/min.

Results. The analytical measurement range was from 31 to 249 mg/dL and 17 to 142 mg/dL for ApoA-I and ApoB, respectively. Within-run CV was 8% for ApoA-I (at 156 mg/dL) and 10% for ApoB (at 103 mg/dL), while between-run CV was between 4-13% for ApoA-I and 8-22% for ApoB. The linear regression between the tandem mass spectrometric method and nephelometry (Dade Behring, BNII) using fetal bovine matrix matched calibrators was y = 2.6445x - 115 (r2 = 0.91) for ApoA-I and y = 1.302x - 9 (r2 = 0.92) for ApoB and was comparable to non-matrix matched calibrators y = 2.8393x - 143 (r2 = 0.91) and y = 1.287x - 9.484 (r2 = 0.92) for ApoA-I and ApoB, respectively. Optimal digestion time of serum (apolipoproteins) was determined to be 22 hr. Comparisons between nano flow and high flow LC gave similar results with a Pearson correlation coefficient for nano flow and high flow of 0.88 and 0.90 for ApoA-I. Nano flow and high flow correlation for ApoB were 0.82 and 0.85, respectively.

Conclusion. We demonstrated the feasibility of quantifying two apolipoproteins in a multiplexed approach. The method is relatively precise, but the measured concentrations differ from the immunoassay. Investigations are underway to understand the cause of these discrepancies.

D-96

Prevalence of elevated high-sensitive C-reactive protein (hsCRP) in subjects with normal low density lipoprotein (LDL).

T. C. Aw, S. K. Pua, P. W. Wong, P. Q. Leow, H. Y. Goh, C. A. Neo, S. P. Tan. *Changi General Hospital, Singapore, Singapore,*

Background In the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) study, 20 mg of statin reduced

LDL by 50% and hsCRP by 37% resulting in a 44% reduction in primary end-points (cardiovascular death, nonfatal stroke, nonfatal MI, hospitalization for unstable angina, or arterial revascularization) and 20% decrease in total mortality (Ridker et al N Engl J Med 2008; 359:2195). It has been estimated that the number of JUPITER eligible patients in US (6.5 million) may be as large as those with elevated hsCRP and LDL (Michos et al J Am Coll Cardiol 2009; 53:911). In view of the implications of the JUPITER study for primary prevention and screening we wondered about the magnitude of apparently healthy subjects with low LDL and elvated hsCRO.

Methods We measured the hsCRP by immunoturbidimetry on an auto-analyzer (Beckman, LX-20) in 391 fasting hospital staff (136 males, mean age 38.0 years; 255 females, mean age 36.4 years) aged 21-68 participating in a health screening exercise with normal LDL (<3.4 mmol/L), glucose and uric acid; LDL was calculated using the Friedewald formula.

Results Fifteen per cent of all the subjects had an elevated hsCRP (> 2.0 mg/L), 12% of the female subjects and 18% of the male subjects.

Conclusion Elevated hsCRP in subjects with normal LDL is quite substantial in our study population. This preliminary finding could have a significant impact on primary prevention for coronary artery disease and the treatment of hyperlipidemias in our patients and merits further study.

D-97

Stability and commutability of a human serum protein-based and human serum lipoprotein-based lipid calibration/quality control material

J. H. Contois, A. R. Farrington, J. D. Nagle. *Maine Standards Company, LLC, Windham, ME,*

Objective: To develop a very stable, liquid, ready-to-use lipid calibrator/QC material for total cholesterol (CHOL), triglycerides (TRIG), LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C). The lipids and apolipoproteins are derived from intact human lipoproteins in a base matrix that also contains human serum proteins in a HEPES buffer with various additives.

Methods: In Study 1, stability was determined according to CLSI EP-25 proposed guidelines; maximum allowable change from baseline was defined as <½ NCEP total allowable error recommendations: CHOL ≤ 4.5%, TRIG ≤ 7.5%, LDL-C ≤ 6.0%, and HDL-C ≤ 6.5% or 2 mg/dL. The material was tested in triplicate on each of three days to establish baseline concentrations and again at periodic intervals on the Beckman DxC800 analyzer. For Study 2, two-levels of quality control material, prepared with the same formulation, was assayed on a Beckman DxC800 analyzer, 72 times over 155 days, including >90 days from a single, open bottle stored refrigerated. Commutability was assessed in Study 3 according to CLSI C53 proposed guidelines by assaying the same material in triplicate along with 20 serum samples on multiple platforms: the Beckman DxC, Roche Integra, Siemens Dimension (in duplicate), and Ortho Clinical Diagnostics Vitros. IRB approval was obtained and volunteer blood donors provided informed consent.

Results: The lipid material showed remarkable stability at refrigerated and ambient temperatures (Study 1). After incubation at room temperature for 103 days, only HDL-C was significantly different from baseline, with 94 days stability. At refrigerated temperature stability was >363 days for CHOL and TRIG; and 258 and 249 days for LDL-C and HDL-C, respectively. In Study 2, designed to assess performance as a QC material, the Levey-Jennings chart shows remarkable consistency. The mean ± SD concentrations after 155 days for the Level 1 control were 171 ± 3, 103 ± 2, 84 ± 2, and 36.7 ± 1.5 mg/dL for CHOL, TRIG, LDL-C, and HDL-C respectively. The mean ± SD concentrations for the Level 2 control were 313 ± 4, 185 ± 3, 162 ± 4 and 66.4 ± 1.4 mg/dL for CHOL, TRIG, LDL-C, and HDL-C respectively. The lipid material was commutable with serum for CHOL, TRIG, and LDL-C on all platforms except for CHOL on the Vitros and LDL-C on the DxC (Study 3), but not for HDL-C.

Conclusion: This lipid material shows exceptional stability at ambient and refrigerated temperatures and performs well as a control material. The commutability data suggests that, with the exception of HDL-C, the material behaves like human serum with most assays.

D-98

An assay for total apolipoprotein B with greater sensitivity and accuracy

J. H. Contois, J. D. Nagle. *Maine Standards Company, LLC, Windham, ME,*

Every very low density lipoprotein (VLDL) and low density lipoprotein (LDL) particle contains one molecule of apolipoprotein B (apoB), and therefore apoB measurement reflects the total number of atherogenic particles in the circulation. Some assays, however, do not appear to accurately measure VLDL-apoB, and many assays lack the sensitivity to accurately measure low concentrations of apoB, an important concern because many patients are treated aggressively with lipid-lowering medications. Therefore, we have developed an immunoturbidimetric assay for total apoB (TAB) without these limitations. IRB approval was obtained and sera were obtained from 20 volunteers who provided informed consent. Enough serum was available to separate VLDL by ultracentrifugation in 19 subjects. The TAB assay uses two reagents, a diluent (R1) and diluted antiserum (R2). Sample is incubated with R1 and a blank measurement is made; then R2 is added and final absorbance is read at endpoint (340 nm). For the TAB assay, within-run precision, assessed with two levels of QC (86 and 146 mg/dL), was ≤3.6%. The limit of detection, assessed with both saline and HDL supernatant (apoB particles removed from serum by precipitation with dextran sulfate-magnesium chloride) was 0.8 and 1.9 mg/dL, respectively. The limit of quantitation, defined as the concentration associated with 20% imprecision, was 10.9 mg/dL. Linearity, assessed by linear regression of observed (y) and expected (x) concentrations derived from dilution of concentrated LDL, extended from about 10-300 mg/dL ($y = 1.02x - 4.3$, $r = 1.00$). Method comparison, using 20 sera measured in triplicate with both the TAB (y) and Roche Hitachi (x) assays, gave the following regression equation: $y = 0.93x + 15.0$, $r = 0.89$; with the bias explained in large part by the greater recovery of VLDL-apo B by the TAB assay. The mean concentrations were 91.8 and 100.4 mg/dL for the Hitachi and TAB assays, respectively. Accuracy was assessed by recovery of IFCC SP3-08 reference material measured in duplicate: The recovered mean concentration, 116 mg/dL, was similar to the target value of 118 mg/dL. We compared VLDL-apoB measured using assay kits from Diasorin, Kamiya, Roche, and Beckman to VLDL-apoB measured with the TAB assay. Assays were performed according to manufacturer's instructions except that sample/reagent volume ratio may have been increased to increase low-end sensitivity. Diasorin, Kamiya, and MSC assays were performed on a Roche Fara analyzer; Roche on the Hitachi 911, and Beckman on the Immage. Mean ± SD concentrations for VLDL-apoB among the 19 subjects were 2.0 ± 1.6, 4.2 ± 3.2, 4.5 ± 3.0, 3.6 ± 3.5, and 10.4 ± 11.8 as measured with the Diasorin, Kamiya, Roche, Beckman, and TAB assays, respectively. The mean VLDL-apo B concentration provided by the TAB assay is consistent with expected values (9.8 mg/dL or ~10% of total apoB *(Sniderman et al, Atherosclerosis 1991; 89:109)*.

Conclusion: The TAB assay appears to more accurately measure the VLDL-apoB component of total apo B and provide a more clinically useful measurement range. These data need to be confirmed with additional data and validation studies.

D-99

Evaluation of Gas Chromatography-Isotope Dilution Mass Spectrometry for High-Density Lipoprotein Cholesterol Measurement

S. D. Pyatt, S. H. Edwards, M. M. Kimberly, G. L. Myers. *Centers for Disease Control and Prevention, Atlanta, GA,*

Background The accepted reference measurement procedure (RMP) for high-density lipoprotein cholesterol (HDL-C) is the Centers for Disease Control and Prevention's (CDC) ultracentrifugation method. In this method, low-density lipoprotein (LDL) and HDL, both with densities greater than 1.006 kg/L, are separated from very low-density lipoprotein (VLDL), triglycerides, and chylomicrons. Analysis of HDL-C in the subfraction containing HDL and LDL is permitted by selective precipitation of lipoproteins containing apoprotein B (apo B). Cholesterol remaining in the supernatant is then directly quantified using the Abell-Kendall (AK) RMP.

In recent years there has been a move towards establishing traceability of RMPs to higher order analytical methods. Mass spectrometry is viewed as the highest order method. In following this trend, the CDC has developed a gas chromatography- isotope dilution mass spectrometry (GC-IDMS) RMP for measuring cholesterol in serum[1]. This procedure was tested for total cholesterol and compared with the AK RMP. Here we report our evaluation of this method for HDL-C measurement after ultracentrifugation and chemical precipitation.

Methods After ultracentrifugation and Heparin-manganese precipitation of apo B-containing lipoproteins, aliquots of the supernatant were diluted with tris-HCl buffer (50 mM, pH 7.4, 0.5% Triton-X 100). 125µL of diluted sample and an equal volume of $^{13}C_2$-cholesterol (50 mg/dL) were mixed together and hydrolyzed with a solution of ethanol and potassium hydroxide (0.33 mol/L). The hydrolyzed cholesterol was extracted with a 5:3 mixture of hexane and water. The organic layer, containing cholesterol, was then evaporated to dryness in a Turbovap evaporator. The cholesterol was converted to the trimethylsilyl ether (TMS) derivative and 1µL was injected on the GC-MS.

The mass spectrometer was operated at 70eV in electron impact ionization (EI) mode. We monitored mass ions 368.4 and 370.4 corresponding to unlabeled and labeled cholesterol, respectively, and the ratios of their abundances were used to prepare a multi-level calibration curve to quantify HDL-C from unknown samples.

Results HDL-C values ranged from 33 mg/dL to 60 mg/dL. The precision of the GC-IDMS method, calculated as % CV, ranged from 0.99% to 2.58 %. The average % CV was 2.0 % for all samples in the sample set. The percent difference from the AK RMP was 2.1%. There was a linear relationship with the AK RMP (slope=1.195, intercept=9.49, r^2=0.95).

Conclusion The CDC GC-IDMS RMP shows satisfactory agreement with the RMP for HDL-C in serum. In addition, imprecision is minimized because of the high selectivity that is characteristic of IDMS methods. This GC-IDMS RMP has demonstrated the robustness

to quantify HDL-C in serum.

[1] Edwards SH, Pyatt SD, Stribling SL, Washburn R, Kimberly MM, Myers GL. Gas chromatography-isotope dilution mass spectrometry method for multi-level serum cholesterol analysis. Clin Chem 2007; 53 (6S), A43 [Abstract]

D-100

Serum lipid profile in patients with cancer

y. zhang. *Chinese PLA General Hospital, beijing, China,*

BACKGROUND AND AIMS: During cancer development, Multiple, complex molecular events have been changed. Many study suggest that the abnormal lipid profile is associated with the occurrence of different cancer. Although changes in lipid profile have been documented in cardiovascular disease, the specific relationship between serum lipid levels and different cancers is not fully understood. The aim of our study was to determine relationships between lipid profile and different cancer.

METHODS AND RESULTS: Preoperative serum lipid concentrations were examined in 163 patients who have histologically confirmed clinically localized cancer and 45 healthy controls (None of them had received radiation therapy, chemotherapy and surgical operation. Also they did not have any concomitant diseases suspected of raising serum lipid levels). We considered the total cholesterol (CH), high-density lipoprotein (HDL)-C, low-density lipoprotein (LDL)-C, triglycerides(TG) , apolipoprotein A-1(ApoA), apolipoprotein B(ApoB) and lipoprotein(Lp(a)).Multiple analyses were carried out to investigate the association of serum lipid levels with different cancer. we found the lipid profile is different in different cancer,In gastrointestinal cancer, the serum levels of ApoA(1.18±0.40 g/L), (HDL)-C(1.03±0.32 mmol/L) and Lp(a)(22.83±20.58 mg/dl) have significantly changed, as for the lung cancer, is ApoA(1.20±0.31 g/L) and Lp(a) (21.59±13.34 mg/dl), but for gynecologic cancer, only Lp(a)(21.15±18.67 mg/dl) has significantly changed. For the neoplasia located at different position of the digestive tract, we found both the gastric and colorectal cancer had the same lipid profile, the changed serum lipids are ApoA(1.20±0.28, 1.10±0.23 g/L), (HDL)-C (1.04±0.28, 0.95±0.23 mmol/L)and Lp(a)(24.82±23.61, 25.03±19.18 mg/dl), as for esophageal cancer, only TG(1.17±0.43 mmol/L) has changed.

CONCLUSION: As we were able to rule out any concomitant diseases such as cardiovascular and kidney diseases as influence factors of serum lipid levels, these alterations can be shown as a specific consequence of the presence of a malignant tumor with a diagnostic and prognostic significance.

D-101

Direct LDL-C Methods do not Significantly Improve Cardiovascular Risk Classification over Calculated LDL-C or Non-HDL-C when compared to the Reference Measurement Procedure

H. E. van Deventer[1], W. G. Miller[2], W. J. Korzun[2], L. M. Bachmann[2], M. M. Kimberly[3], G. L. Myers[3], A. T. Remaley[1]. *[1]National Institute of Health, Bethesda, MD, [2]Virginia Commonwealth University, Richmond, VA, [3]Centers for Disease Control and Prevention, Atlanta, GA,*

Introduction: The objective was to compare cardiovascular disease (CVD) risk classification using direct LDL-C (dLDL-C), Friedewald calculated LDL-C (cLDL-C), and non-HDL-C.

Methods: 138 (79 %) of 175 subjects examined had known CVD or other conditions that may affect LDL-C measurement. The reference measurement procedure (RMP) for LDL-C (rLDL-C) and HDL-C was performed at the CDC and was used to establish correct risk classification. dLDL-C and dHDL-C were measured using Denka, Kyowa, Roche, Sekisui, Serotec, Sysmex, UMA and Wako reagents on a Hitachi-917 analyzer. Total cholesterol and triglyceride were measured by enzymatic methods. Patients were classified into CVD risk categories based on NCEP ATP III guidelines.

Results: For subjects with TG <2.26 mmol/L (200 mg/dL), Pearson correlation coefficients between cLDL-C, dLDL-C and non-HDL-C for each method compared to the RMP were ≥0.96, except for Wako dLDL-C (0.85). Weighted Deming regression showed no significant constant or proportional error (P >0.05) except for Wako dLDL-C which had intercept 0.05 (95% CI 0.02-0.09) mmol/L. The mean Kappa score for concordance with the RMP for CVD risk was 0.81 (95% CI 0.74-0.87) by dLDL-C, 0.86 (95% CI 0.82-0.91) by cLDL-C and 0.91 (95% CI 0.87-0.94) by non-HDL-C. The mean misclassification rate was 14.6% (95% CI 10.0-19.2%) for dLDL-C, 10.5 % (95% CI 7.0-14.0%) for cLDL-C and 7.6 % (95% CI 5.7-9.4%) for non-HDL-C. Misclassification based on cLDL-C using Roche (6.2 %) and Serotec (6.2 %) dHDL-C was smaller than their respective dLDL-C methods (17.9% and 24.8% respectively (P <0.05)). Only for Wako was dLDL-C significantly better than cLDL-C for CVD risk classification (9.6 % vs. 17.2 % (P <0.05)). For subjects with TG ≥2.26 mmol/L (200 mg/dL), Pearson correlation coefficients were ≤0.85 for all dLDL-C and cLDL-C methods, except Denka (R^2 = 0.93) and Sekisui (R^2 = 0.97). All cLDL-C methods had a negative bias vs. rLDL-C, producing under-classification of CVD risk. In contrast, the dLDL-C methods, except for Serotec and Sysmex, over-classified CVD risk. The mean Kappa score for concordance with rLDL-C for CVD risk classification was 0.60 (95% CI 0.50-0.70) by dLDL-C methods, 0.48 (95% CI 0.42-0.54) by cLDL-C and 0.92 (95% CI 0.86-0.97) by non-HDL-C. The mean misclassification rate was 31.0 % (95% CI 21.4-40.7%) for dLDL-C methods, 39.4 % (95 % CI 36.0-42.6%) for cLDL-C methods and 6.7 % (95% CI 2.3-11.0%) for non-HDL-C. Using the Wilcoxon signed rank test, the percent differences between dLDL-C and rLDL-C were significantly lower than between cLDL-C and rLDL-C for all methods except Sysmex. Pearson correlation coefficients for all non-HDL-C methods were ≥ 0.96, and, for hypertriglyceridemic samples, Denka, Kyowa and Wako methods showed, using McNemar's test, significantly fewer misclassifications than their corresponding dLDL-C or cLDL-C results, and Sekisui less than its corresponding cLDL-C results (all P<0.05).

Conclusions: Except for patients with hypertriglyceridemia, dLDL-C methods did not improve the accuracy of CVD risk classification compared to cLDL-C. Non-HDL-C exhibited the best concordance with the reference measurement procedure for CVD risk classification for both normal and hypertriglyceridemic subjects.

D-102

Establishment of practical procedure for measurement of total cholesterol by Isotope Dilution/Gas Chromatography/Mass Spectrometry at the Osaka Medical Center for Health Science and Promotion (CRMLN lipid reference laboratry)

I. Koyama[1], H. Iso[1], M. Kiyama[2], T. Shimamoto[2], Y. Ishikawa[2], M. Nakamura[2]. *[1]Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan, [2]Osaka Medical Center for Health Science and Promotion, Osaka, Japan,*

Traceability of total cholesterol (TC) measurements can be verified by comparing manufacturers' and clinical laboratories' methods with the Abell-Kendall (AK) reference measurement procedure (RMP) in the CDC/CRMLN certification program. CDC previously presented a RMP for cholesterol using Isotope Dilution/Gas Chromatography/ Mass Spectrometry (ID/GC/MS)[1]. We have also completed to set up a practical measurement system for cholesterol using ID/GC/MS for purpose of more accurate TC standardization in Japan. Sample preparation is the same as for the AK method except a preliminary dilution with tris-HCl buffer (50mM, pH7.4) and addition of $^{13}C_2$-cholesterol as an internal standard. Diluted samples including the internal standard $^{13}C_2$-cholesterol are hydrolyzed with ethanolic potassium hydroxide (EtOH/KOH) at 50C for 1 hr followed by hexane extraction and evaporation under vacuum. The cholesterol is derivatized with O-Bis(trimethylsilyl)-acetamide before GC/MS analysis. A magnetic sector-type mass spectrometer (JMS GC mateII) is operated in electron impact ionization mode and mass ion fragments m/z 368.4 and m/z 370.4 corresponding to the native and labeled cholesterol fragment ions, respectively, are used for selective ion monitoring. The total run time from injection to MS detection is less than 5 minutes. Three quality control pools (MQ10, Q27, Q28) from CDC and SRM1951b from the National Institute of Science and Technology (NIST) were analyzed in quadruplicate in 20 analytical runs. The data were compared with the assigned values of CDC and NIST. As a result, r^2 for the standard calibration range (0-400mg/dL) was 0.9998. The within run (n=4) CV and the among run (20 assays) CV ranged from 0.08% - 1.57% and 0.20% - 0.51%, respectively. The average bias from CDC and NIST ID/GC/MS methods was 0.39% and 0.03%, respectively. These results demonstrate sufficient precision and accuracy that is required for the method to be considered as a potential RMP.

1. S.H. Edwards, S.D. Pyatt, S.L. Stribling, R. Washburn, M.M. Kimberly, G.L. Myers. Gas chromatography-isotope dilution mass spectrometry method for multi-level serum cholesterol analysis. Clin Chem 2007; 53(6S), A43 [Abstract]

Wednesday PM, July 28

Poster Session: 2:00 pm – 4:30 pm
Cancer/Tumor Markers

D-103

Antioxidants and lipid peroxidants in patients with upper aerodigestive tract cancers in Taiwan

C. Chen, S. Tsai, K. Lee, L. Tsai. *Kaohsiung Medical University, Kaohsiung, Taiwan,*

Background: The upper aerodigestive tract cancer (UATC) is the fourth cause of male cancer death in Taiwan. Reactive oxygen species (ROS) plays an important role in cancer carcinogenesis. However, the human body has developed different antioxidant systems to fight against ROS attacks. To delineate the involvement of oxidative stress in the promotion of UATC, we investigated the changes of the antioxidative capacity and lipid peroxidants in the patients with UATC.
Methods: We analyzed the superoxide anion ($O_2^{\cdot\square}$) generation and the levels of malondialdehyde (MDA) as an index of lipid peroxidation along with the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRx), and glutathione status (including reduced glutathione [GSH] ,oxidized glutathione [GSSG], and GSH/GSSG ratio) in 109 patients with UATC and 102 controls.
Results: Our data showed that the levels of MDA, the activities of antioxidant enzymes (including SOD and GRx), and the GSH/GSSG ratio in the blood of the patients were significantly higher than those in the controls ($p < 0.01$). Conversely, the levels of GSH and GSSG and the GPx activity in the blood of patients were significantly decreased compared with those in the controls ($p < 0.01$).
Conclusion: These findings suggest oxidative stress may be involved in UADT cancer. The increased activities of erythrocyte antioxidant (SOD, GRx) enzymes may be a compensatory upregulation in response to the increased oxidative stress and the loss of the large amount of erythrocyte GSH levels may be due to the increased detoxification capacities and defense against oxidative stress.

D-104

Association of oxidative stress-related enzyme gene polymorphisms with breast cancer in Taiwanese women

S. Wu[1], Y. Chen[2], M. Hou[2], S. Tsai[2], Y. Tseng[3], L. Tsai[2]. *[1]Taipei Veterans General Hospital, Taipei, Taiwan, [2]Kaohsiung Medical University, Kaohsiung, Taiwan, [3]Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan,*

Background: Because mitochondrial manganese superoxide dismutase (MnSOD) converts superoxide anion into H_2O_2, which is neutralized by catalase (CAT) or glutathione peroxidase 1 (Gpx 1) and converted into highly reactive hypochlorous acid by myeloperoxidase (MPO), we hypothesized that gene variants could be associated with risk of breast cancer.
Methods: Genotyping analyses of oxidative stress-related enzymes (MnSOD, GPx1, MPO, and catalase [*CAT* -15A>T, and *CAT* -262C>T]) were analyzed in 260 patients with breast cancer and 224 controls by PCR-based methods.
Results: When comparing the relationship between a single genetic polymorphism and risk of breast cancer, we found those with the *MnSOD* 1183T>C C carrier (p<0.005) or those with the *GPx1* Pro198Leu CT (p<0.001) were predisposed to having a decreased risk of breast cancer. Furthermore, when comparing the relationship between combined genetic polymorphisms and risk of breast cancer, we found that those with the combined genotypes of the *MnSOD* 1183T>C C carrier and *CAT* -262C>T CC, *CAT* -15A>T, or *MPO* -463G>A (p<0.001, p<0.05, p<0.001, respectively); or those with the combined genotypes of the *GPx1* Pro198Leu CT and *CAT* -262C>T CC, *CAT* -15A>T, or *MPO* -463G>A (all p<0.05); or those with the combined genotypes of *GPx1* Pro198Leu CT and *MnSOD* 1183T>C (p<0.001) were predisposed to having a decreased risk of breast cancer.
Conclusion: The results indicate that oxidative stress-related enzyme genetic variants, especially *GPx1* Pro198Leu CT, may be associated with decreased risk of breast cancer in Taiwan.

D-105

Altered Activity and Expression of Transcription Factor AP-1 in Esophageal Squamous Cell Carcinoma During Human Papillomavirus Infection

M. M. Mir[1], I. Salam[1], N. Dar[1], S. Hussain[2], B. C. Das[2]. *[1]SKIMS, Srinagar, Jammu and Kashmir, India, [2]ICPO, Noida, Delhi, India,*

Background: Esophageal squamous cell carcinoma (ESCC) is a leading cause of cancer related deaths in Jammu and Kashmir region of India. A substantial proportion of esophageal carcinoma is associated with infection of high-risk HPV type 16 and HPV18, the oncogenic expression of which is controlled by host cell transcription factor Activator Protein -1 (AP-1). We, therefore, have investigated the role of DNA binding and expression pattern of AP-1 in esophageal cancer with or without HPV infection.
Methods: Seventy five histopathologically-confirmed esophageal cancer and an equal number of corresponding adjacent normal tissue biopsies from Kashmir were analyzed for HPV infection, DNA binding activity and expression of AP-1 family of proteins by PCR, gel shift assay and immunoblotting respectively.
Results: A high DNA binding activity and elevated expression of AP-1 proteins were observed in esophageal cancer, which differed between HPV positive (19%) and HPV negative (81%) carcinomas. While JunB, c-Fos and Fra-1 were the major contributors to AP- 1 binding activity in HPV negative cases, Fra-1 was completely absent in HPV16 positive cancers. Comparison of AP-1 family proteins demonstrated high expression of JunD and c-Fos in HPV positive tumors, but interestingly, Fra-1 expression was extremely low or nil in these tumor tissues.
Conclusion: Differential AP-1 binding activity and expression of its specific proteins between HPV - positive and HPV - negative cases indicate that AP-1 may play an important role during HPV-induced esophageal carcinogenesis.

D-106

Relationship between cathepsin D and CA15-3 antigen for breast cancer

A. González-Sistal[1], J. Arias[2], A. Baltasar Sánchez[1], A. Ruibal[3]. *[1]Department of Physiological Sciences II, University of Barcelona. Faculty of Medicine, Hospitalet de Llobregat (Barcelona), Spain, [2]Surgery Service. Hospital Monte del Naranco, Oviedo, Spain, [3]Nuclear Medicine Service. Complejo Hospitalario Universitario, Santiago de Compostela, Spain,*

Background: Cathepsin D belongs to a class of acidic lysosomal proteases that are found in all cells. It is over-expressed and hyper-secreted by epithelial breast cancer cells. Studies in breast cancer have shown a statistically significant association between the presence of cathepsin D and the extension of the tumor. This protease is an independent marker of poor prognosis in breast cancer being correlated with the incidence of clinical metastasis. CA15-3 has been shown to be a useful marker in the follow-up of breast cancers. Its main clinical applications include diagnosing and monitoring metastatic disease and assessing response to therapy
Objective: To study the relationship between preoperative CA15-3 serum levels and cathepsin D histopathologic concentration in sections from breast cancer patients.
Methods: 122 women with infiltrating ductal carcinoma of the breast, aged between 27 and 83 with no prior treatment were studied. Preoperative CA15-3 serum levels were determined by an immunoradiometric method (Di-Sorin, Italy). Cathepsin D was determined given dosages in cytosols by immunoradiometric analysis of International CIS (France). The values were referred to mg of proteins and dosed by the Bradford method. The median value for the cathepsin D (50 pmol/mg prot.) was used as reference cut-off.
Results: The CA15.3 values were as follows:

Cathepsin D	n	range (CA15-3 U/ml)	median (CA15-3 U/ml)	p value
>50	56	6-186	20	
				0.023
≤50	66	6.3-162	24	

The results indicate that there are significant differences in median values when comparing cathepsin D ≤50 and > 50 pmol/mg prot (p: 0.023).
Conclusion: Our results show that there is an inverse relation between CA15.3 and cathepsin D values. To further understand the role of cathepsin D as potential marker of breast cancer additional research would be advisable.

D-107

Comparison Of Minimal Residual Disease Data, Assessed By Flow Cytometry And Pcr Of Fusion Gene Transcripts In Childhood B-Cell Precursor Acute Lymphoblastic Leukemia

A. Popov[1], G. Tsaur[2], T. Verzhbitskaya[2], T. Riger[3], Y. Yakovleva[2], A. Ivanova[3], A. Druy[1], E. Shorikov[2], L. Saveliev[1], L. Fechina[2]. *[1]Ural State Medical Academy, Yekaterinburg, Russian Federation, [2]Regional Children's Hospital #1, Yekaterinburg, Russian Federation, [3]Research Institute of Cell Technologies, Yekaterinburg, Russian Federation,*

Minimal residual disease (MRD) monitoring by flow cytometry (FC) or polymerase chain reaction (PCR) is a strong tool for risk-adapted treatment in childhood acute lymphoblastic leukemia (ALL). IgH/TCR rearrangements monitoring by real-time quantitative PCR (RQ-PCR) is well-standardized but very laborious and costly approach. Hence other methods - FC and PCR of fusion gene transcripts - can be used for long-term MRD monitoring.

Aim - to evaluate qualitative and quantitative concordance between MRD data assessed by flow cytometry (FC) and fusion gene transcript (FGt) copy number (CN) measured by RQ-PCR and reverse-transcriptase PCR (RT-PCR) in children with B-cell precursor ALL (BCP-ALL) during treatment.

Methods. Concurrent detection of MRD by multicolor FC, RQ-PCR and RT-PCR was performed in 132 follow-up bone marrow samples from 35 children with BCP-ALL. Among them 15 patients (pts) had *ETV6-RUNX1* FGt, 14 pts - *MLL-AF4* FGt, 2 pts - *MLL-MLLT1* FGt, 2 pts - *MLL-EPS15* FGt, 2 pts - *E2A-PBX* FGt.

Results. 42 of 132 samples were MRD-negative by both methods, 8 (6.06%) - were negative by FC but positive by RT-PCR. Remaining 82 samples were MRD-positive by both methods. Despite high qualitative concordance (93.94%) between FC and RT-PCR data, low quantitative concordance between FC and RQ-PCR results was found (R^2=0.291). Significant quantitative difference in FC and RQ-PCR data could be associated with variability of FG expression during treatment that does not correspond to the cells' number. Moreover, percentage of tumor blasts among all nucleated cells is calculated during FC MRD detection, while MRD value in RQ-PCR of FGt is corresponded to the initial FGt and control gene levels. FC appears to be better for the quantitative *MRD* assessment however FGt detection by RT-PCR is more appropriate for MRD qualitative detection due to higher sensitivity. Hence, FC is more applicable for *MRD* monitoring during early treatment phases while PCR of FGt - for later time-points.

Conclusion. Tandem application of FC at early time-points and FGt detection by PCR at later time points seems to be a useful tool for long-term MRD monitoring in childhood BCP-ALL.

D-109

Evaluation of Liaison Calcitonin II Gen, a new kit for Calcitonin determination.

E. Cavalier, A. Carlisi, A. Bekaert, O. Rousselle, P. Delanaye, J. Chapelle. *University Hospital of Liège, University of Liège, Liège, Belgium,*

Introduction: Calcitonin, a 32 aminoacid peptide produced by the parafollicular C cells of the thyroid is the classical clinical marker for medullary thyroid carcinoma (MCT). In clinical practice, a patient with calcitonin concentration >10 ng/L should undergo a pentagastrin test to exclude MCT. It is important to note, however, that these interpretive guidelines have been developed using the Cisbio international reagent set for calcitonin measurement. Recently, DiaSorin launched an improved version of the Liaison Calcitonin kit. The aim of that work was to evaluate this new kit.

Material and methods: We evaluated the precision with a modified protocol based on CLSI EP-5A2: 14 serum pools were assayed in six replicates per day on five different days. Linearity was evaluated based on CLSI EP-6A. We established the limit of quantification (for a total error of 20%), measurement uncertainty and the accuracy profile of the method. We evaluated the reference range in a healthy population (122 women and 145 men), selected on basis of the absence of anti-thyroglobulin antibodies, and normal levels of TSH and fT4. We studied the fidelity to the 2nd IS 89/620. With samples enriched with high amounts of this IS, we tried to observe if hook and carryover effects occurred. We studied the susceptibility of HAMA interference with selected samples, known to present an interference and finally, we compared the results with Cisbio on 267 consecutive and 45 samples obtained after pentagastrin stimulation.

Results: The LOQ was established at 5.3 ng/L. Repeatability and intermediate precision were <10% in the studied range (2.9-1159 ng/L). The method was found to be linear until the 1/10 dilution. The kit was correctly calibrated against the IS, 1µUI corresponding to 5.5pg of Calcitonin Liaison (expected: 4.8-5.7 pg). Measurement uncertainty ranged from 25% at 2.9 ng/L to 6.5% at 1159 ng/L. The accuracy profile built with the predictive tolerance interval method shows that, on average, 95% of the future results that will be generated by this method will be included in the computed tolerance intervals of ±20% in the 5.3-1159 ng/L studied range. Ninety-five percent of the healthy male and female presented calcitonin levels <10 ng/L. We did not observe any hook effect with samples presenting values up to 1million ng/L, but there was a slight carry-over, with a blank sample giving an amount of 8 ng/L after being assayed after the 1million pg/L sample. Samples presenting spurious high levels due to HAMA interference with the 1st generation kit unfortunately gave the same erroneous profile. In the "normal" population, the Bland-Altman graph showed a mean difference of 0.1±2.0 ng/L between Liaison and Cisbio. In samples obtained after pentagastrim stimulation (range: 9.3-838 ng/L), the mean difference was 11.1±49,3 ng/L. No statistical difference was observed between the methods in these two populations.

Conclusions: Liaison Calcitonin II Gen is a very robust method. The analytical performances have greatly improved compared to the 1st version of the kit. The comparison with Cisbio is remarkable, which allows the use of the "10 ng/L" cut-off for the screening of MCT.

D-110

The ratio of PTH as measured by third and second generation assays as a marker for parathyroid carcinoma

E. Cavalier[1], A. F. Daly[1], D. Betea[1], P. Pruteanu Apetrii[1], P. Delanaye[1], P. Stubbs[2], A. R. Bradwell[2], J. Chapelle[1], A. Beckers[1]. *[1]University Hospital of Liège, University of Liège, Liège, Belgium, [2]University of Birmingham, Birmingham, United Kingdom,*

Introduction: Parathyroid carcinoma (PCa) is a rare disease, comprising only 0.005% of all cancers. In the US National Cancer Database, only 286 cases have been described over 10 years. PCa accounts for less than 1% of sporadic primary hyperparathyroidisim (PHP) and is associated with more severe clinical features than parathyroid adenomas. The severe hypercalcemia due to unchecked recurrent PTH hypersecretion is the main causes of morbidity and death in these patients. Differentiating PCa from parathyroid adenoma is challenging, particularly as the histopathology of parathyroid tumors can be equivocal. Indeed, the definitive diagnosis of PCa is largely made only when recurrence or metastasis occurs. As surgery remains the only curative treatment for PCa and as better outcomes are associated with complete resection of the tumor at the time of initial surgery, it is important to make a correct diagnosis at the time of first occurrence. Hence, a biological marker that could help to reliably distinguish PCa from parathyroid adenoma would be useful. It has recently been demonstrated that PCa over-secreted the amino form of parathyroid hormone (PTH), which is recognized by 3rd generation but not by 2nd generation ("intact") PTH assays. In normal individuals, the 3rdgeneration/2ndgeneration PTH ratio should always be <1.

Material and methods: We studied the utility of the 3rdgeneration/2ndgeneration PTH ratio as a means of distinguishing PCa patients (n=24) from control groups with and without disorders of calcium secretion, including patients on renal hemodialysis (n=73), post-renal transplantation (60), elderly healthy (n=82) and PHP (n=30). Second ("intact") and 3rd generation PTH were assayed with the PTH Duo kit from Scantibodies. The CV of these assays was <8%.

Results: The mean 3rdgeneration/2ndgeneration PTH ratio was 0.58±0.10 in the dialysis patients, 0.54±0.10 in the renal transplant group, 0.54±0.12 in the elderly healthy patients and 0.68±0.11 in the PHP group. All 245 of these patients presented a 3rdgeneration/2ndgeneration PTH ratio of <1. In contrast, we observed an inverted ratio >1 in 20 PCa patients, whereas only 4 PCa patients had a "normal" ratio of <1.

Conclusions: An inverted 3rdgeneration/2ndgeneration PTH ratio occurred in the majority of patients with advanced PCa and was absent in all 245 relevant controls. A 3rdgeneration/2ndgeneration PTH ratio >1 had a sensitivity of 83.3% and a specificity of 100% amongst PHP patients as a marker for PCa; among all published cases, the sensitivity was 75.8% and the specificity was 98.9%. Our results, based on a large cohort of PCa patients, shows that an inverted ratio may indeed have clinical utility as a tumor marker for PCa, as suggested in previous smaller series. Future investigations will be needed to assess if the dual determination of PTH with 2nd and 3rd generation PTH assays could be proposed in treated patients suspected of having PCa or as a pre-operative screening test to detect patients in whom an elevated suspicion for PCa exists. Alternatively, the 3rdgeneration/2ndgeneration PTH ratio could also be used as a new tool in the follow-up of the operated patients to identify those with persistent disease or later relapse.

D-111

The Value of Tumor Markers CA 125, CA 15-3, and CA 19-9 in Assessing the Response to Treatment in Pulmonary and Pleural Tuberculosis

M. A. Serdar, M. Ozkan, C. Tasci, A. Balkan, B. Ozkara, M. Erbil, H. Bilgic. *Gulhane Military Medical School, Ankara, Turkey,*

Backgrounds: Tuberculosis activation criteria vary according to the involved organs. Active tuberculosis treatment is terminated after the end of the classical six-month

tuberculosis treatment. We aimed to examine the changes in tumor markers such as CA 125, CA 15-3 and CA 19-9 during tuberculosis treatment.
Methods: Tumor markers CA 125, CA 15-3 and CA 19-9 were measured before and after the treatment in 67 patients with lung and pleural tuberculosis by using Roche E170 analyser. Patients with lung tuberculosis were grouped according to the degree of sputum smear positivity. All values were compared with the results of the healthy control group.
Results: CA 125 and CA 15-3 were significantly high when compared with healthy control group and there were significant decrease in both after treatment ($p<0.001$) and $p<0.004$ respectively). However, difference in CA 19-9 was not statistically significant ($p <0.08$). There was significant correlation between the sputum positivity degree and CA 125 level ($r = 0341$, $p = 0.012$). In patients with pleural tuberculosis CA 125, CA 15-3 and CA 19-9 values did not changed significantly after treatment ($p <0.06$, $p <0.2$, $p <0.18$ respectively). Because there was a significant decrease in especially CA 125 level with the treatment.
Conclusion: we claim that this marker can be used to evaluate the efficacy of the tuberculosis treatment.

D-112

Serum Free Light Chain Anomalies in a Case of Light-Chain Cast Nephropathy

J. R. Tate[1], S. Bazeley[1], S. Sykes[1], C. Hawley[2], P. Mollee[3]. *[1]Chemical Pathology, Pathology Queensland, Royal Brisbane & Women's Hospital, Brisbane, Australia, [2]Renal Unit, Princess Alexandra Hospital, Brisbane, Australia, [3]Haematology Dept., Pathology Queensland, Princess Alexandra Hospital, Brisbane, Australia,*

Background: Clinical studies with serum free light chains (FLC) assays have demonstrated their clinical usefulness. However, antigen excess and non-linearity on sample dilution can cause FLC underestimation and mislead result interpretation, particularly when monitoring patients with clonal plasma cell diseases. A case of grossly underestimated FLC in light-chain cast nephropathy is presented.
Materials and Methods: Case: a 62-year-old male presented with IgA Kappa multiple myeloma, Kappa Bence Jones protein (KBJP), elevated serum creatinine of 996 µmol/L and light-chain cast nephropathy. Treatment was with bortezomib, adriamycin and dexamethasone chemotherapy and haemodiafiltration (HDF), utilising the Gambro HCO (high cutoff) 1100 dialyzer. HDF was performed with a single filter daily for 5 days then alternate daily for a total of 12 treatments. During therapy monitoring, daily KFLC and lambda (L) FLC were measured by BNII analyser using Freelite™ (The Binding Site Ltd., UK) on pre- and post-dialysis specimens.
Results: FLC results at presentation were KFLC 56 mg/L (reference range: 3-19), LFLC 6 mg/L (6-26), and K/L FLC ratio 9.3 (0.26-1.65). Although KFLC concentration was within the initial measuring range of 6 to 203 mg/L, it is our practice to perform additional dilutions on all samples with KFLC >50 mg/L. Subsequent higher sample dilutions to 1 in 8,000 indicated a final KFLC of 3,780 mg/L which was approximately 70-fold higher than the starting concentration. All subsequent samples used to monitor pre- and post-HDF KFLC concentration were also underestimated at the initial 1 in 100 sample dilution. Although several post-HDF samples were below our dilution cut-off, final KFLC values at higher dilution ranged from 48.6-fold higher (36 mg/L resulted at 1,750 mg/L) on Day 6 to 1.9-fold higher (78 mg/L resulted at 149 mg/L) on Day 31. Poor correlation was observed between KFLC concentrations measured at starting and higher sample dilutions ($r^2=0.349$; n=35; Days 1-97). A similar starting KFLC concentration did not always result in the same final result at higher dilution, e.g. initial KFLC of 182 mg/L resulted at 436 mg/L (Day 31; trace KBJP) compared with KFLC of 186 mg/L gave 779 mg/L (Day 97; 80 mg/24 h KBJP). 24-hour urine KBJP excretion correlated with changes in KFLC concentration ($r^2=0.669$; n=5; Days 24-97).
Conclusions: We present a case of light-chain cast nephropathy which exhibited a range of anomalous FLC measurements during chemotherapy and haemodialysis treatments ranging from gross antigen excess to non-linear KFLC reaction on dilution. The clinical implications of not recognising such dilutional anomalies are that incorrect patient management decisions could be made and unnecessary therapies introduced. If the 70-fold higher KFLC concentration had not been recognised, the high cut-off dialysis would have appeared ineffective in removing the FLC. Response to chemotherapy could still be monitored by serum and urine protein electrophoresis, but no reliable temporal guide to the effectiveness of dialysis would be available. KFLC concentration at 1 in 100 starting dilution appeared to increase after Day 15 in pre- and post-dialysis samples, which could be misinterpreted as a rising KFLC concentration and lack of disease response.

D-113

Evaluation of some tumor markers in diagnosis and prognosis of hepatocellular carcinoma

N. A. Gohar, S. El Kateb, D. Gayar, I. Hamdy. *Cairo University, Cairo, Egypt,*

Background:Early detection of hepatocellular carcinoma (HCC) is critical for successful treatment. . The aim of the work was to assess the usefulness of serum AFP, PIVKA-II and TGF-β1 in the diagnosis and prognosis of HCC and to evaluate the role of serum VEGF in prediction of venous invasion and metastasis in HCC patients.
Methods:This study was conducted on 50 patients divided into 4 groups; Group I: 10 patients with liver cirrhosis, Group II: 20 patients with benign hepatic focal lesion (HFL), Group III: 10 HCC patients without metastasis before and after treatment in the form of percutaneous alcohol injection , and Group IV: 10 HCC patients presenting with metastasis. Ten apparently healthy subjects were taken as a control group. Patients as well as the control subjects were submitted to the following: A) Full clinical examination.B) Imaging techniques including abdominal US and CT scan. Patients with HCC were subjected to bone scan and MRI brain, chest and abdomen to detect distant metastasis. C) Laboratory investigations including: complete LFTs, PT and concentration, serological examination for hydatid disease, cytological examination of fluid aspirated from infectious cyst, serodiagnosis of HCV and HBV infection, serological markers including AFP, PIVKA-II, VEGF and TGF-β1. Lastly, histopathologic examination of liver biopsies obtained from focal lesions of HCC patients.
Results:A statistically significant difference in the median serum level of AFP, PIVKA-II, VEGF and TGF-β1 was found on comparing the HCC groups (with and without metastasis) with the control, benign focal lesions and the cirrhotic groups. The median serum PIVKA-II level was significantly lower in the HCC group without metastasis (16.2 ng/ml) compared to that with metastasis (33.6ng/ml) (P=0.001). The median serum VEGF level was significantly higher in the HCC group without metastasis (2580 pg/ml) compared to that of the HCC group with distant metastasis (1840 pg/ml)(P=0.008). There was a significant decrease of median serum level of all parameters in HCC patients without metastasis after ablation therapy. A negative correlation was observed between serum VEGF and serum PIVKA-II in the HCC group without metastasis. Regarding sensitivity and specificity of the four serological markers: at a cut-off level of 28 ng/ml, AFP yielded a sensitivity of 80% and a specificity of 97%, at a cut-off level of 12.5mAU/ml for PIVKA-II, the sensitivity was 80% and the specificity was 97%, VEGF revealed a sensitivity of 96.7% and a specificity of 85% at a cut-off level of 780 pg/ml, and lastly TGF-β1 yielded a sensitivity of 76.7% and a specificity of 97% at a cut-off level of 32.4 ng/ml. Combination of these markers improved both sensitivity and specificity, as combination of AFP and PIVKA-II yielded a sensitivity of 93.3% and a specificity of 98.2%, and for AFP with TGF-β1, the sensitivity was 87.5% and the specificity was 99%.
Conclusion: Combined determination of serological markers could be used as a highly valuable tool for screening and diagnosis of HCC. They could also be used as prognostic markers hence decreasing the need for more invasive procedures such as liver biopsy

D-114

Specimen Type Comparison for Circulating ProGRP Concentration in Various Lung Diseases

H. J. Choi[1], H. R. Kim[2], M. G. Shin[1], J. Park[3], D. Cho[1], S. J. Kee[1], S. H. Kim[1], J. H. Shin[1], D. W. Ryang[1], S. P. Suh[1]. *[1]Chonnam National University Hospital, Gwangju, Korea, Republic of, [2]Brain Korea 21 Project, Center for Biomedical Human Resources at Chonnam National University, Gwangju, Korea, Republic of, [3]Abbott Diagnostics, Seoul, Korea, Republic of,*

Background: Measuring circulating progastrin-releasing peptide (proGRP) levels has been shown to be helpful in distinguishing small cell lung cancer (SCLC) from other cancers. However, recent investigations have revealed that the stability of serum proGRP was worse than that of other cancer markers, likely due to the thrombin activity in serum. Therefore, it is recommended to use plasma to achieve more stable proGRP measurement. To date, most clinical data on proGRP have been based on concentrations in serum. This study evaluated the agreement between proGRP in fresh serum and plasma from healthy individuals and patients with various lung diseases including lung cancers.
Methods: From January 2009, pairs of serum and EDTA plasma were collected from 49 apparently healthy individuals (62.1±8.5 years, M:F = 27:22). At the same time, EDTA plasma of 178 patients with pulmonary diseases were prospectively collected at their first hospital visit (65.2±9.2 years, M:F = 137:41). ProGRP concentrations were measured using the Abbott ARCHITECT ProGRP. For healthy individuals, squamous cell carcinoma antigen (SCC) and neuron-specific enolase (NSE) concentrations in serum and plasma were also compared. The final diagnoses of the pulmonary patients were SCLC (N=37), non-SCLC (N=116) and benign lung diseases (N=23).
Results: ProGRP concentrations were higher in plasma than in serum by an average of 44.4%. The correlation coefficient between plasma and serum proGRP was lower than

that of SCC. But no significant difference was found between proGRP and NSE in terms of the correlation coefficient of the 2 types of specimens. Plasma proGRP was higher in malignancy (336.4±925.4 pg/mL) than in benign conditions (40.1±11.5 pg/mL). The SCLC patients showed higher levels of proGRP (1256.3±1605.6 pg/mL) compared to the other types of lung cancer. Among the SCLC cases, the limited stage diseases had lower levels of plasma proGRP (33.0±759.6 pg/mL) than the extensive diseases (1940.1±1947.7 pg/mL).

Conclusion: Higher levels of proGRP in plasma than in serum agreed with previous studies, and that was thought to reflect the enhanced stability of plasma proGRP. The difference between proGRP values obtained in serum versus plasma was not significantly different from that of NSE which was not claimed by the manufacturer. Considering the different concentrations between benign diseases, non-SCLC and SCLC, plasma proGRP is thought to be capable of aiding in the initial diagnosis of a patient with SCLC.

Key words: ProGRP, serum, plasma, lung disease, SCLC.

* Hyun-Jung Choi and Hye-Ran Kim made equal contribution.

D-115

Allele-specific PCR tests for the detection of therapy-related EGFR mutations

R. Dahse[1], A. Berndt[2], H. Kosmehl[1]. *[1]HELIOS Clinic Erfurt, Erfurt, Germany, [2]Friedrich Schiller University, Jena, Germany,*

Background: The activation of the epidermal growth factor receptor (EGFR) signaling cascades is an important pathway in cancer development. EGFR transmits signals to the nuclei instructing cancer cells to proliferate and to metastasize. EGFR signal blocking is an important goal for targeted anticancer therapy strategies in two ways: competitive inhibition of growth factor binding by an antibody or of the receptor tyrosine kinase by small molecules. Recently, EGFR activating mutations were identified in patients with non-small-cell lung cancer (NSCLC). These activating mutations result in ligand-independent tumor cell dependence on EGFR signaling and simultaneously provide the patients with an improved responsiveness to tyrosine kinase inhibitors. The vast majority of activating EGFR mutations are either a deletion of a conserved sequence in exon 19 or a single point mutation in exon 21 (L858R). Because responses to tyrosine kinase inhibitors are almost entirely limited to mutation-positive patients whereas mutation-negative patients more benefit from chemotherapy, molecular testing of the EGFR status is needed for an individualized selection of treatment.

Methodology: Two simple and sensitive assays based on allele-specific PCR were designed for the initial screening on the both most common therapy-related EGFR mutations in clinical tumor samples. For detecting the exon 19 E746_A750 deletion, a so-called Bi-PASA (Bidirectional PCR Amplification of Specific Alleles) was developed. Bi-PASA contains 4 primers: i) two allele-specific primers complementary to the sequence variation in a bi-directional orientation such that both primers terminate at the variation site and ii) two so-called outer primers that anneal at different distances from the sequence variation to differentiate the downstream and upstream reactions on an agarose gel. In this way, the assay has an inherent amplification control. For the exon 21 L 858R point mutation assay, allele-specific primers, A and B, with mismatches at their 3` end - A complements the normal allele (2573T) and B which complements the mutant allele (2573G) and a consensus primer were designed. Before testing clinical samples, both allele-specific PCR assays were validated in known genomic samples (wild type control DNA, and mutant control DNA from cell lines NCI-H-1650 and NCI-H-1975).

Results: The predicted amplification pattern for normal and mutant genotypes could be obtained with high specificity (i. e. detection of only the normal or only the mutant allele), high sensitivity (i.e. no spurious PCR fragments) and acceptable yield. For clinical diagnostics, screening assays must be able to detect mutations in heterogeneous genomic samples - tumor specimen may contain a high percentage of non-neoplastic cells. In serial dilution experiments, the mutant alleles were detectable in mixed samples with an at least 6-fold excess of normal DNA. To validate the assays for use in clinical diagnostics, 35 microdissected lung adenocarcinoma and 65 salivary gland carcinoma were analyzed. Five exon 19 deletions were identified and confirmed by genomic sequencing.

Conclusions: Both PCR protocols are appropriate for initial EGFR mutation analysis and provide a rapid, sensitive, and cost-effective screening method. The tests select patients who have maximal benefit from EGFR tyrosine kinase inhibition.

D-116

Clinical value of Serum CYFRA21-1, neurone-specific enolase and carcinoembryonic antigen in the diagnosis of Lung Cancer

L. cheng. *Department of Laboratory Medicine, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China,*

Background: CEA, CYFRA 21-1 and NSE are the commonly used tumor markers in lung cancer. We try to assess the value of serum CYFRA21-1, NSE and CEA for diagnosis of lung cancer and discuss the relation of serum levels of the three tumor markers with the clinical stage of Non-Small Cell Lung Cancer (NSCLC) in Chinese population.

Methods: The concentrations of CYFRA21-1, NSE and CEA in serum of 199 lung cancer patients, 98 patients with benign lung diseases and 116 healthy persons were tested. CEA was tested with micro-particle enzyme chemiluminescence immunoassay while CYFRA21-1 and NSE were tested with electrical chemiluminescence immunoassay (ELC).

Results: With the specificity of 95%, the cutoff values were CYFRA21-1 3.26μg/L, CEA 5.51μg/L and NSE 26.14μg/L respectively when benign lung diseases were used as control. For lung cancer, the sensitivities of the three markers were CYFRA21-1 44.7%, NSE 22.6% and CEA 38.7% respectively. The sensitivity would be increased to 71.9% when three markers were used in combination. CYFRA21-1 was most sensitive to squamous cancer (66.2%). Sensitivity was high for all stages of squamous cancer, stage I: 61.1%, stage II: 47.1%, stage IIIA: 78.6%, stage IIIB: 70.0%, stage IV: 80.0%, while for Small Cell Lung Cancer (SCLC), the sensitivity was only 9.5%. CEA was most sensitive to adenocarcinoma (53.3%). The sensitivities at various stages were: stage I: 23.1%, stage II: 36.4%, stage IIIA: 66.7%, stage IIIB: 64.3% and stage IV: 74.9%. NSE sensitivity to SCLC was 76.2% at maximum, while only 16.3% to NSCLC. CYFRA21-1 and CEA were positively relevant to the clinical stage of NSCLC respectively. NSE serum level showed no significant deviation among various stages of NSCLC.

Conclusion: CYFRA21-1 is the most valuable marker for the diagnosis of NSCLC and squamous cancer, CEA is highly valuable for the diagnosis of advanced adenocarcinoma, and NSE is the most valuable for the diagnosis of SCLC. Combination of the three TMs greatly increases the diagnostic sensitivity to lung cancer. Differential diagnosis of NSCLC and SCLC can be made by using CYFRA21-1 and NSE. CYFRA21-1 and CEA are positively relevant to the clinical stage of NSCLC. High concentration predicts a late stage while low concentration does not provide much information for determining the stage.

D-117

Levels and clinical Significance of circulating endothelial progenitor cells in human ovarian cancer

y. su, l. zheng, q. wang, w. li, z. cai, j. bao. *Nanfang Hospital, guangzhou, China,*

Background: Since advancing idea that tumors are angiogenesis-dependent, angiogenesis is considered to play an important role in metastasis of malignant tumor. So antiangiogenetic therapy as a novel alternative approach to treat tumor has been developed, which was different from traditional methods. Ovarian cancer is one of the most aggressive gynecological malignancies and its high mortality is most often a direct result of delays delayed in diagnosis. Only 25% of ovarian cancers are diagnosed while the malignancy is still confined to the ovary. Thus, anti-angiogenesis therapy has become a new strategy for ovarian cancer treatment.

Circulating bone marrow-derived endothelial progenitor cells (EPCs) have been reported to participate in tumor angiogenesis and growth. However, the role of circulating EPCs in tumor progression is controversial. Whether circulating EPCs are involved in ovarian cancer progression and angiogenesis has not yet been investigated.

The aim of this study was to determine the correlation between EPCs levels and disease progression and angiogenesis in ovarian cancer.

Methods 42 patients with histologically proven ovarian cancer were included along with a control group of 25 healthy women. These patients had no additional malignant, inflammatory, or ischemic disease, wounds, or ulcers that could influence the number of circulating EPCs. The number of circulating EPCs in the peripheral blood in healthy volunteers and ovarian cancer were determined by flow cytometry. EPCs were defined by co-expression of CD34 and vascular endothelial growth factor receptor[[Unsupported Character - Codename]] 2 (VEGFR2). In addition, we determined CD34 and VEGFR2 mRNA levels of the same patients by real-time reverse transcription-polymerase chain reaction. Plasma levels of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) were determined by enzyme-linked immunosorbent assay. Statistical analyses were performed with Statistical Package for Social Sciences 13.0 software. The Mann-Whitney U test and Student's *t*-test was used to compare variables between the two groups. Overall survival analyses were performed using the Kaplan-Meier method. Because of skewed distributions, VEGF and MMP-9 levels are described using median values and ranges. The comparison between EPCs level and VEGF/MMP-9 levels was assessed with the log-rank statistic. Data are expressed as mean ± standard error (SE). $P < 0.05$ was considered statistically significant.

Results Circulating levels of EPCs were significantly increased in ovarian cancer patients, correlating with tumor stage and residual tumor size. Higher levels of EPCs were detected in patients with stage III and IV ovarian cancer than patients with stage I and II disease. After excision of the tumor, EPCs levels rapidly declined. Residual tumor size greater than 2 cm was associated with significantly higher levels of EPCs. In addition, high circulating EPCs correlated with poor overall survival. Pretreatment CD34 mRNA levels were not significantly increased in ovarian cancer patients compared with healthy controls;

however, VEGFR2 expression was increased, and plasma levels of VEGF and MMP-9 were elevated.

Conclusions Our results demonstrate the levels and clinical relevance of circulating EPCs in ovarian cancer. EPCs may be a potential biomarker to monitor ovarian cancer progression and angiogenesis or anti-angiogenesis therapy response.

D-118

Epidermal Growth Factor-induced Epithelio-Mesenchymal Transition in Human Esophageal Carcinoma Cells - The Establishment of an I*n vitro* Mestastasis Model

Z. Cai[1], Q. Wang[1], L. Zheng[1], Y. Qiu[1], Y. Zhou[2], Q. He[3]. *[1]Nanfang Hospital, Guang zhou, China, [2]Department of Anatomy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, Hong Kong, [3]Institute of Life and Health Engineering, Jinan University, Guang zhou, China,*

BACKGROUD: Deciphering the molecular basis of esophageal cancer metastasis requires adequate experimental models which resemble the complicated cascade as closely as possible. The purpose of the present study was to establish an *in vitro* model for the following metastasis-related studies. Epithelio-Mesenchymal Transition (EMT) is a necessary step of metastasis process and generally be accepted as an *in vitro* correlate of tumor metastasis. Epidermal growth factor (EGF) has been indicated could induce EMT in several cell lines. Since the high expression of EGFR was detected in more than half of esophageal squamous cell carcinoma (ESCC) patients and has been reported to be confirmatively correlated with poor prognosis and nodal metastasis, EGF was presumed a potential promoter for malignant progression of ESCC. We therefore examined the effects of EGF on esophageal carcinoma EC109 cells. METHODS: EC109 cells were treated with vairous concentration of EGF. Various cell bioactivities were investigated. RESULTS: The results showed that EGF at low concentration (below 20ng/ml) promoted primaryly cell proliferation, while EGF at high concentration induced the cells undergo EMT-like changes. The treated cells changed morphology from cobble-stone to spindle shape, exhibited higher cell invasive and metastatic capacity, and occurred expression alteration of lineage markers. CONCLUSION: EGF at high concentration could induce the EC109 cells emergy EMT. This *in vitro* EMT model may facilite the studies on the fundational molecular basis of metastatic process of ESCC.

D-119

Changes of lectin-binding ratios of serum alkaline phosphatase in liver diseases

G. Chen. *Wuxi People's Hospital, Wuxi, China,*

Objective To investigate the changes of lectin-binding ratios of serum alkaline phosphatase in liver diseases.

Methods L-PHA(leukoagglutinating phytohemagglutinin),LCA (lentil lectin) and DSA(Datura stramonium agglutinin)-binding ratios of serum alkaline phosphatase were determined in 39 healthy individuals, 41cases of primary hepatic cancer and 98 cases of other related diseases, using lectin affinity precipitation method.

Results L-PHA,LCA and DSA-binding ratios of serum alkaline phosphatase in primary hepatic cancer were significantly increased as compared with control group or other related disease groups.

Conclusion The lectin-binding ratios of serum alkaline phosphatase are potential indexes for the laboratory diagnosis of primary hepatic cancer.

Table: Results of lectin-binding ratios of serum alkaline phosphatase in liver diseases(%) ($x \pm s$)

Groups	n	L-PHA	LCA	DSA
Healthy individuals	39	9.59±3.61	37.51± 8.74	18.23±5.65
Primary hepatic cancer	41	20.24±6.93*	54.89±13.21*	31.04±7.82*
Metastatic hepatic cancer	12	11.91±3.40$^{\Delta}$	41.11± 7.32$^{\Delta}$	24.75±6.92*$^{\Delta}$
Hepatocirrhosis	18	15.32±4.07*$^{\Delta}$	45.07± 8.18*$^{\Delta}$	26.53±7.80*
Chronic hepatitis	22	12.38±3.42*$^{\Delta}$	39.36± 9.24$^{\Delta}$	24.33±5.21*$^{\Delta}$
Acute hepatitis	23	12.21±3.51$^{\Delta}$	35.06± 7.44$^{\Delta}$	19.11±5.04$^{\Delta}$
Digestive tract tumor	16	13.12±3.02*$^{\Delta}$	35.67± 8.11$^{\Delta}$	24.08±5.89*$^{\Delta}$
Pregnant woman	7	10.68±3.43$^{\Delta}$	39.83± 8.70$^{\Delta}$	19.31±6.13$^{\Delta}$

* Compared with control group, P<0.01

$^{\Delta}$ Compared with primary hepatic cancer group, P <0.01

D-120

Pancreatic Cancer Induced Diabetes Mellitus: Relevance Of The S100a8 N-Terminal Peptide (Nt-S100a8)

D. Basso[1], E. Greco[1], A. Padoan[1], P. Fogar[1], M. Scorzeto[2], E. Fadi[1], D. Bozzato[1], S. Moz[1], F. Navaglia[1], C. Zambon[1], R. Seraglia[3], A. Valerio[1], C. Reggiani[2], S. Pedrazzoli[1], M. Plebani[1]. *[1]University Hospital of Padova, Padova, Italy, [2]University of Padova, Padova, Italy, [3]CNR, ISTM, Padova, Padova, Italy,*

Background: After isolating NT-S100A8 from pancreatic cancer (PC) tissue of diabetic patients, we verified whether this peptide alters PC cell growth and invasion and/or insulin release and [Ca2+]i oscillations of insulin secreting cells and/or insulin signalling. We verified also whether this peptide is treaceble in serum to detect pancreatic cancer induced diabetes mellitus.

Methods: BxPC3, Capan1, MiaPaCa2, Panc1 (PC cell lines) cell growth and invasion were assessed in the absence or presence of 50, 200 and 500 nM NT-S100A8. In NT-S100A8 stimulated b-TC6 (insulinoma cell line) culture medium, insulin and [Ca2+] were measured at 2,3,5,10,15,30 and 60 minutes, and [Ca2+]i oscillations were monitored (epifluorescence) for three minutes.

Results: 500 nM NT-S100A8 stimulated BxPC3 cell growth only and dose dependently reduced MiaPaCa2 and Panc1 invasion. 500 nM NT-S100A8 induced a rapid insulin release and enhanced b-TC6 [Ca2+]i oscillations after both one ($F=6.05$, $p<0.01$) and two minutes ($F=7.42$, $p<0.01$). In the presence of NT-S100A8, [Ca2+] in b-TC6 culture medium significantly decreased with respect to control cells ($F=6.3$, $p<0.01$). NT-S100A8 did not counteract insulin induced phosphorylation of the insulin receptor, Akt and IkB-a, but it independently activated Akt and NF-kB signalling in PC cells. To verify whether NT-S100A8 is traceable in serum as a potential index of pancreatic cancer induced diabetes mellitus, sera from 42 pancreatic patients (17 with and 25 without diabetes mellitus) and from 11 healthy controls were subjected to discontinuous SDS-PAGE in the same conditions which allowed NT-S100A8 to be isolated from pancreatic cancer tissues. At analysis of the low molecular weight (from about 15,000 to about 1,000 Da), nine protein bands were identified in sera. Only the band with an approximate molecular weight of 14,200 Da was correlated with the diagnosis of pancreatic cancer, being present in 10/11 controls and absent in 37/42 cancer cases ($\chi2=26.8$, $p<0.001$). Two bands with an approximate molecular weight of 10,000 and 1,000 Da respectively, were correlated with each other ($\chi2=4.8$, $p<0.05$) and, in cancer cases, their disappearance was closely correlated with the presence of diabetes mellitus ($\chi2=8.6$, $p<0.01$ and $\chi2=6.6$, $p<0.05$).

Conclusion: NT-S100A8 exerts a mild effect on PC cell growth, while it reduces PC cell invasion, possibly by Akt and NF-kB signalling, NT-S100A8 enhances [Ca2+]i oscillations and insulin release, probably by inducing Ca2+ influx from the extracellular space, but it does not interfere with insulin signalling. New potential serum biomarkers for pancreatic cancer and pancreatic cancer induced diabetes mellitus diagnosis were also identified.

D-121

Analytical Characteristics of CA15-3, CA19-9, CA 125, Carcinoembryonic Antigen and Alpha fetoprotein Assays Utilizing Luminescent Oxygen Channeling Immunoassay (LOCI®) Technology on the Dimension Vista® System.

W. E. Kelley[1], J. Sterner[2], D. Cervelli[2], J. Gray[3], R. E. Christenson[3]. *[1]Univ of Maryland Medical Center, Baltimore, MD, [2]Siemens Healthcare Diagnostics, Newark, DE, [3]Univ of Maryland School of Medicine, Baltimore, MD,*

Objective Characterize analytic performance of LOCI® assays for CA19-9*, CA15-3*, CA125*, CEA, and AFP on the Dimension Vista® System.

Relevance Monitoring biomarkers in patients with pancreatic (CA19-9), breast (CA15-3), ovarian (CA125), colorectal (CEA), and non-seminomatous testicular (AFP) cancer correlates with clinical response to treatment. Characterizing these assays on the Vista will assist laboratorians with decisions regarding this technology.

Methodology: Limit of blank(LOB) and Limit of detection(LOD) were determined following CLSI-EP17A, with n=60 replicates of analyte-free calibrator and low analyte samples, respectively. Analytical measurement range (AMR) was determined with serial dilution mixtures of high and low serum pools run in triplicate and examined visually and analyzed with linear regression. Repeatability and total reproducibility were assessed using CLSI protocol EP5-A2 in which three controls and one serum pool were run in duplicate, twice daily for twenty days (total n=80). Lot-to-lot variability and method comparison to a predicate method was evaluated using linear regression.

Results: LOD, LOB, AMRs and reproducibility, see table.

Tumor Marker	Analytical Characteristic	Reproducibility		
		Mean	Repeatability, %CV	Within-Lab, %CV
	Limit of Blank	Level 1		
CA 15-3	0.3 U/mL	24.52 U/mL	2.8	3.9
CA 19-9	1.0 U/mL	58.48 U/mL	2.8	4.0
CA 125	0.3 U/mL	24.88 U/mL	2.0	2.7
CEA	0.12 ng/mL	2.02 ng/mL	1.7	2.4
AFP	0.2 ng/mL	12.09 ng/mL	1.1	1.9
	Limit of Detection	Level 2		
CA 15-3	0.5 U/mL	66.59 U/mL	2.6	3.8
CA 19-9	2.0 U/mL	170.56 U/mL	1.3	2.6
CA 125	1.0 U/mL	61.85 U/mL	1.5	3.2
CEA	0.2 ng/mL	15.92 ng/mL	1.1	1.5
AFP	0.5 ng/mL	83.92 ng/mL	1.2	2.3
	AMR	Level 3		
CA 15-3	0.5-300 U/mL	164.05 U/mL	2.4	3.8
CA 19-9	2-1000 U/mL	420.68 U/mL	1.3	2.3
CA 125	1-1000 U/mL	165.42 U/mL	1.8	3.4
CEA	0.2-1000.0 ng/mL	36.55 ng/mL	0.8	1.5
AFP	0.5-1000.0 ng/mL	55.82 ng/mL	1.5	2.1
		Serum Pool A		
CA 15-3 (serum)		13.59 U/mL	3.0	5.3
CA 19-9 (serum)		11.40 U/mL	4.1	6.4
CA 125 (serum)		11.04 U/mL	2.6	4.0
CEA (serum)		229.26 ng/mL	1.2	1.9
AFP (serum)		237.59 ng/mL	1.0	2.4

Lot-to-lot comparisons were: CA125, Lot1=1.01*Lot2-1.69, r=0.999; CA15-3, Lot 1=1.00*Lot2+1.37, r=0.996; CA19-9, Lot 1= 1.08*Lot 2-1.50, r=0.999; CEA, Lot1 = 1.06*Lot2-2.05, r=0.999; AFP, Lot 1=1.00* Lot 2+0.33, r=1.000. Method comparison data were: CA125, Vista =1.11*Centaur+5.13, r=0.987, Sy/x =14.6, n=241; CA15-3, Vista = 0.967*Centaur+1.90, r=0.99, Sy/x=4.68, n=233; CA19-9, Vista = 0.937*Centaur +1.34, r=0.923, Sy/x=33.1, n=161; CEA, Vista =1.02*ACCESSII+5.6, r=1.0, Sy/x=21.2, n=114; AFP, Vista = 0.90*Centaur+1.43, r=1.00, Sy/x=14, n=114.

Conclusion: LOCI technology for CA19-9, CA15-3, CA125, CEA and AFP on the Dimension Vista demonstrated acceptable characteristics including LOB, LOD, AMR, reproducibility, lot-to-lot variability and agreement with accepted methodology, and is an attractive alternative for clinical laboratory use.

*Under review by US FDA; not available for sale

D-122

Clinical Concordance of the Cancer Biomarkers CA 15-3, CA 19-9, CA 125, Carcinoembryonic Antigen (CEA), and Alpha-Fetoprotein (AFP) on the Dimension Vista® Systems

W. E. Kelley[1], D. Cervelli[2], H. Rebuck[3], J. Sterner[2], R. Christenson[3]. *[1]Univ of Maryland Medical Center, Baltimore, MD, [2]Siemens Healthcare Diagnostics, Newark, DE, [3]Univ of Maryland School of Medicine, Baltimore, MD,*

Objective Assess the clinical accuracy of monitoring cancer patients with CA 19-9*, CA 15-3*, CA 125*, carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) LOCI® assays on the Dimension Vista® Systems by assessing concordance of temporal monitoring with commercially available comparative methods.

Relevance Monitoring of cancer biomarkers in patients with breast (CA 15-3), pancreatic (CA 19-9), ovarian (CA 125), colorectal (CEA), and non-seminomatous testicular (AFP) cancer correlates with clinical response to treatment. Characterizing these assays on the Vista will assist laboratorians with decisions regarding utilization for patient monitoring.

Methodology: Clinical concordance (accuracy) of serial Vista measurements for monitoring CA 19-9, CA 15-3 and CA 125 concentrations were compared with the predicate ADVIA Centaur® assays; the Beckman-Coulter Access® 2 CEA and Abbott Axsym® AFP assays were the additional predicate assays. Patients were monitored with 2 to 8 serial specimens, mean 3.5. Patient demographics, see table. Clinical concordance was defined as agreement of the Vista and predicate assay in temporal monitoring pattern; patterns were categorized as Rising, Falling or Stable. Tumor marker values for the assays were categorized as Rising or Falling if they exceeded the reference change value (RCV), expressed as ; Z is the z-score, CV_A is the analytical variation (total CV), and CV_I is biological variation obtained from the literature. Stable tumor marker values did not exceed the RCV. Total concordance (Rising, Falling or Stable) and 95% CI were calculated for each cancer type.

Validation: Clinical concordance, see table.

Variable	CA 15-3	CA 19-9	CA 125	CEA	AFP
Number of patients	75	75	75	74	70
Type of Cancer	Breast	Pancreatic	Ovarian	Colorectal	Testicular
(Stage)	(I-IV)	(I-IV)	(I-IV)	(I-IV)	(I-IV)
Age in years	22 to 76	41 to 86	20 to 86	36 to 86	1 to 54
Samples for monitoring	258	196	255	217	244
Number Rising (Concordant, %)	115 (106, 92%)	26 (22, 85%)	66 (64, 97%)	64 (55, 86%)	34 (34, 100%)
Number Falling (Concordant, %)	69 (58, 84%)	5 (4, 80%)	39 (38, 97%)	29 (24, 83%)	66 (54, 82%)
Number Stable (Concordant, %)	74 (65, 88%)	165 (155, 94%)	150 (147, 98%)	124 (116, 94%)	144 (131, 91%)
Overall Concordance (95% CI)	89% (84.9-92.6)	92% (87.7-95.6)	98% (95.0-99.1)	90% (85.1-93.5)	94% (90.1-96.5)

Conclusion: Dimension Vista LOCI technology for CA19-9, CA15-3, CA 125, CEA, and AFP measurements demonstrated acceptable concordance with predicate methods for monitoring appropriate cancer patients.

*Under review by US FDA; not available for sale

D-123

Circulating biomarkers in advanced pancreatic cancer

P. J. Hamer, S. Brown-Shimer, S. P. Bradley, K. Pierce, J. Ramirez, D. Trivedi, D. Newman, W. P. Carney. *Oncogene Science, Cambridge, MA,*

Background: Pancreatic cancer is one of the deadliest forms of cancer as its five year survival rate is only 4%. There is currently a dearth of circulating biomarkers that have been investigated for pancreatic cancer. Our group has been interested in exploring various circulating biomarkers in pancreatic cancer since these are tests that can be used in real time to monitor patient response or lack of response to therapy. With the increase in the number of therapeutic options, circulating biomarkers can provide a continuous way of assessing patient status and signal the need for additional therapeutic interventions. Therefore, we analyzed plasma and serum samples with a variety of circulating biomarkers for potential significance in this cancer or biomarkers known to be targets for new therapeutics in development.

Methods: EDTA plasma samples from 52 pancreatic patients were analyzed for TIMP-1 and PDGFR-β levels. In addition, serum samples from 25 different pancreatic cancer patient sera were analyzed for TIMP-1 and PDGFR-β. Of the 52 patients where EDTA plasma was available, 20 pancreatic cancer patients were analyzed for circulating Ras p21, VEGF-165, VEGF-R2 and EphA2. The majority of these patients (70%) had stage 3 pancreatic cancer while the remainder had stage 2 or stage 4 disease. All ELISA tests were developed and manufactured by Oncogene Science (Cambridge, MA). Normal ranges and cutoff values were established for each analyte.

Results: Data from this study showed that several putative biomarkers were elevated in a large percentage of the advanced pancreatic patients. Of the 52 patients with plasma samples, 89% had elevated TIMP-1 levels whereas 67% of the 20 patients with serum samples had elevated TIMP-1 levels. Of the 20 patient subset where plasma samples were analyzed , 35% of the patients had increased EphA2 levels and 90% were increased for VEGF-165 while 77% of the patients had elevated Ras p21 levels.

Conclusions: A high percentage of pancreatic cancer patients have elevated levels of several circulating biomarkers such as Ras p21, VEGF-165, EphA2 and TIMP-1. It is known that a high percentage of pancreatic cancer patients have mutated K-ras genes and the introduction of an assay for circulating ras p21 may assist in deciphering what role Ras p21 plays in pancreatic cancer. In addition, VEGF-165 is the target of many current and future anti-angiogenesis drugs. A panel of markers such as those analyzed in this study of pancreatic cancer has the potential for use as a selection tool for targeted therapies such as EphA2 targeted therapies under development. In addition, biomarker panels, which may include traditional tumor markers, may have utility in monitoring patient therapy.

D-124

Effect of race and age on prostate cancer detection using a combination [-2]proPSA, PSA, and Free PSA using the Beckman Coulter Access Immunoassay Systems. A multi-center prospective clinical study.

W. L. Roberts[1], G. G. Klee[2], L. S. Marks[3], C. H. Bangma[4], W. J. Catalona[5], A. W. Partin[6], M. G. Sanda[7], K. M. Slawin[8], D. W. Chan[6], L. J. Sokoll[6], R. H. N. van Schaik[4], D. L. Broyles[9], A. B. Cruz[9], I. A. Mizrahi[9], S. S. Shin[9], J. T. Wei[10]. *[1]University of Utah, Salt Lake City, UT, [2]Mayo Clinic, Rochester, MN, [3]University of California Los Angeles, Los Angeles, CA, [4]Erasmus University Medical Center, Rotterdam, Netherlands, [5]Northwestern University Feinberg School of Medicine, Chicago, IL, [6]Johns Hopkins University School of Medicine, Baltimore, MD, [7]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, [8]Vanguard Urologic Institute and Texas Prostate Center, Houston, TX, [9]Beckman Coulter, Inc., Carlsbad, CA, [10]Unviersity of Michigan School of Medicine, Ann Arbor, MI,*

Introduction and Objectives: Age- and race-adjusted PSA reference ranges have been suggested to improve the utility of PSA testing. Screening algorithms that remain constant with respect to age and race would be simpler and easier to use. The influence of age and race on a mathematical combination of [-2]proPSA (p2PSA)**, PSA and fPSA was evaluated to determine the effect on prostate cancer (PCa) detection.

Methods: 892 subjects (430 PCa and 462 benign by biopsy) with PSA ranging from 2-10 ng/mL were enrolled from 7 clinical centers. Subjects were ≥50 years of age, with non-suspicious DRE. 98% had ≥10 core biopsies. [-2]proPSA, PSA and fPSA were analyzed on the Beckman Coulter Access 2 Immunoassay Analyzer***. We used a formula combining PSA, fPSA, and [-2]proPSA (called Beckman Coulter Prostate Health Index or *phi*) to evaluate the effect of race and age on PCa detection. Receiver operating curve (ROC) analyses and PCa prevalence adjusted probability (age only) of PCa analyses were conducted.

Results: No correlation between *phi* and age were noted (r = 0.008, p = 0.810). Within each age decade, PCa subjects had a higher *phi* than benign subjects (p<0.001 for each category). At a constant *phi* of 21.3, the clinical sensitivity was 95.9%, 93.8%, and 97.3% for the 50-59, 60-69, and ≥70 years age groups. Comparable median ages (62.0 vs. 63.0; p=0.838) were obtained above and below the 21.3 *phi* level. Comparable probability of PCa was observed across the three age decades and *phi* ranges. The probability of PCa for the 50-59 years of age group were 10.9%, 15.6%, 33.4%, and 53.5% for *phi* ranging from <25, 25.0-34.9, 35.0-54.9 and ≥55, respectively (with relative risks, relative to phi <25, of 1.0, 1.4, 3.1, and 4.9) . The probability of cancer for the 60-69 age group were 12.5%, 18.0%, 34.4% and 54.6% for the same four *phi* ranges, respectively (with relative risk of 1.0, 1.4, 2.8, and 4.4). Similarly, the probability of cancer for the ≥70 years of age group were 5.8%, 23.1%, 26.1% and 44.8% for the four *phi* ranges, respectively (with relative risks of 1.0, 4.0, 4.5, and 7.7). No significant differences in *phi* were found between Caucasian (N=726) and African-American (N=46) men for the PCa (p=0.149) and benign groups (p=0.306). At a *phi* of 21.3, similar clinical sensitivity was obtained for the Caucasian (95.3%) and African-American (95.5%) groups (p=1.00).

Conclusion: The lack of trends for age and probability of cancer do not support the use of age-adjusted *phi* values for PSA-based PCa detection. In addition, *phi* performs similarly in both Caucasians and African-American men. These results suggest that Beckman Coulter *phi* may be applied to a broad spectrum of men without the use of age or race-specific reference ranges.

*Not intended as off-label promotion of any BCI product

**Pending FDA approval

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D-125

Evaluation of an ELISA for Quantifying CYFRA 21-1 Protein in Serum and Correlation with an Automated Chemiluminescence Enzyme Immunoassay

J. A. Erickson[1], D. G. Grenache[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]University of Utah Health Sciences Center, Department of Pathology, Salt Lake City, UT,*

Background: Cytokeratins are intermediate filament structural proteins found in the cytoskeleton of epithelial cells. A soluble fragment of cytokeratin 19, CYFRA 21-1, has clinical utility in the prognosis, treatment monitoring and staging in patients with non-small cell lung cancer (NSCLC). Recently, the automated Lumipulse® S CYFRA 21-1 chemiluminescence enzyme immunoassay (CLEIA, Fujirebio, Inc., Tokyo, Japan), previously validated and employed in our laboratory, was removed from the US market. Therefore, we have evaluated the newer CanAg® CYFRA 21-1 EIA (Fujirebio Diagnostics AB, Göthenburg, Sweden), a manually performed ELISA, as a possible replacement.

Objectives: To perform an analytical validation of the CanAg CYFRA 21-1 ELISA for the quantitative determination of CYFRA 21-1 in human serum.

Methods: CYFRA 21-1 protein was measured according to the assay manufacturer's testing protocol. Performance characteristics, including analytical sensitivity, linearity, imprecision, and analyte stability, were performed. A split-sample comparison study was completed using the previously validated automated Lumipulse S CYFRA 21-1 CLEIA as the comparative method. The 97.5th percentile, non-parametric reference interval was established from 150 healthy adult volunteers (75 males, 75 females). The project was approved by the University of Utah's Institutional Review Board.

Results: The limit of blank was determined to be 0.4 ng/mL by measuring 10 replicates of the zero calibrator, calculating the mean and adding three standard deviations. Linearity was determined by combining serum pools with high (57.8 ng/mL) and low (0.0 ng/mL) CYFRA 21-1 concentrations to create six specimens with an expected concentration range of 0.5-56.4 ng/mL. Linear regression generated a slope of 0.997 with an intercept of -2.26 and an R^2 of 0.993. Low end linearity was evaluated similarly (high pool 11.6 ng/mL, low pool 0.3 ng/mL, expected range 0.5-11.0 ng/mL, n=6) producing a slope of 0.996, intercept of -0.237 and R^2 of 0.999. Within-run imprecision studies produced CVs of 6.9, 2.7 and 1.5% at concentrations of 1.3, 6.5, and 37.5 ng/mL, respectively (n=12 per concentration), and between-run CVs of 12.8, 7.1 and 6.1% at 1.5, 6.8, and 38.6 ng/mL, respectively (n=12 per concentration over 60 days). CYFRA 21-1 stability at ambient temperature was determined at a minimum of two days (CVs of 9.3, 1.6 and 2.7% at 2.2, 12.5, and 14.2 ng/mL, respectively, n=6 per concentration) and for a minimum of 14 days at 4-8 °C (CVs of 6.6, 1.5 and 2.2% at 2.5, 7.4, and 14.3 ng/mL, respectively, n=6 per concentration). Utilizing Deming Regression analysis, a split sample comparison study of the CanAg ELISA vs. the Lumipulse CLEIA resulted a slope of 1.781, a y-intercept of -0.536 with an R^2 of 0.826 (n=80). An upper reference interval limit at the 97.5th percentile was established at 2.3 ng/mL CYFRA 21-1 with no significant differences identified by either gender (p=0.12) or age (p=0.98).

Conclusions: The CanAg CYFRA 21-1 EIA demonstrates acceptable performance for quantifying CYFRA 21-1 protein in serum. Although the manual ELISA and automated Lumipulse assays correlate adequately, there is significant disagreement between the two assays. Therefore, results cannot be used interchangeably.

D-126

Laboratory Detection of a Nonsecretory Multiple Myeloma Using Free Light Chain Assay and Anti-Free Light Chain Immunofixation Electrophoresis

L. Song, N. Mistry, B. Riviere. *Quest Diagnostics Nichols Institute, Chantilly, VA,*

Introduction: Nonsecretory multiple myeloma(NSMM) accounts for 1-5% of all cases of multiple myeloma(MM). Traditionally, protein electrophoresis(PE) and immunofixation electrophoresis(IFE) are the laboratory tests for MM. Patients with NSMM normally have symptoms of MM but their laboratory results are negative for monoclonal gammopathy because no monoclonal proteins can be detected using PE and IFE. The diagnosis of MM in patients with NSMM is normally delayed for 1 to 12 month. We report a case of NSMM detected by serum free light chain(FLC) assays and IFE when anti-free light chain antisera was used.

Method: PE and IFE were performed on Helena's SPIFE 3000. FLC assays were performed using the FLC reagent from The Binding Site put on Dade Behring BNII. Concentration of immunoglobulin IgG, IgA, and IgM were measured on the Roche Cobas Integra 800. Beta-2 microglobulin was determined using Dade Behring BNII.

Case Report: Patient is a 48 year old male. Both serum PE and urine PE were negative for monoclonal gammnpathy. IFE results for gamma, alpha, mu heavy chains, and kappa and lambda light chains were all negative. Quantitative immunoglobulin results for IgG was 76 mg/dL (Reference range(RR): 694-1618 mg/dL), for IgA was <5 mg/dL (RR: 81-463 mg/dL), and for IgM was 6 mg/dL (RR: 48-271 mg/dL), indicating hypogammaglobulinemia. On the other hand, Beta-2 microglobulin was elevated with a result of 5.16 mg/dL (RR: <=2.51mg/L). The serum kappa free light chain was significantly elevated with a result of 680 mg/L (RR: 3.3-19.4 mg/L). The free kappa to free lambda ratio (κ/λ ratio) was 261.5 (RR:0.26 -1.65). These findings pointed to the possibility of NSMM. We decided to test for kappa free light chains using the Helena's anti free κ antisera in IFE test. We detected a band of free κ light chain using anti free κ light chain antisera although this band did not show up when regular anti κ light chain antisera was used in the IFE test. This patient was diagnosed with NSMM.

Discussion: This case shows that FLC assay is more sensitive in detecting the abnormalities of free light chains in NSMM. It also shows that the abnormal free light chains in NSMM patients detected by FLC and by IFE using the anti-free light chain antisera may have the nature of monoclonal gammopathy. It is possible that the anti free light chain antisera used in the Helena IFE can recognize the free light chain present in this particular patient with NSMM. Further studies on NSMM cases will provide more data to reveal the nature of these light chains in NSMM. When the results of PE and IFE are negative but FLC is significantly elevated, NSMM should be considered. IFE using

anti-free light chain antisera may be of value to study the nature of the free light chains. It is also helpful to clinicians to add a comment on the possibility of NSMM on the report.

D-127

A Fully Automated Chemiluminescent Microparticle PIVKA II Assay for the ARCHITECT® Instrument System

E. Yoshida[1], K. Fujita[1], R. Chiba[1], M. Azetaka[1], E. Takahashi[1], B. Dowell[2], T. Yoshimura[1]. *[1]Abbott Japan Co., Ltd., Chiba, Japan, [2]Abbott Laboratories, Abbott Park, IL,*

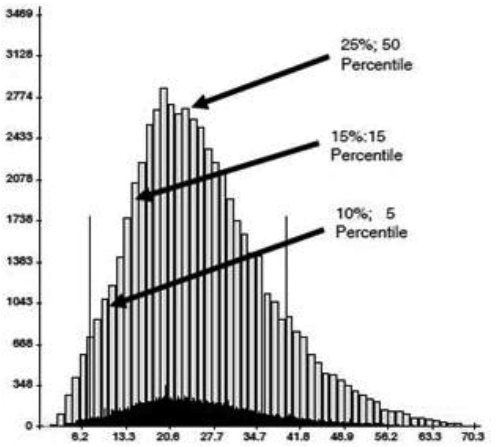
Objective: The purpose of this study is to evaluate the performance characteristics of the prototype ARCHITECT PIVKA-II assay on the Abbott ARCHITECT instrument.
Principle: PIVKA-II (protein induced by Vitamin K absence or antagonist - II), also known as des-carboxy prothrombin (DCP), is a tumor marker useful for aiding in the diagnosis, treatment monitoring and determination of the prognosis of patients with hepato-cellular carcinoma (HCC). PIVKA-II is measured in human serum or plasma using a PIVKA-II specific monoclonal antibody, 3C10, and an antibody to human prothrombin.
Methods: The prototype ARCHITECT PIVKA-II assay is a fully automated two step assay utilizing paramagnetic microparticles coated with a monoclonal antibody 3C10 specific to PIKVA-II and an acridinium-labeled antibody to human prothrombin. The ARCHITECT PIVKA-II assay has a short assay time of 29 minutes and a high throughput of 200 test/hour. Assay sample diluent, the microparticle coated with 3C10, and the serum or plasma sample are incubated for 18 minutes in the first step to capture PIVKA-II on the 3C10 microparticles. Following a wash, the anti-prothrombin conjugate is added to the mixture in the second step and incubated for 4 min. Following a wash, Pre-trigger and Trigger solutions are added to the reaction mixture. The produced chemiluminescence is measured as relative light units (RLUs), which are directly proportional to the amount of PIVKA-II antigen in the sample.
Results: The assay has an analytical range of 0 to 30,000 mAU PIVKA/mL. Calibration curves are stable for 30 days with reagent on-board the instrument. Dilution linearity went up to 30,000 mAU/mL. Method comparison at 10-1000 mAU/mL range to the Picolumi PIVKA-II (semi-automated immunoassay) showeda correlation coefficient of r = 0.981 and a slope = 0.95 with 169 specimens. Spike recovery gave a mean of 95.6 % recovery. No interference by HAMA / RF / Heterophilic Abs was observed. Precision determined by CLSI (EP5-A2) showed %CVs of 2.4 - 6.1. The limit of blank (LoB) and limit of detection (LoD) of the assay are ≤ 1.4 mAU/mL and 2.5 mAU/mL, respectively. Reagent accelerated stability (40ºC, 7days) showed 100.2 % recovery compared to day 0.
Conclusions: The fully automated prototype ARCHITECT PIVKA-II assay is an accurate, sensitive and precise assay for the measurement of PIVKA-II levels in human sera and plasmas.

D-128

Audit of the proper cut-off value of Free to Total PSA Ratio using Modular E170 Roche immunoassay analyzer

R. M. Dorizzi, P. Maltoni, C. Sgarzani, M. Zappulla, V. Ventura. *Laboratorio Unico di AVR, Cesena, Italy,*

Background. Free/Total PSA Ratio is lower among prostate cancer patients than among benign prostate disease patients. The correct measure of FPSA is critical for subjects with total PSA concentration falling between 2.5 and 10 ug/L and without abnormal findings upon DRE.
The aim of the study was to audit the cut-off of FPSA and TPSA proposed by the manufacturer for the results obtained using an automated immunoassay analyzer in order to assess the applicability in our region of the proposed cut-off of the F/T PSA (25%).
Methods. We downloaded all the F/T PSA results obtained between March 2009 and January 2010 stored in the LIS of the Corelab Unit of Laboratorio Unico di Area Vasta Romagna, serving about one million inhabitants. All the results have been obtained using the automated analyzer Modular E170 (Roche, Mannheim, Germany). We analyzed the 61219 obtained results using the software GraphROC.
Results. The lowest value was 0.33%, the highest 93%, the mean 25.8% [95% Confidence Interval (CI) 25.74-25.93]), median 24.5% (CI = 24.4-24.6). Percentiles 2.5th, 5th, 10th, 25th, 75th, 90th, 95th and 97.5th were respectively 7.2, 9.4, 12.4, 17.7, 40.9, 46.8 and 52. Figure 1 summarizes the results showing the F/TPSA ratio corresponding to 50th percentile (25%); to 15th percentile (15%) and 5th percentile (10%);
Conclusions. The dramatic effect of changing the method in the measuring values of TPSA, FPSA and F/TPSA with different analyzers and in different regions has been extensively debated (Stephan C et al, Clin Chem 2006; 52: 59-64; Stephan C et al, BJU International 2007; 99: 1427-31¸Young-Jae I et al, Yonsei Medical Journal 2004; 45: 873-8; Thakur V et al Disease Markers 2004; 19: 287-92). The large number of results collected in ten months allowed to easily demonstrate that the cut-off proposed by the manufacturer is not proper for our area.



D-129

Correlation between plasma and serum sample types for measurement of the tumor markers CA19-9, CA125 and CA15-3 on the Dimension Vista® System.

L. M. Bachmann[1], T. D. Givens[1], D. Cervelli[2]. *[1]Virginia Commonwealth University, Richmond, VA, [2]Siemens Healthcare Diagnostics Inc., Newark, DE,*

CA125, CA19-9 and CA15-3 are utilized for serial monitoring of cancer therapy and recurrence. Cancer patients commonly exhibit low hematocrit and validation of plasma for tumor marker analysis may decrease blood requirements. The relationship between plasma and serum measurements was investigated for the LOCI® CA125[a], CA19-9[a] and CA15-3[a] assays on the Dimension Vista® Systems from Siemens. EDTA(E) and/or Li-Heparin(L) plasma and serum(S) were obtained from residual laboratory specimens, each from the same collection event. Data were evaluated by Deming regression analysis. Precision was evaluated by CLSI EP5-A2. Between-run CVs for serum pools ≤ 13.1 U/mL were < 9%. Between-run CVs for all sample types were ≤ 4.0% at concentrations >100 U/mL. Regression results are shown (**Table 1**). All R^2 values were ≥ 0.996. The Sy.x for CA19-9 suggested a potential for clinically significant variation. Regression was performed over a range more useful for residual disease monitoring [2.0 - 50 U/mL] with slope of 1.04 and 1.01; intercept of -0.91 and 0.06; and Sy.x of 1.2 and 1.8 U/mL for CA19-9(E) and (L), suggesting plasma is suitable for residual disease monitoring compared with serum. For concentrations that exceeded AMR and required dilution, the slopes and intercepts were similar to Table 1 and did not differ from 1.00 and 0.00, except for CA125(L) with slope 0.97(p <0.05). The maximum %residual was 18% for CA19-9(L) at 8,982 U/mL. CA19-9(S) vs. CA19-9(S) (replicates) resulted in maximum %residual of 7% at 13,069 U/mL. EDTA and Li-Heparin plasma measurements for Siemens Dimension Vista CA19-9, CA125 and CA15-3 were comparable to serum over the assay AMRs. Sy.x was increased at higher concentration ranges, but maximum differences among sample types were clinically insignificant. [a] Under review by FDA; not available for sale. *Statistically significant difference of slope vs. 1.00 or
intercept vs. 0.00 (p < 0.05).

Table 1

TM	T	N	Range (U/mL)	Slope (95% CI)	Intercept (95%CI)	Sy.x (U/mL)
CA19-9	E	56	2.3 - 971	*0.96 0.93 - 0.99	1.30 -1.6 - 4.2	13.3
CA19-9	L	53	2.7 - 971	0.99 0.96 - 1.02	0.70 -2.4 - 3.8	16.9
CA125	E	66	1.7 - 896	1.00 0.89 - 1.11	-0.38 -2.8 - 2.0	6.6
CA125	L	87	1.7 - 921	*0.93 0.91 - 0.96	0.49 -0.4 - 1.4	8.1
CA15-3	E	70	3.0 - 240	0.97 0.90 - 1.04	0.50 -0.8 - 1.8	2.1
CA15-3	L	66	3.0 - 240	0.99 0.93-1.05	0.46 -0.5 - 1.4	1.2

D-130

Novel monoclonal antibodies for determining levels of imatinib in biological fluids

A. Volkov, J. B. Courtney, S. J. Salamone. *Saladax Biomedical, Inc., Bethlehem, PA,*

Objective: Imatinib is a potent and selective inhibitor of the Bcr-Abl and KIT protein tyrosine kinases. It is widely used for treatment of Philadelphia chromosome positive chronic myeloid leukemia (Ph+ CML) and KIT-positive gastrointestinal stromal tumor (GIST). Studies have shown a significant inter-patient variability in imatinib trough concentrations (from 181 to 2947 ng/mL) and a correlation between systemic levels of the drug and clinical outcome. The objective of this work was to develop monoclonal antibodies for imatinib in order to develop a rapid immunoassay for determining levels of imatinib in the plasma of patients. The current physical methods for measuring imatinib require specially trained personnel, are expensive, time consuming and not amenable for widespread use. An automated immunoassay would provide a rapid, simple and inexpensive method to routinely measure levels of imatinib in patients. Imatinib is metabolized in the liver by cytochrome CYP3A4 isozyme. The main plasma metabolite of imatinib is the N-demethylated piperazine derivative (N-desmethylimatinib). It has potency similar to imatinib and a plasma AUC (area under the concentration vs. time curve) approximately 10-20% of that of imatinib. Since the dose-response relationship for this drug has been established for plasma levels of imatinib only, one of the main requirements for antibodies was low cross-reactivity with N-desmethyl imatinib.
Method: The immunogen used in this work was N-hydroxysuccinimide-activated butyric acid derivative of imatinib coupled to keyhole limpet hemocyanin (KLH) in the presence of 66% dimethyl sulfoxide (DMSO). Upon completion of the reaction DMSO concentration was reduced stepwise by dialysis against phosphate buffer. The efficiency of imatinib coupling was evaluated by ultraviolet spectrometry and was found to be 90%. Due to low solubility of imatinib-KLH conjugate in phosphate buffer it was dissolved in 50% DMSO, emulsified in Complete Freund's adjuvant and used to immunize female BALB/c mice with 100 µg/mouse. The mice were boosted once four weeks after the initial injection with 100 µg/mouse of the same immunogen emulsified in Incomplete Freund's Adjuvant. Monoclonal antibodies were obtained by fusion of spleen cells isolated from selected mice and SP2/0 cells.
Results: This immunogen was successful in eliciting two highly selective monoclonal antibodies for imatinib with IC_{50} of 30 ng/mL and cross-reactivity with N-desmethyl imatinib of 1.3%.
Conclusion: These monoclonal antibodies are the first report of antibodies able to measure imatinib levels. They will be useful for rapid and cost effective monitoring of imatinib in the plasma of patients and better adjustment of dosing for individual patients.

D-131

Polymorphisms in apoptotic genes, Caspases 8 and 9 and their role in prostate cancer susceptibility in cohort of North India

G. George[1], R. K. Mandal[1], P. kesarwani[1], A. Mandhani[1], S. N. Sankhwar[2], R. Mittal[1]. *[1]Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India, [2]Chhtrapati Shahuji Maharaj Medical University, Lucknow, India,*

Background: Despite the potential importance of apoptosis pathways in prostate tumor etiology, little has been published regarding prostate tumor risk associated with common gene variants in Caspases.
Methods: Using data from a hospital-based case-control study conducted by Sanjay Gandhi Post Graduate Institute of Medical Science, India between 2007-2009. We evaluated risk of prostate cancer (n=165) patients and 205 cancer-free controls age-matched, unrelated and of similar ethnicity. We genotyped the functional IVS12-19G/A, D302H, -678del and -652 6Nins/del polymorphisms in the promoter of Caspase 8 and -293del, -1263A/G in Caspase 9.
Results: A significant increased risk for prostate cancer was found for the CASP8 IVS12-19G/A heterozygous genotype (p=0.02; OR=1.69) as well as for the variant allele carriers (p=0.04; OR=1.56). Also the Caspase 9 -1263A/G showed lower risk for both heterozygous and variant allele carrier genotypes (p=<0.001; OR=0.45 and p=0.05; OR=0.66 respectively). Caspase 9 -1263A/G was also found to be associated with increased risk in bone metastasis. Furthermore, a significant additive interaction between *Caspase 8* IVS12-19G/A polymorphism and tobacco smoking was observed with prostate cancer risk.
Conclusion: These results suggested that the CASP8 IVS12-19G/A and Caspase 9 -1263 polymorphism may be involved in etiology of prostate cancer and thus could be implicated as a marker for genetic susceptibility in North Indian population. The study also suggested that CASP activation may be synonymous with apoptosis and that activation of caspases may be involved in inducing cell death.

D-132

Monitoring Cancer Patients: Clinical Performance of the Access® BR Monitor (CA 15-3 Antigen), GI Monitor (CA 19-9 Antigen) and OV Monitor (CA 125 Antigen) Assays on the Unicel® DxI800 Immunoassay System from Beckman Coulter: A European Multicenter Study

P. Steiber[1], R. Molina[2], M. Gion[3], A. Gressner[4], F. Troalen[5], J. Auge[2], S. Holdenrieder[1], M. Zancan[3], M. Wycislo[4], D. Nage[1], J. Blanchet[6]. *[1]Institut für Klinische Chemie, Klinikum der Universität München-Campus Großhadern, Munich, Germany, [2]Laboratory for Clinical Biochemistry, Hospital Clinic, Barcelona, Spain, [3]ABO Association c/o the Center for the Study of Biological Markers of Malignancy, General Regional Hospital, Venice, Italy, [4]Institut für Klinische Chemie, Universitätsklinikum der RWTH Aachen, Aachen, Germany, [5]Departement de Biologie Clinique, Institut Gustave Roussy, Villejuif, France, [6]Beckman Coulter, Nyon, Switzerland,*

Background: The Access BR, GI and OV Monitor immunoassays (Beckman Coulter) use antibodies different from the original Centocor* antibodies. It is of special relevance to compare their diagnostic capacity not only at primary diagnosis but also during follow-up care of cancer patients.
Methods: Using Access BR Monitor we investigated serial samples from 19 patients with breast cancer, using Access OV Monitor serial samples of 33 ovarian cancer patients and using Access GI Monitor serial samples of 43 pancreatic cancer patients. All results were run on a UniCel DxI 800 and compared with other commercially available methods (Roche Elecsys*).
Results: The serial values were summarized for each marker and the corresponding reference method in three groups: 1) increase >25%, 2) decrease >25% and 3) stable marker for increase <25% and decrease <25%. Concerning the correlations, we observed for BR Monitor and CA 15-3 in 67%, for GI Monitor and CA 19-9 in 84% and for OV Monitor and CA 125 in 92% a complete coincidence of the 3 groups. For OV Monitor and CA 125 as well as for GI Monitor and CA 19-9 there were no discrepancies in measurement in group 1 or in group 2, for BR Monitor and CA 15-3 this situation occurred in one case (2 serial samples within 128 samples).
Conclusion: Serial measurements of the BR Monitor, GI Monitor and OV Monitor assays with other assays showed a high degree of comparability.
*All trademarks are property of their respective owners.

Elecsys CA 19-9

Access GI Monitor	Decrease > 25%	Stable	Increase > 25%	Total
Decrease > 25%	50 20.0%	11 4.4%	0 0%	61 24.4%
Stable	10 4.0%	75 30.0%	4 1.6%	89 35.6%
Increase > 25%	0 0%	12 1.8%	88 35.2%	100 40.0%
Total	60 24.0%	98 39.2%	92 36.8%	250 100%

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The Beckman Coulter prostate health index (phi) improves diagnostic accuracy in prostate cancer detection

S. Vincendeau[1], J. Blanchet[2], J. Ramirez[3], X. Durand[4], E. Deligne[4], F. Guille[1], A. Houlgatte[4]. *[1]Deptartment of. Urology, Hospital Pontchaillou, Rennes, France, [2]Beckman Coulter, Nyon, Switzerland, [3]Deptartment of Clinical Chemistry, HIA du Val de Grace, Paris, France, [4]Deptartment of Urology, HIA du Val de Grace, Paris, France,*

Introduction The benefit of screening for prostate cancer (PCa) using total prostate-specific antigen (tPSA) as biochemical marker is a matter of intense debate due to the relatively low clinical specificity of tPSA leading to serious drawbacks such as overdiagnosis and overtreatment. New biomarkers that could improve the specificity for PCa detection are highly desirable. Previous studies showed that a molecular isoform of PSA ([-2]proPSA) could improve the clinical specificity for the detection of PCa, compared to tPSA and %free PSA (%fPSA). Beckman Coulter recently developed an innovative "prostate health index" or "*phi*" which combines tPSA, fPSA and [-2]proPSA (Hybritech p2PSA**) results. The clinical performance of phi for the detection of PCa is under evaluation in a two-center study.
Methods After 3 months of recruitment, 129 men (79 with, 50 without PCa, all confirmed with a >10 core biopsy) with tPSA levels between 2 - 10 ng/mL and non-suspicious digital rectal examination (DRE), from the Hospital Pontchaillou in Rennes and the Hospital Val de Grace in Paris, were enrolled in the study. The serum concentrations of tPSA,

fPSA and [-2]proPSA were measured with Beckman Coulter Access immunoassays on a DxI800 instrument. Interim analysis using ROC curve was used to compare the clinical performances of phi with the %fPSA taken as a reference for the detection of PCa.
Results Combining the two PCa cohorts for tPSA-range 2 - 10 ng/mL, the area-under-the-ROC curves (AUC) analysis shows that phi (AUC=0.67) provided significantly better (p=0.05) clinical performance relative to %fPSA (AUC=0.57). The data analysis also demonstrates that the probability of a positive biopsy is continuously increasing with the *phi* score. Men with a *phi* score below 30 showed a positive biopsy rate of 45% whereas 87% with a *phi* score of greater than 70 were biopsy positive.
Conclusions Using this data set, and regrouping a limited number of patients, analysis of this two-center study indicates that *phi* may have superior clinical performance in detecting PCa in the tPSA range of 2 - 10ng/mL compared to current reference biomarker of %fPSA. These results will have to be confirmed using the entire cohort of subjects (n>200).
*Not intended as off-label promotion of any Beckman Coulter product
**In development for US market, pending FDA approval

D-134

[-2]proPSA as Biomarker to Detect Aggressive Prostate Cancer

C. Stephan[1], J. Blanchet[2], K. Miller[1], K. Jung[3]. *[1]Department of Urology, Charité - Universitätsmedizin, Berlin, Germany, [2]Beckman Coulter, Nyon, Switzerland, [3]Berlin Institute for Urologic Research, Berlin, Germany,*

Objective: Current serum tumor markers like PSA or %fPSA are not effective in identifying aggressive prostate cancer (PCa). The objective was to evaluate the new automated Access Hybritech p2PSA ([-2]proPSA) assay for the ability to distinguish between Gleason score < 7 (less aggressive) and ≥ 7 (aggressive) PCa.
Method: Sera from 311 PCa patients were measured with Access Hybritech p2PSA**, total PSA (tPSA) and free PSA (%fPSA) assays on Access Immunoassay Systems (Beckman Coulter, Brea, CA) within the 0-30 ng/mL tPSA range. Within the clinically relevant tPSA range 2-10 ng/mL, p2PSA data from 264 PCa patients were further analyzed for their discriminative power to differentiate between Gleason score < 7 and ≥ 7 and between pT2 and pT3 tumors. Different ratios between p2PSA and fPSA (named as %p2PSA), and p2PSA and %fPSA (p2PSA/%fPSA), were compared with p2PSA and %fPSA alone.
Results: Compared with tPSA and %fPSA (P=0.011 to 0.028), p2PSA was also significantly different in both tPSA ranges between pT2 and pT3 tumors (P=0.023 and 0.027), while %p2PSA and p2PSA/%fPSA showed improved significance levels (P from <0.0001 to 0.001). The comparison of Gleason score < 7 and ≥ 7 tumors revealed no difference for tPSA in both tPSA ranges (P=0.08 and 0.66), while p2PSA was only different in the group of all patients (P=0.028). The %fPSA could significantly discriminate between Gleason score < 7 and ≥ 7 tumors (P=0.004 and 0.008). However, %p2PSA (P<0.0001, 0.0008) and p2PSA/%fPSA (P<0.0001, 0.0003) again showed the best significance levels.
Conclusion: The ratios %p2PSA and p2PSA/%fPSA revealed the strongest association to differentiate between pT2 and pT3, as well as between Gleason score < 7 and ≥ 7 prostate cancer, compared with %fPSA or p2PSA alone. Therefore, p2PSA ratios may especially indentify aggressive PCa.
*Not intended as off-label promotion of any Beckman Coulter product
**In development for US market, pending FDA approval

D-135

Autoantibody Signatures and Immune Complexes as Potential Biomarkers in Ovarian Cancer with Low Serum CA-125.

D. J. O'Rourke[1], D. A. DiJohnson[1], A. F. Vitonis[2], D. W. Cramer[2], B. C. S. Liu[2]. *[1]Brigham and Women's Hospital, and Northeastern University, Boston, MA, [2]Brigham and Women's Hospital, Harvard Medical School, Boston, MA,*

Background: CA-125 is widely used as a biomarker in ovarian cancer patients to monitor response to therapy and disease recurrence. However, CA-125 is not elevated (≤ 35 U/ml) in about 20-30% of patients that have ovarian cancer and thus cannot be used for monitoring. Therefore, there are needs to 1) discover new biomarkers that perform better than CA-125 for monitoring, and 2) explain why some cases, even those with tumors expressing CA-125, have low serum CA-125 levels.
Methods: To discover new biomarkers, we developed a whole proteome native antigen microarray platform to identify tumor-associated signatures that might be used for monitoring disease recurrence and/or treatment response. In brief, we separated whole proteome tumor antigens from a cancer cell line into distinct fractions using a 2-D liquid chromatography fractionation strategy where the 1st dimension is separation by isoelectric points and the 2nd dimension is separation by hydrophobicity. Following the 2nd dimension, the fractions were arrayed onto nitrocellulose coated microscope slides. The slides were then incubated with Cy3/Cy5 fluorescent dye-labeled serum antibody isolated from cases and controls, respectively. The informative spots on the arrays and their corresponding fractions containing the associated antigens were identified.
To address why some cases have low serum CA-125, we determined whether immune complexes may exist in some cancer patients (i.e., anti-CA-125 antibodies may form complexes with the CA-125 antigen and mask its detection). To test this possibility, we developed an antigen capture approach to measure CA-125 antigen and the corresponding immune complexes in biofluids. The platform utilized mouse monoclonal antibodies to human CA-125 arrayed onto nano-particle slides to capture the CA-125 antigen and associated immune complexes. After washing, the slides were followed with Cy5-tagged goat anti-human antibodies, and subsequently scanned for fluorescence intensities using ScanArray.
Results: For autoantibody signatures, we identified specific autoantibody reactivity in the cancer cases when compared with normal healthy controls in 8 of 8 ovarian cancer cases with serum CA-125 of ≤ 35 U/ml. Autoantibodies from ovarian cancer patients were labeled with Cy3 dyes and autoantibodies from control samples were labeled with Cy5 dyes. Consistent Cy3 spots representing autoantibody reactivity from ovarian cancer samples were seen on the antigen microarrays. There were 34 Cy3 reactive fractions and only 2 Cy5 reactive fractions following analysis. For immune complexes, we identified 7 of 10 ovarian cancer cases that have immune complexes to CA-125. When we compared the fluorescent intensity of the immune complexes, there was approximately a 100% increase in Cy5-tagged goat anti-human antibodies levels for cases with low serum CA-125 level when compared to the cases with a high serum CA-125 level.
Conclusions: Our results demonstrate the feasibility of using our whole proteome native antigen fractionation microarray as a platform to potentially identify biologically relevant signatures, even in ovarian cancer cases that have low serum CA-125. In addition, our preliminary results also suggest that some women who present with ovarian cancer and low serum CA-125 levels may actually have CA-125 which is hidden from conventional assays due to the presence of immune complexes.

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Performance evaluation of the Access Hybritech® p2PSA† immunoassay.

L. J. Sokoll[1], D. W. Chan[1], G. G. Klee[2], W. L. Roberts[3], R. H. N. van Schaik[4], D. A. Arockiasamy[5], D. L. Broyles[6], C. M. Carlson[5], I. A. Mizrahi[6], T. B. Pierson[5], J. E. Tam[5]. *[1]Johns Hopkins Medical Institutions, Baltimore, MD, [2]Mayo Clinic, Rochester, MN, [3]ARUP Laboratories, Salt Lake, UT, [4]Erasmus University Medical Center, Rotterdam, Netherlands, [5]Beckman Coulter Inc, Chaska, MN, [6]Beckman Coulter Inc, Carlsbad, CA,*

Introduction: Prostate Specific Antigen (PSA) assays are widely used to aid in the detection of prostate cancer. Currently, total PSA and free PSA assays are available to the clinician for this purpose. Total PSA assays measure both complexed and non-complexed forms of PSA while free PSA assays measure only non-complexed PSA. Free PSA is a mixture of PSA isoforms including immature PSA (proPSA) and nicked forms of PSA (BPSA). The proPSA isoforms have retained portions of the leader sequence (e.g. [-7], [-4], and [-2]proPSA) and their presence in male sera may have utility in the detection of prostate cancer.
Objective: We describe the development of a **chemiluminscent** immunoassay to measure serum levels of the PSA isoform [-2]proPSA. This immunoassay was adapted to run on the Beckman Coulter Access Immunoassay Systems and this study characterized the assay's analytical performance.
Methods: Access Hybritech p2PSA assay is a sandwich immunoassay using an anti-[-2]proPSA monoclonal antibody attached to paramagnetic beads, and an anti-PSA monoclonal antibody conjugated to alkaline phosphatase. The assay was calibrated using recombinant [-2]proPSA. The purity of the [-2]proPSA was established by N-terminal amino acid sequencing, and amino acid composition. Molecular mass of the purified [-2]proPSA was determined using MALDI-mass spectrometry, and the assay was calibrated based on this determination. This assay uses only 50 uL of serum and results are obtained within 20 minutes. Sensitivity and imprecision were determined as outlined in the CLSI guidance documents EP17-A and EP5-A2, respectively. Imprecision testing was performed at multiple test sites.
Results: Access Hybritech p2PSA has a dynamic range of 0.5 to 5,000 pg/mL. The LOB is 0.50 pg/mL, the LOD is 0.69 pg/mL, and the LOQ is 3.23 pg/mL (20% CV). The assay shows no hook effect up to 15,000 pg/mL [-2]proPSA. The total CV of the assay is <7% for [-2]proPSA concentrations between 20 and 1000 pg/mL. The assay is robust with < 5% difference between lots of calibrators and reagents. There is no detectable interference from normal serum components or commonly prescribed drugs. Access Hybritech p2PSA requires the same sample type (serum) and handling conditions as described for the Access Hybritech PSA and Access Hybritech free PSA assays, and the same patient specimen can be used for all three assays.
Conclusion: Access Hybritech p2PSA assay is a sensitive immunoassay for measuring the levels of [-2]proPSA in male serum.
†In development for US Market, pending FDA approval
*Not intended as off-label promotion of any Beckman Coulter product

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Validation of a Laboratory Developed Kinetic (kPCR) Assay for Detection of Activating *KRAS* Mutations in the Siemens Clinical Laboratory

D. Maghakian[1], J. Turczyn[1], S. Cruz[1], P. Joseph[1], M. Albalos[2], A. Ying[2], L. Dolores[2], J. Detmer[2], A. Uzgiris[2]. *[1]Siemens Clinical Laboratory, Siemens Healthcare Diagnostics Inc., Berkeley, CA, [2]Siemens Healthcare Diagnostics Inc., Berkeley, CA,*

Background: Colorectal cancer (CRC) mortality approaches 33% in the developing world. In the United States, CRC accounts for almost 9% of cancer-related mortality, with only 39% early stage diagnosis (localized tumor) and markedly decreased survival when distant metastasis has occurred. The *KRAS* oncogene (gene ID 3845) encodes an intracellular GTPase binding protein of the epidermal growth factor (EGF) signal transduction pathway. Specific mutations result in constitutive activation promoting cell growth, differentiation, and survival. Monoclonal antibodies (mAb) cetuximab (mouse anti-human chimeric IgG1), and panitumumab (human IgG2) have therapeutic utility to increase progression-free survival and decrease tumor burden in CRC patients lacking *KRAS* activating mutations. Although prognostic value of *KRAS* genotyping is not determined and additional biomarkers are under investigation, *KRAS* genotyping provides ≥99% negative predictive value (NPV), allowing evidence-based benefit stratification. In this study, the Siemens Clinical Laboratory's *KRAS* genotyping by kinetic PCR (kPCR) is described as a companion diagnostic to assist future drug development and CRC patient therapeutic management.

Objective: To validate a kPCR method in the clinical laboratory for the detection of specific *KRAS* activating mutations in a background of wild-type DNA.

Methods: Twenty nanograms extracted genomic DNA were amplified using eight individual kPCR reaction mixes on a single 96-well plate to detect seven clinically relevant *KRAS* mutations. One homozygous wild-type (GGT GGC) reaction was prepared per sample as an internal amplification control. A 1% mutant or homozygous wild-type (cell-to-cell) control was included on each run. Detection utilized the VERSANT® kPCR Molecular System (Siemens, Deerfield, IL, US) and TaqMan chemistry (Life Technologies, Carlsbad, CA, US). Run and individual reaction acceptance criteria and quality management practices were implemented specific to CLIA-certified or CAP-accredited clinical laboratory guidelines. Two comparator methods were used in the validation: a laboratory-developed *KRAS* OpenGene® DNA sequencing assay (Siemens Clinical Laboratory) and the DxS K-RAS Mutation Kit RUO (DxS Ltd, Manchester, UK). All validation runs were conducted in the Siemens Clinical Laboratory.

Results: Analytical sensitivity and specificity (mutant/normal cell ratio) was 100% at 1 in 100, with sensitivity decreasing to 91.7% at 1 in 250 using DNA extracted from human A549 cells (n = 72). Total analytical agreement to the reference method using a cohort of paired tumor and normal FFPE extracts (n = 58) was 94.8%, exceeding the performance of both sequence analysis and the DxS kPCR method (93.0% and 93.1%, respectively). Although three wild-type FFPE samples were incorrectly classified as "mutant," thirteen known "mutant" FFPE samples were correctly classified by this assay, corresponding to 93.3% (05% CI: [0.817, 0.986]) diagnostic specificity and 100% diagnostic sensitivity (95% Clopper-Pearson CI: [0.753, 1]).

Conclusion: The negative predictive value of *KRAS* mutation analysis in CRC patients has been established in peer-reviewed literature. Accordingly, the American Society of Clinical Oncology (ASCO) recommends *KRAS* genotyping be completed in an accredited clinical laboratory prior to anti-EGFR-targeted therapy for CRC patients. The *KRAS* kPCR assay provides a qualitative, cost-effective solution.

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Alpha-methylacyl-CoA racemase as a new marker of prostate cancer

R. Prusa[1], M. Masarik[2], J. Gumulec[2], S. Kuchtickova[2], V. Kudlackova[2], V. Tanhauserova[2], A. Rovny[3], R. Hrabec[3], V. Adam[4], R. Kizek[5]. *[1]2nd Faculty of Medicine and Faculty Hospital Motol, Prague 5, Czech Republic, [2]Faculty of Medicine, Masaryk University, Brno, Czech Republic, [3]Dept of Urology, St. Anne´s Faculty Hospital, Brno, Czech Republic, [4]Faculty of Agronomy, Mendel University, Brno, Czech Republic, [5]Faculty of Agronomy, Mendel University, Prague 5, Czech Republic,*

Background. Prostate cancer is one of the most common forms of cancer among men. Currently, prostate cancer is screened using serum prostatic specific antigen (PSA) value and digital rectal examination. Gene expression profiling of prostate cancers has lead to the discovery of many novel genes associated with cancers. Alpha-methylacyl-CoA racemase (AMACR) is one such gene which is highly expressed in prostate carcinoma cells compared with normal prostate epithelial cells. AMACR is a peroxisomal and mitochondrial enzyme involved in the β-oxidation of branched fatty acids. It was recently reported that AMACR has very high sensitivity and specificity to prostate adenocarcinoma. Therefore it is very important to find new approaches in AMACR detection. The aim of this study was to detect AMACR in tumour prostate cell lines by SDS-PAGE, capillary chip electrophoresis and electrochemical method.

Methods. *Biological samples.* Prostatic cell lines derived from prostate adenocarcinoma (LNCaP-FGC, PC-3, and 22RVL) and prostatic cell line derived from normal epithelium (PNT1A - human immortalized prostatic cell line) were used. *Protein detection.* SDS-PAGE and Western-blot with subsequent immunodetection were used. *Chip capillary electrophoresis:* Experion system from Bio-Rad was used with chemicals supplied by manufacturer according to the user's manual. *Immunohistochemical detection:* Immunohistochemical detection of AMACR was performed by R.T.U Vectastain universal ABC kit (Vector Laboratories, Burlingame, CA, USA).

Results. Firstly we detected AMACR in lysates of tumour and non-tumour cell lines by SDS PAGE and western blotting with subsequent immunodetection with polyclonal rabbit antibody. The results show relatively big differences between tumour and non-tumour cell lines in AMACR content. In case of tumour cells, the expression of AMACR was up-regulated in comparison with non tumour ones. Then we cultivated cells on glass slides for immunodetection of AMACR *in situ* and we obtained similar results. We also investigated the influence of zinc ions on AMACR expression in tumour or non-tumour prostatic cell lines. We added 20 μM of zinc ions to the cultivation medium and after 5 days we demonstrated up regulation of AMACR expression in prostate tumour cells (LNCaP, PC-3, 22RVL) but no changes in PNT1A cells. Further measurements were carried out with electrochemical instruments and Experion capillary chip electrophoresis. Using both systems we were able to detect AMACR in subnanomolar concentrations.

Conclusion. We proposed new methods for detection and determination of AMACR in cell lines exctract. Our results can be used for further casting light on AMACR role in prostate cancer. AMACR can be considered as a tumour marker with almost 100 % specificity and sensitivity to prostate carcinoma but simple and fast methods for its detection is still lacking.

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Cathepsin B in the diagnosis of transitional cell cancer of the bladder

P. Dusek, R. Prusa, K. Kotaska, S. Vesely, M. Bunesova, M. Babjuk. *2nd Faculty of Medicine and Faculty Hospital Motol, Prague 5, Czech Republic,*

Background: Cathepsin B is lysosomal enzyme that belongs to the group of cystein proteases. Cathepsins play an important role in the process of protein degradation. Activity of cathepsin B was investigated especially in cancer of liver, colorectal cancer, lungs cancer and breast cancer. In transitional cell carcinoma (TCC) only limited data have been published.

Methods: Using immunoassay technique (ELISA) we measured urine and serum concentration of cathepsin B in 40 patients with TCC of the bladder (29 males and 11 women). Average of age was 68.1 years (46 - 91). Superficial tumor and invasive tumor was diagnosed in 23 and 17 patients, respectively. Low grade cancer was found in 18 patients and high grade in 22 patients. We measured concentration of cathepsin B in urine and serum in a control group of healthy persons (n = 25, the average age 52,4 years, 17 women, 8 males). We compared results in both groups and in subgroups of patients by grading of malignity and staging of tumor. We evaluated results by Mann-Whitney statistic test.

Results: Median of concentrations of cathepsin B in serum in bladder cancer group was 54.2 ng/ml (range 22.6-184.66) and in control group was 45.74 ng/ml (range 4.3-102.38). Concentrations of cathepsin B in serum of patients with bladder cancer were significantly higher than in control group (p=0.0451). Median of concentrations of cathepsin B in urine in bladder cancer group was 3.868 ng/ml (0.032-33.07) and in control group was 0.874 ng/ml (0-35.428). Concentrations of cathepsin B in urine of patients with bladder cancer were significantly higher than in control group (p=0.0006). Median of concentrations of cathepsin B in serum in subgroup with low grading of malignity was 49.3 ng/ml (range 22.6-136.04) and subgroup with high grading of malignity was 69.91 ng/ml (range 23.58-184.66). Concentrations of cathepsin B in serum of patients with low high grading of malignity of bladder cancer were significantly higher than in patients with low grading malignity of cancer (p=0.0167). Median of concentrations of cathepsin B in urine in subgroup with low grading of malignity was 2.957 ng/ml (range 0.144-20.00) and subgroup with high grading of malignity was 5.274 ng/ml (range 0.032-33.07). Differences of concentrations of cathepsin B in urine in subgroups by grading of malignity were not significant (p=0,1763). Median of concentrations of cathepsin B in serum in subgroup with superficial bladder cancer was 50.7 ng/ml (range 23.48-184.66) and subgroup with invasive cancer was 65.12 ng/ml (range 22.6-145.4). Difference of concentrations of cathepsin B in serum of patients with superficial and invasive bladder

cancer was not significant (p=0.1756). Median of concentrations of cathepsin B in urine in subgroup with superficial bladder cancer was 1.98 ng/ml (range 0.032-9.896) and subgroup with invasive cancer was 6.235 ng/ml (range 1.534-33.07). Concentrations of cathepsin B in urine of patients with invasive bladder cancer were significantly higher than in patients with superficial cancer (p=0.0001).
Conclusion: These results suggest that elevated levels of cathepsin B in serum and urine in patients with bladder cancer offer valuable diagnostic and prognostic information.

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The effect of zinc ions on metallothionein and specific prostate antigen content in prostatic cancer cell lines

R. Prusa[1], M. Masarik[2], J. Gumulec[2], S. Kuchtickova[2], V. Kudlackova[2], V. Tanhauserova[2], A. Rovny[3], R. Hrabec[3], S. Krizkova[4], V. Adam[4], T. Eckschlager[1], R. Kizek[4]. *[1]2nd Faculty of Medicine and Faculty Hospital Motol, Prague 5, Czech Republic, [2]Faculty of Medicine, Masaryk University, Brno, Czech Republic, [3]Dept of Urology, St. Anne´s Faculty Hospital, Brno, Czech Republic, [4]Faculty of Agronomy, Mendel University, Brno, Czech Republic,*

Introduction. Metallothioneins (MT) belong to the group of intracellular, low-molecular mass cysteine-rich proteins. Due to their high affinity to heavy metal ions (Zn, Cd, As, etc.), their main function in a cell is maintaining homeostasis of essential heavy metals and detoxification of toxic ones. The role of MT in cancerogenesis remains still unclear, but MT can be considered as a new promising tumour disease marker.
Objective. The main aim of this paper is to study MT in tumour prostate cell lines. Particularly, we aimed at the comparison the differences in MT expression between cells treated with zinc ions and control ones.
Materials and methods. *Biological samples.* Prostatic cell lines derived from prostate adenocarcinoma (LNCaP-FGC, PC-3, and 22RVL) and prostatic cell line derived from normal epithelium (PNT1A - human immortalized prostatic cell line) were used. Prostatic cells were grown in 75 cm^2 flasks up to 80% confluence of the culture in either RPMI 1640 (PNT1A, LNCaP, 22RVL) or Ham´s F12 (PC-3) modified medium with added foetal bovine serum to 7 - 10% and/or zinc ions up to 20 µmol/L concentration. The cells were then treated with RIPA buffer or thermally denaturated extract for cell. *Protein detection.* SDS-PAGE and Western-blot with subsequent immunodetection were used.
Results. We investigated the influence of zinc ions on MT expression in prostatic cell lines derived from prostate carcinoma. For MT determination we used SDS-PAGE and western blot with subsequent immunodetection. Moreover we used modern electrochemical methods (Brdicka reaction) for detection of MT. We demonstrated up-regulation of prostatic specific antigen expression in prostate tumour cells (LNCaP, PC-3, 22RVL) and down-regulation of MT expression. This fact can be explain by decreased concentration of zinc ions in prostatic tumour cells and therefore lower concentration of MT in tumour cell lines in comparing with non-tumour cells. Besides immunodetection we measured MT by adsorptive transfer stripping technique coupled with differential pulsed voltammetry Brdicka reaction and we obtained similar results.
Conclusions. This study provides new insight on expresion of MT in prostate tumour cell lines. Evidently MT expression is significantly down regulated in 22RVL, and PC-3 cells due to the significant lower zinc concentration. We observed significant (α=0.05) difference in MT content between cell lines treated/nontreated with zinc ions by using immunohistochemical methods and electrochemical methods as well.
Acknowledgements. *Financial support from GACR 301/09/P436, IGA MH 10200-3 and GA AV IAA401990701 is highly acknowledged.*
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D-142

Clinical Evaluation Of Tumoral Markers Determination In Serum And Serous Effusions

A. García Claver, J. Carretero Gómez, L. Rodelgo Jiménez, R. Ramos Corral, C. Tapia-Ruano Díaz-Quetcuti, M. Gómez-Serranillos Reus. *Hospital Virgen De La Salud, Toledo, Spain,*

Background: Approximately 10 to 30 % of serous effusions are of neoplastic etiology. Cytology is, nowadays, the standard method for the diagnosis of malignant effusion, having a high specificity with a sensitivity around 50%. Biochemical analysis of serous effusions are usually carried out to evaluate the etiology and provide a result in a short period of time.
Objectives: In this study, we evaluate the clinical utility of measuring three tumoral markers (CA15-3, CA125 and CEA) simultaneously in serum and fluid.
Material and methods: A total of 93 serous effusions (57 ascites and 36 pleural effusions) and their respective serum samples were analyzed by an electrochemoluminiscence immunoassay on a Modular E170 analyzer (Roche Diagnostics). Benign effusions (54) were mainly due to hepatic disease or pneumonia, while malign effusions (39) were due to lung or hepatic cancer in most cases. Statistical analysis was made using SPSS 15.0 and MedCalc 9 for Windows.
Results: Tumoral marker levels were higher in fluid and serum of neoplastic etiology, finding levels of CA15-3 and CEA in fluid and their ratio fluid-serum statistically significant (P<0.05), using the Mann-Whitney U test. Receiver operator characteristic (ROC) curves were used to determine the best cut-off for the diagnosis. For CA 15-3 ROC curve showed a cut-off of 10.17 UI/ml, with a sensitivity of 53.8% and a specificity of 87% (AUC=0.735 (0.633-0.821) 95% CI, P=0.0001). The cut-off for CEA was 1.07 ng/ml, with a sensitivity of 56.4% and a specificity of 90.7% (AUC=0.735 (0.633-0.821), 95% CI, P=0.0001). Therefore, we reclassified the samples as malign when CEA and/or CA15.3 were higher than the cut-off value obtained and made a ROC curve, having a better AUC (0.748 (0.647-0.832), 95% CI, P=0.0001) with a sensitivity of 71.8% and a specificity of 77.8%. Besides, negative predictive value improved attending both tumoral markers, 79.2%, being 72.3% for CA15.3 and 74.2% for CEA, individually.
Conclusions: The simultaneous determination of CEA and CA15.3 in serous effusions is a useful tool in the differential diagnosis. It allows us to rule out a malignant etiology of the effusion in nearly 80% of the samples with levels below the proposed cut-off, in a shorter period of time and with higher sensitivity than cytology.

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GP73 (GOLPH 2) is a fine serum marker for liver disease progression by quantitative analysis

L. Tian[1], Y. Wang[1], C. Lin[2], Y. Tian[1]. *[1]Chinese PLA general hospital, Beijing, China, [2]Hotgen Biotech Co., Ltd, Beijing, China,*

Objective: Golgi protein-73 (GP73) is found to be up-regulated in the impaired liver tissue; however, serum GP73 is detected to be a new marker for hepatocellular carcinoma (HCC) by semiquantitive western bloting. The aims of this study were to quantify expression of serum GP73 in the progression of liver diseases, and to determine the relationship between serum GP73 and cancer progression and liver injury and identify its clinical application.
Methods: Serum GP73 was detected by quantitative double-antigen sandwich ELISA method.
Results: A total of 279 patients were studied, 85 cases were HCC, 39 cases liver cirrhosis (LC), 46 cases hepatitis and 109 cases normal control. Serum GP73 (sGP73) levels were significantly higher in patients with LC compared to those with HCC, hepatitis and control group (P<0.000). sGP73 had a sensitivity of 76.92% and a specificity of 78.82% at the optimal cutoff point of 189.6 µg/L with an area under the receiver operating curve (AUROC) of 0.801 vs. 0.782 for AFP (P=0.525), sGP73 had significantly higher sensitivity and specificity for differentiating HCC from LC; sGP73 levels were elevated in the serum of 16.2% (6/37) of HCC individuals who had serum AFP levels less than 20 µg/L and 25% (12/48) of HCC individuals with AFP more than 20 µg/L; sGP73 was positively correlated with total bilirubin (TBIL) (r=0.257, P=0.018), AST (r=0.25, P=0.021), and negatively correlated with ALB (r=-0.43, P<0.000), A/G (r=-0.32, P=0.003) which can roughly estimate the extent of liver damage.
Conclusions: Higher level of GP73 could be found in the serum of patients with hepatic injury. This hinted that serum GP73 may be a good marker for differentiating HCC and liver diseases. Further validation studies are needed to confirm the role of serum GP73 in the liver diseases.
Table 1 Characteristics of 279 subjects

Variables	HCC (n=85)	LC (n=39)	Hepatitis (n=46)	Control (n=109)
Age	56.19±10.80	52.63±10.51	36.98±11.89	58.34±11.44
Gender(M/F)	72/13	26/13	33/13	70/39
Etiology% (HBV/ HCV/Other)	71/5/9	30/3/6	46/1[c]/0	N
ALT (U/L)	35.80 (10.70-208.70)	29.25 (9.20-502.30)	41.55 (7.60-480.10)	19.40 (9.90-82.00)
AST (U/L)	36.30 (12.20-138.40)	35.60 (15.20-284.20)	30.70 (14.00-300.80)	19.70 (10.80-65.60)
Albumin (g/L)	39.50 (21.70-48.80)	35.90 (21.40-48.50)	35.64±6.64	43.11±4.36
Albumin/Globulin (A/G)	1.12±0.45	1.11±0.30	1.40±0.24	1.51±0.21
Total Bilirubin (umol/L)	15.05 (5.40-87.40)	22.80 (8.00-390.0)	16.39 (7.10-56.80)	14.95±4.56
AFP (ug/L)	40.25 (1.12-24200.00)	3.275 (0. 79-430.00)	3.32 (0.91-1000)	2.52 (0.75-11.25)
<20/20-100/>100	37/11/37	33/3/0	45/0/1	109/0/0

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Sensitive Quantification of Vimentin Methylation with a Novel Methylation Specific qInvader Technology

H. Zou[1], H. Allawi[2], X. Cao[1], M. Domanico[1], J. Harrington[1], W. Taylor[3], T. Yab[3], D. Ahlquist[3], G. Lidgard[1]. *[1]Exact Sciences Corporation, Madison, WI, [2]King Hussein Institute for Biotechnology & Cancer, Amman, Jordan, [3]Mayo Clinic, Rochester, MN,*

Background: Colorectal cancer is the second most common cause of cancer related deaths in the United States, yet it is potentially the most treatable and preventable cancer with effective screening. Stool DNA testing offers a noninvasive, accurate, affordable, and user-friendly approach to achieve effective detection of both colorectal cancer and precancerous adenomas. Exact Sciences is working on a next generation stool DNA screening test and has developed a multiplexed quantitative Invader® assay for the simultaneous detection of methylated and unmethylated sequences in the promoter region of the vimentin gene, a known colorectal cancer marker. We herein report the performance of this DNA assay on colorectal tissues from cancers, pre-malignant adenomas, and normal epithelia.
Objective: To develop a qInvader approach for sensitive detection of methylated markers based on real-time fluorescence measurement, and to evaluate its performance in detection of methylated vimentin in colorectal neoplasms.
Methodology: qInvader combines PCR with Invader chemistry and detects Invader signal during each amplification cycle in a similar fashion to real-time PCR. A two dye multiplexed qInvader assay was designed to detect both methylated and unmethylated vimentin. The dynamic range of this assay for quantifying methylated and unmethylated vimentin was determined using 10-fold serially diluted plasmids. Analytical sensitivity was measured by detecting methylated vimentin spiked in unmethylated vimentin and vice versa at 10, 1.0, 0.1, and 0.01% ratios. In addition, detection of vimentin methylation in tissues from 22 colorectal cancers, 10 pre-malignant advanced adenomas (i.e. size >/= 1cm), and 12 normal colorectal epithelia was also performed, with all patients and normal controls age and gender matched. Methylation level was defined as the ratio of methylated vimentin copies to unmethylated copies multiplied by 10,000.
Results: qInvader linearly detected methylated or unmethylated vimentin down to <10 copies. No cross reactivity was observed when methylated and unmethylated assays were used to amplify 10^5 copies of unmethylated and methylated genes, respectively. The multiplex assay detected methylated vimentin spiked in unmethylated vimentin at 0.01% ratio and vice versa. Median methylation level was 76 (range; 0~1,089,202) in cancers, 4766 (range; 0~7,329,650) in adenomas, and 0 (range; 0~0.1) in normal epithelia. Vimentin methylation level was significantly higher in cancer and adenoma than in normal epithelia ($p<0.01$). At a specificity cutoff of 100%, methylated vimentin was detected in 68% (15/22) of cancers and in 90% (9/10) adenomas. Notably, methylated vimentin was not detected in 11 of 12 normals.
Conclusions: Methylation specific qInvader is a promising approach for quantifying methylated DNA markers. The vimentin qInvader assay discriminated between colorectal neoplasia and normal samples at high sensitivity and specificity.

D-145

Application of Quantitative Multiplex Immunoassays in Solid Tissue Diagnosis

F. G. Strathmann, B. R. Huber, G. S. Baird. *University of Washington, Seattle, WA,*

Background: Hormone-secreting pituitary adenomas are subclassified by either pre-operative quantitative serum hormone testing or post-operative qualitative immunohistochemistry (IHC) of tumor tissue. This classification is made based on the identity of the secreted hormone(s) and is essential in predicting prognosis and guiding therapy. In this study, pituitary hormones were quantitated in formalin-fixed, paraffin-embedded (FFPE) adenoma tissue extracts to test the hypothesis that the results of a quantitative immunoassay performed on tissue homogenates correlate with standard pathologic IHC diagnosis.
Methods: Pituitary adenoma samples were collected from FFPE pathology archives with standard diagnostic immunohistochemistry performed in parallel. Tissue proteins were extracted from 3 x 10 µm sections using a commercial protein extraction kit (Qiagen qProteome). Hormones were quantitated with the Millipore HPT-66K Human Pituitary Kit on a Luminex 200 instrument. Total pituitary hormone concentrations were normalized to total extract protein concentrations.
Results: IHC classification and extract hormone concentrations were 100% concordant. Hormone concentrations were at least 4- to 322-fold higher in the appropriate tumor subclass (i.e. ACTH in IHC-classified ACTH-expressing adenoma) than in any other subclass. IHC-classified null adenomas had significantly lower total hormone content than hormone-expressing adenomas (P=0.02). Some IHC-classified null adenomas had biologically plausible quantitative hormone results (detectable Follicle Stimulating Hormone and Luteinizing Hormone, but no other detectable hormone), which indicated that IHC may be too insensitive to detect physiologically meaningful hormone expression.
Conclusions: These results provide promising data for the application of bead-based immunoassays in the classification of human solid tissue neoplasms as adjuncts, or even possibly replacements for morphologic examination. The entire assay, from pre-analytical specimen preparation to multiplex immunoassay analysis, is amenable to automation, indicating that some currently manual anatomic pathology diagnostic practices could be performed in the automated clinical laboratory.

D-146

Serum concentrations of human epididymis protein 4 (HE4) in women with ovarian cancer

Y. M. Kim[1], D. H. Whang[2], J. Park[3], H. A. Park[2], S. W. Lee[1], S. H. Kim[1]. *[1]Asan Medical Center University of Ulsan College of Medicine, Seoul, Korea, Republic of, [2]Inje University College of Medicine, Seoul Paik Hospital, Seoul, Korea, Republic of, [3]Abbott Diagnostics, Seoul, Korea, Republic of,*

Background: A reliable noninvasive means of discriminating between ovarian cancer and a benign mass is important because about 20% of women will develop a pelvic mass at some point in their lives. In that sense, measuring serum human epididymis protein 4 (HE4) levels may have a potential to be a valuable diagnostic tool for ovarian cancer. It was determined that HE4 may be superior to cancer antigen 125 (CA 125) for distinguishing ovarian cancer because of increased specificity of the HE4 for ovarian cancer versus nonmalignant conditions. This study evaluated the diagnostic performance of HE4 in a clinical setting and the possible synergy between HE4 and CA 125 for diagnosis of ovarian cancer.
Methods: During a 4-month period, 122 healthy premenopausal women, 102 healthy postmenopausal women, 73 women with a benign ovarian mass (64 premenopausal and 9 postmenopausal), and 61 women diagnosed ovarian cancer (26 premenopausal and 35 postmenopausal) were enrolled in a consecutive manner. Menopausal status was determined based either on the serum FSH level or the patient's history. All of the diseased cases were diagnosed histologically using surgical specimens. Serum samples were obtained from each individual at the time of their initial presentation at the hospital and were stored frozen until they were tested. Serum HE4 and CA 125 were measured with the Abbott ARCHITECT i2000sr instrument.
Results: The healthy postmenopausal individuals showed higher HE4 levels (95% CI: 36.6~40.0 pmol/L) than the healthy premenopausal individuals (95% CI: 33.3~36.5 pmol/L). Both the premenopausal group and postmenopausal group showed no difference of HE4 concentrations between the healthy individuals and the patients with a benign ovarian mass. In contrast, CA 125 showed significantly elevated levels in the premenopausal women with a benign ovarian mass compared to the healthy women (mean±SD: 52.5±65.8 U/mL vs. 15.5±6.9 U/mL). The diagnostic sensitivity for ovarian cancer in the premenopausal women was equal (65.0%) between HE4 and CA 125 at the concentrations of 95% specificity. However, combining HE4 and CA 125 was shown to enhance the diagnostic sensitivity in the premenopausal women to 76.9% at the same level of the specificity. Elevation of HE4 tended to be confined to serous-type malignancies but there was no correlation between the HE4 level and the FIGO stage. HE4 and CA 125 showed comparable AUCs with the ROC curve analysis at diagnosing ovarian cancer among the postmenopausal women, and the synergy between the 2 markers was not apparent in this group.
Conclusion: Serum HE4 was found to be a sensitive and specific marker for diagnosing ovarian cancer, especially in premenopausal women. In this study, adding HE4 to CA 125 yielded a higher diagnostic accuracy in premenopausal women compared to using either marker alone. In postmenopausal women, HE4 and CA 125 exhibited comparable diagnostic accuracy for ovarian cancer.

D-147

A new GenoArray DNA test for cervical cancer screening and clinical management

Y. Tong, Y. Li, J. Gu, F. Li. *Renmin Hospital of Wuhan University, Wuhan, China,*

Objective Flow-through hybridization, a simple rapid method for carcinogenic human papillomavirus (HPV) genotyping (*Hybrimax*; Hybribio Limited Co.) has been developed to detect 15 high-risk types(16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) and 6 low-risk types(6, 11, 42, 43, 44, CP8304) of in HPV about 3 h, to screen women in developing regions for cervical intraepithelial neoplasia (CIN). We did a cross-sectional study to assess the clinical accuracy of *Hybrimax* as a rapid screening test in the Wuhan city.
Methods 790 cervical swab samples were collected consecutively from women aged 26 to 58 years in Department of Gynaecology and Obstetrics in Renmin Hospital of Wuhan

University from February 15 to October 30, 2008. 63 women had CIN2+, and 31 had CIN3+. Use the CIN2+ as the reference standard and area-under-the-curve analysis with a two-sided alpha error level. All women were assessed by visual inspection with acetic acid (VIA), liquid based cytology, HPV Hybrid Capture 2 DNA Test (HC2), and colposcopy with directed biopsy and endocervical curettage as necessary.
Results Complete data were available for 742 (93.92%) women. HPV DNA was detected in 304(40.97%) samples by Hybrimax. 231(75.99%) women were infected with single-type HPV and 73(24.01%) women were infected with multiple-type HPV. The sensitivity and specificity of Hybrimax for cervical specimens were 93.61% and 97.25%, respectively ,for vaginal specimens were 86.25% and 89.42%, respectively, compared with 48.9% and 89.4% for VIA. The ROC for VIA were significantly different for cervical and vaginal specimen comparisons for the Hybrimax(p=0.0003 and p=0·0047, respectively).The sensitivity and specificity of HC2 for cervical specimens were 93.5% and 79.8%, respectively, The correlation between the Hybrimax and HC2 was good (Kappa index=0.79).
Conclusion The Hybrimax is a rapid simple system for HPV genotyping as a primary screening method for cervical cancer prevention.

D-150

Hepatocellular Carcinoma Recurrence Confirmation after Hepatectomy - Evaluation of AFP, Sensitive AFP-L3% on μTAS Wako i30-
H. Hanada[1], K. Takeoka[1], I. Maeda[1], Y. Hidaka[2], H. Nagano[3], Y. Iwtani[4]. *[1]Division of Laboratory for Clinical Investigation, Department of Medical Technology, Osaka University Hospital, Suita, Japan, [2]Laboratory for Clinical Investigation, Osaka University Hospital, Suita, Japan, [3]Department of Surgery, Osaka University Graduate School of Medicine, Suita, Japan, [4]Department of Biomedical Informatics, Division of Health Science, Osaka University Graduate School of Medicine, Suita, Japan,*

Background: AFP (α-Fetoprotein) is a glycoprotein used as a tumor marker for hepatocellular carcinoma (HCC), but elevation of AFP is sometimes observed even in a case of hepatitis or liver cirrhosis. This non-specific elevation makes it difficult to differentiate HCC from benign liver diseases by the marker. Moreover, recent improvement of imaging has contributed to detection of HCC at smaller tumor sizes where elevation of AFP is not found. AFP-L3 is a fucosylated isoform of AFP and is produced specifically in HCC and is not dependent on the size of tumor. Therefore, AFP-L3%, the fraction of AFP-L3 in total AFP, provides useful information for diagnosis and prognosis of HCC patients. PIVKA-II, or DCP (des-γ-carboxy prothrombin), is reported to be specific to HCC and is complementary to AFP or AFP-L3%. Therefore combination assays for these markers are recommended and widely used for diagnosis and prognosis of HCC patients in Japan. Objective and Result: A fully automated immunoanalyzer, μTASWako i30 (Wako Pure Chemical Industries, Osaka, Japan), has been launched by employing newly developed microfluidics technology, called the LBA-EATA method[1),2)] (Liquid-phase Binding Assay-Electrokinetic Analyte Transport Assay method). Application of the new technology enables the determination of AFP and AFP-L3% with higher sensitivity (LoD of 0.2 ng/mL of AFP-L3) and good accuracy (CV=0.1- 2.9% for AFP-L3%). We evaluated the system for detection of the tumor markers in HCC patients (n=52) treated with hepatectomy. DCP was measured by using our routine chemiluminescent immunoassay method in Osaka University Hospital. Among these patients, recurrence of HCC was defined in 27 cases by using imaging. Elevation of AFP (cut off = 20 ng/mL), AFP-L3% (cut-off = 10 %) and DCP (cut off = 40 mAU/mL) were observed in 16 (59.3 %), 18 (66.7 %) and 17 (62.9 %) cases, respectively before recurrence was identified by imaging. The specificity of the assays for detecting HCC recurrence were 92.0 %, 100 % and 60 % for AFP, AFP-L3% and DCP, respectively. Combination of these assays resulted in 81.5% sensitivity to detect recurrence (Specificity: 68.0 %).
When cut-off value of 5% for AFP-L3% was used, AFP-L3% alone became positive in 5 cases (18.5 %) before the diagnosis by imaging. AFP levels (cut off 20 ng/mL) were still negative in 3 out of the 5 cases at the time of diagnosis.
Conclusion: The improved assay sensitivity of AFP-L3% on μTASWako i30 and combination assay with DCP will contribute to the early prediction of HCC recurrence and the disease prognosis.
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D-151

hTERT Polymorphisms in patients with liver cirrhosis and primary carcinoma
Y. Guo, J. DONG, H. Jiang, Y. Tian. *Chinese PLA General Hospital, BeiJing, China,*

Background: Primary carcinoma of liver is a kind of common malignant carcinoma. It has been found that hepatitis B or C virus infection is the main cause of primary carcinoma. And etiopathological process might be: hepatitis -cirrhosis-carcinoma. It has been confirmed that activation of telomerase expression is one of cause of tumor. Telomerase reverse transcriptase catalytic subunit (hTERT) is the important component of telomerase, which serves as a template for the addition of telomeric repeats and have an important role in the development of tumor.
Objective: This study was to study the difference of hTERT polymorphisms in patients with liver cirrhosis and primary carcinoma.
Materials and Methods: There were 4 groups in the study: liver primary carcinoma group, liver cirrhosis group, primary carcinoma and metastasis group, Control group. rs2853691, rs2736122, rs10069690, rs2853676, rs2853677, rs2075786, rs2736098, rs2853690, rs6554743, rs2736118, rs2736114 were investigated by using Beckman Coulter SNP stream system.
Result: 1 There were difference in rs2736114, rs2075786, rs2736122, rs10069690 (p<0.01). 2 There were no significant difference in the distribution of rs6554743, rs2736098, rs2853690, rs2736114, rs2075786, rs2853677, rs2853676 among groups.
Conclusion: Some polymorphisms of hTERT in different group indicate the potential effect on tumor pathogenesis of hTERT.rs2736122, rs2075786, rs10069690 differ in groups. Especially rs10069690 [C/C] allele which are 69.5% and 80.0% in healthy and cirrhosis testees , while there are increased ratios of 85.6% and 90.6% in liver primary carcinoma and complications of metastasis.

D-152

Comparison and correlation of CA19-9 and liver function indexes between pancreatitis and pancreatic cancer
X. G. Liu. *Chinese PLA General Hospital, Beijing, China,*

0bjective To study the comparision of CA19-9 and liver function index between acute pancreatitis(AP), chronic pancreatitis(CP), pancreatic cancer and the correlation of CA19-9 and Liver function index in three disease groups.
Methods CA19-9 and liver function indexes was detected in 70 patients with pancreatic cancer, 43 patients with AP, 25 patients with CP and 26 matched healthy volunteers with Electrochemiluminescence immunoassay and Colofimetry of Biochemistry.
Results The results showed that the serum TBIL and ALT level had no statistically difference(*P*>0.05). However, the serum ALB levels were significantly lower (*P*<0.01) and CA19-9,TBA,DBIL,AST,γ-GT,ALP levels were significantly higher (*P*<0.01) in patients with pancreatic cancer , AP, CP than that in control group(*P*<0.01).The CA19-9 and ALB levels were were significantly higher(*P*<0.01)and γ-GT levels were significantly lower(*P*<0.05) in patients with pancreatic cancer than that in patients with AP. Compared with CP, only the CA19-9 level were significantly higher (*P*<0.01) in patients with pancreatic cancer. There was a positive correlation between CA19-9 and γ-GT levels in patients with pancreatic cancer (r=0.339, P=0.004) and patients with CP(r=0.570, P=0.006). In patients with AP, CA19-9 levels had positive correlations with TBA, DBIL, ALT, AST, γ-GT and ALP.
Conclusion The results indicate that it maybe helpful to the diagnosis of AP,CP and pancreatic cancer by detecting CA19-9 and liver function indexes at one time.

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Auxiliary Screening Value of the relative Biomarker in Pancreatitis and Pancreatic cancer
x. dong. *Chinese PLA General Hospital, Beijing, China,*

Objective To investigate the screening value of LIPASE,AMS,GGT,CA199,CA125 and CEA in pancreatitis and pancreatic cancer,we detected the concentration of the six biomarkers.
Method we analyzed the data of 216 patients with pancreatitis and pancreatic cancer(PC) all diagnosed by pathological test retrospectively with SPSS.
Result Compared with the control group,,GGT,CA199,CEA and CA125 had a higher concentration in pancreatic cancer group,especially CA199;Compared with the chronic pancreatitis(CP)group,the concentration of the same four biomarker is higher in pancreatic cancer group(p<0.01),while AMS has a lower concentration than that of pancreatitis group(*p*<0.01).But the difference of LIPASE concentration between the two groups was not significant. Compared with acute pancreatitis(AP)group,CA199 and CEA is higher significantly in pancreatic cancer group(*p*<0.01)while CA125 and GGT had no significant difference between the two groups. The tumor-markers of CP and AP group had been compared with control group. As a result,for CP group,only CA199 is higher significantly than that of control group;for AP group,both CA199 and CA125 are .The sensitivity of CA125 to PC is 37.8%, CEA 38.2%,which has no significance. The sensitivity of CA199 is 69.4%,which is higher significantly than that of CEA and CA125. Secondly,the detective rate of LIPASE to AP and CP is higher than that of AMS(□2=13.493,10.708 repectively, *P*<0.01).
Conclusion Although the positivity rate of CEA to PC is low,it can be used to screen PC from CP; The combined detection of CA199,CA125,CEA and AMS can be used

to distinguish the PC from CP,while CA199,CEA,AMS and LIPASE can be used to distinguish the PC from AP .It can reduce the rate of misdiagnosis; Otherwise the value of LIPASE to AP and CP was significantly higher than that of AMS, and the value of LIPASE and AMS to CP was higher than that to AP.

D-154

Validation and Clinical Performance of CEA and CA19-9 assays in Pleural Effusions

W. Reilly, J. Hackbarth, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN,*

Introduction: Analysis of tumor markers in pleural effusions has been considered as an adjunct to cytology for differentiating between malignant and non-malignant effusions, especially in cases where cytology examination is inconclusive. Carcinoembryonic antigen (CEA) and Carbohydrate Antigen 19-9 (CA19-9) are often ordered in the clinical laboratory for this purpose.
Objective: To validate the analytic performance and diagnostic value of the Beckman-Coulter® Access CEA2 (CEA) and GI -Monitor (CA 19-9) reagent systems in pleural fluid samples.
Methods and materials: All studies were conducted using standard IVD-reagents on the Beckman-Coulter UniCel DxI 800. Two hundred thirteen residual pleural fluid samples that were collected for cytology examination at the Mayo Cytopathology Laboratory were used for validation. Samples were collected by thoracentesis at Mayo Clinic Rochester between December, 2007 and August, 2009. For establishing the assay clinical performance specimens were classified as malignant (n=107) or non-malignant (n=106) effusion based on cytology, pleural biopsy, thoracoscopy, and long-term follow-up of medical records. The Mayo Clinic Institutional Review Board approved this study.
Results: For CEA, intra-(N=20) and inter-(N=20) assay imprecision, expressed as CVs, were <5% at CEA concentrations of 10 and 100 ng/mL. Functional sensitivity was 0.5 ng/mL and the linear measurement-range extends to 990 ng/mL. The average dilution linearity was 96% (88-103%) and the average spike recovery was 104% (88-124%). For CA19-9, intra-(N=20) and inter-(N=20) assay imprecision, expressed as CVs, were <5% at CA19-9 concentrations of 25 and 60 U/L. Functional sensitivity was 5.0 U/L and the linear measurement-range extends to 2000 U/L. The average dilution linearity was 101% (94-104%) and the average spike recovery was 109% (102-111%). Neither assay was affected by bilirubin up to 60 mg/dL or hemoglobin up to 1100 mg/dL. ROC curve analysis was performed to select a cut-off that provided the highest specificity for each analyte. A CEA cutoff of 3.5 ng/mL yielded a sensitivity of 52% and specificity of 96% for identifying effusions caused by any malignancy. The sensitivity improved to 75% when only effusions caused by malignancies known to secrete CEA and/or CA19-9 were considered. A CA19-9 cutoff of 20 U/mL yielded a sensitivity of 37% and specificity of 95% for identifying effusions caused by any malignancy. The sensitivity improved to 52% when only effusions caused by malignancies known to secrete CEA and/or CA19-9 were considered. CEA was superior to CA19-9 in identifying malignant pleural effusions.
Conclusion: We have validated the Beckman-Coulter Access CEA2 (CEA) and GI -Monitor (CA 19-9) for use in pleural effusions. Performance of the assays in pleural fluids is similar to the performance in serum samples. Measurement of CEA and CA19-9 in pleural fluid is useful as a complement to cytology for classifying pleural effusions. Performance of these markers is specifically enhanced in effusions caused by malignancies that show elevations of these markers in serum.

D-155

Evaluation Of Serum Human Telomerase Reverse Transcriptase Messenger RNA As A Novel Tumor Marker For Hepatocellular Carcinoma

A. A. Abdel Hady, M. M. Hassan, F. El Shanawany, A. Anas, E. Mostafa, A. M. Abdel Hadi. *Theodor Bilharz Research Insitute, Giza, Egypt,*

Objectives: Liver cancer is the most common neoplasm and the most common cause of cancer death within the world. Early detection of hepatocellular carcinoma (HCC) will increase the potential for curative treatment and improves survival. Diagnostic accuracy of serum telomerase reverse transcriptase messenger RNA (hTERT mRNA) and other correctional tumor markers such as α-fetoprotein (AFP) prothrombin induced by vitamin K antagonist (PIVKA-II) to select the most reliable diagnostic and prognostic markers in HCC. This study was conducted on 25 healthy controls, 25 cirrhotic and 30 patients with HCC. All patients had been diagnosed with HCV-associated chronic liver disease. Methods: serum PIVIK-II and AFP were measured by enzymatic immunoassay (ELISA) while determination of serum telomerase concentration was performed using real quantitative RT-PCR. Results: mean telomerase, PIVKA-II and AFP were significantly higher in HCC patients as compared to both cirrhotic patients and controls with a significant elevation in cirrhotic patients as compared to controls. Positive correlation was found between serum telomerase and size of hepatic focal lesions. Also, a positive correlation was found between both serum telomerase and PIVIKA-II and between the pathological grades of HCC. In HCC the sensitivity/specificity (88.2/79.6) of telomerase was much higher than both PIVIKA-II (80.5/69.3) and AFP (72.6/61.5). Conclusion: The usefulness of serum telomerase assay in HCC diagnosis and it's to other tumor marker were shown. Therefore, serum telomerase is a novel, available detector and prognostic marker for HCC diagnosis.

D-156

Clinical Evaluation of the Dimension Vista® LOCI® Percent Free PSA Ratio and Diagnostic Concordance of the Dimension Vista Percent Free PSA with Beckman ACCESS® Percent Free PSA

J. A. Snyder[1], P. Volkir[2], K. Garvey[1], J. Lyons[1], J. Gray[3], R. H. Christenson[3]. *[1]Siemens Healthcare Diagnostics Inc., Newark, DE, [2]Siemens Healthcare Diagnostics Inc., Elkhart, IN, [3]University of Maryland School of Medicine, Baltimore, MD,*

Introduction: A clinical study of men aged ≥50 years was performed to determine the clinical utility of the Dimension Vista LOCI FPSA assay* when used as a ratio of percent fPSA (Dimension Vista LOCI FPSA/Dimension Vista LOCI FPSA) to detect prostate cancer (PCa) in men with a nonsuspicious digital rectal exam (DRE) and LOCI TPSA between 4 and 10 ng/mL. Receiver operator characteristic (ROC) curve analysis examined diagnostic performance at various percent free PSA cutoffs. Additionally, to compare concordance with another commercially available free PSA assay, diagnostic concordance was performed on a subset of samples.
Methods: Banked serum samples from 645 patients were included that had been prospectively collected from men referred to a urologist for evaluation of PCa at 28 clinical sites across the United States under IRB-approved protocols. Only men with a nonsuspicious DRE and a Dimension Vista TPSA result between 4 and 10 ng/mL were included in the study. Men with a history of PCa or who had undergone a DRE <5 days prior to sample collection were excluded. Serum samples were tested on a Dimension Vista System. Samples from a subset of 116 patients were also tested for free PSA and total PSA on the Beckman ACCESS system (Hybritech calibration).
Results: Two hundred thirty-five of the patients (36.4%) had PCa as determined by biopsy. ROC curve analysis demonstrated an area under the curve (AUC) of 0.66, with a 95% confidence interval (95% CI) of 0.62-0.71 for Dimension Vista percent free PSA. At a cutoff of 19%, the diagnostic sensitivity and specificity (95% CI) for Dimension Vista percent free PSA were 90.2% (85.7%-93.7%) and 17.6% (14.0%-21.6%), respectively; at a cutoff of 18%, the diagnostic sensitivity and specificity were 88.5% (83.7%-92.3%) and 22.9% (18.9%-27.3%), respectively. At a percent free PSA cutoff of ≤19% for Dimension Vista and ≤25% for ACCESS, clinical concordances for percent free PSA result and biopsy were 45.7% and 43.1%, respectively. At a percent free PSA cutoff of ≤18% for Dimension Vista, the clinical concordance for percent free PSA result and biopsy was 46.6%.
Conclusions: The Dimension Vista LOCI percent free PSA ratio demonstrates comparable clinical performance to that of an FDA-approved free PSA assay. A cutoff of 19% results in the detection of 90.2% of prostate cancers and could avoid unnecessary biopsy in 18.1% of patients; a cutoff of 18% results in the detection of 88.5% of cancers and may avoid unnecessary biopsy in 22.9% of men without prostate cancer.
* PMA submission under review by US FDA; not available for sale in the US.

D-157

Clinical Evaluation of the Dimension Vista® LOCI® TPSA Method and Comparison to Beckman Coulter PSA assays

J. A. Snyder[1], P. Volkir[2], K. Garvey[1], J. Lyons[1], J. Gray[3], R. H. Christenson[3]. *[1]Siemens Healthcare Diagnostics Inc., Newark, DE, [2]Siemens Healthcare Diagnostics Inc., Elkhart, IN, [3]University of Maryland School of Medicine, Baltimore, MD,*

Introduction: A clinical study involving 1592 men aged ≥50 years was performed to determine the clinical reliability of the Dimension Vista TPSA assay to detect prostate cancer (PCa). Additionally, a subset of clinical samples were tested with the Beckman Coulter HYBRITECH® PSA method to determine the impact of standardization on clinical result.
Methods: Serum samples were obtained retrospectively from a specimen bank. Samples in the bank had been prospectively collected at 29 clinical sites across the United States under one or more IRB-approved protocols with informed consent from men who had been referred to a urologist for diagnosis of PCa. Men with a history of PCa or who had undergone a DRE less than 5 days prior to sample collection were excluded from the study. Serum samples were tested on a Dimension Vista system at Siemens Healthcare Diagnostics, Glasgow, DE, and a separate aliquot of select samples was tested for PSA on the Beckman Access® system using the HYBRITECH calibration at the University of Maryland, Baltimore, MD. PSA results from the Beckman system were multiplied by 0.8

to obtain an estimate of the PSA values with the Beckman WHO calibration.[1]

Results: A total of 580 patients (36.4%) had PCa as determined by biopsy. Receiver operator curve (ROC) analysis was performed to determine the diagnostic sensitivity and specificity of the LOCI TPSA method at various cutoffs. ROC analysis demonstrated an area under the curve (AUC) of 0.65, with a 95% confidence interval (95 % CI) of 0.62-0.68. At a cutoff of 4.0 ng/mL, the diagnostic sensitivity and specificity (95% CI) for the LOCI TPSA method were 86.6% (83.5%-89.2%) and 32.6% (29.7%-35.6%), respectively; while at a cutoff of 3.1 ng/mL, the diagnostic sensitivity and specificity were 93.1% (90.7%-95.0%) and 22.2% (19.7%-24.9%), respectively. A Wilcoxon rank-sum test was performed using the Analyse-it® application for Microsoft Excel® for 116 samples with a range of approximately 4-15 ng/mL tested with both the LOCI and HYBRITECH PSA methods. The median difference in ng/mL (95% CI) between results with the Dimension Vista LOCI TPSA vs. Beckman HYBRITECH, LOCI TPSA vs. Beckman WHO, and Beckman HYBRITECH vs. Beckman WHO were 0.62 (0.47-0.79), 2.14 (1.97-2.34), and 1.55 (1.44-1.64), respectively. Similar results were obtained regardless of the clinical diagnosis (benign vs. PCa).

Conclusions: The Dimension Vista LOCI TPSA method demonstrated clinical performance comparable to that of the Beckman HYBRITECH PSA assay. Optimum sensitivity and specificity were obtained with the 4.0 ng/mL cutoff with calibration traceable to the WHO standard.

1. Jansen FH, Roobol M, Bangma CH, van Schaik RH. Clinical impact of new prostate-specific antigen WHO standardization on biopsy rates and cancer detection. Clin Chem. 2008 Dec;54(12):1999-2006.

* PMA submission under review by US FDA; not available for sale in the US.

D-158

Cytokines and cell adhesion molecules exhibit distinct profiles in health, ovarian cancer, and breast cancer.

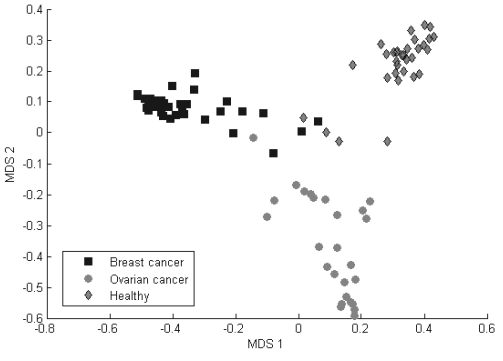
M. P. A. Henderson, H. Hirte, S. J. Hotte, P. A. Kavsak. *McMaster University, Hamilton, ON, Canada,*

Background: Clinical proteomics and bioinformatics have spurred increased research in the field of cancer biomarkers. The ideal biomarker would indicate both the presence of malignancy as well as the identity of the tissue of origin. We examined a panel of cytokines and cell adhesion molecules in an attempt to identify cancer specific profiles.

Methods: EDTA plasma samples were obtained from healthy women (n= 32), and the Ontario Tumour Bank (OTB). All cases were pathologically verified and have clinical information, such as histology, patient history, systemic therapy. The EDTA plasma samples obtained from the OTB were from women with a histological diagnosis of ovarian cancer (n = 42) or breast cancer (n= 61). These samples were measured for cytokines (IL-8, VEGF, MCP-1, EGF, IL-6, IL-4, IL-10, TFN-α) and adhesion molecules (VCAM-1, ICAM-1, P-, E-, L-selectins) using the evidence investigator™ (Randox). The patients were randomly assigned to the test groups (healthy n = 10, breast cancer n= 15, ovarian cancer n = 15) the remaining patients were included in the training groups. A random forest decision tree (Matlab R2009b) was created using the training group and a combination of markers were identified that could accurately classify the test group.

Results: The random forest procedure ranked the markers in descending order of importance for classifying the training samples as follows: VCAM-1, IL-2, TNF- α, ICAM-1, IL-4, IL-8, IL-1α, L-selectin. Multidimensional scaling was used to display the proximity of the training groups after classification (see Figure). Five hundred classification trees were generated using the training data for the 8 markers. The test samples were then classified as healthy (AUC = 1), ovarian cancer (AUC = 0.93) or breast cancer (AUC = 0.96).

Conclusion: Cytokine and cell adhesion molecule arrays can be used to differentiate between ovarian cancer, breast cancer and cancer free patients.



D-159

Circulating levels of EGFR, TIMP-1, CAIX and EphA2 in ovarian cancer

S. Brown-Shimer, P. J. Hamer, K. Pierce, D. Newman, J. Ramirez, D. Trivedi, S. P. Bradley, W. P. Carney. *Oncogene Science, Cambridge, MA,*

Background: Survival rates for ovarian cancer patients have been improving, however, management of patients with advanced ovarian cancer continues to be a difficult clinical problem. Due to a variety of reasons, patients often receive expensive therapies with dangerous side-effects to which their tumors do not respond. Soluble biomarkers which provide the real time status of a particular biomarker have the potential to aid clinicians in selecting and monitoring appropriate therapies. Circulating levels of these 4 biomarkers have been reported in many cancers, and 3 of the 4 are targets for novel cancer therapeutics. To address the clinical utility of these circulating biomakers in ovarian cancer, we determined serum and plasma levels of all four biomarkers.

Methods: Plasma and sera from stage I -IV ovarian cancer patients were analyzed using the TIMP-1, EGFR, CAIX and EphA2 ELISA tests developed and manufactured by Oncogene Science (Cambridge, MA). Levels were then compared to those found in healthy, normal females. Statistical methods used included Kruskal-Wallis test and Dunn's multiple comparison test.

Results: Circulating levels of all four biomarkers were measured in the serum and/or plasma of ovarian cancer patients as compared with levels found in normal healthy, females. For our analysis of serum results, stages I and II were combined and stages III and IV were combined. Serum levels of TIMP-1 and CAIX were significantly elevated in stages I/II ($p = < 0.001$ for both markers) and in stages III/IV ($p = <0.001$ and <0.01, respectively). Serum levels of EGFR were significantly decreased in stages I/II and III/IV ($p= < 0.01$ and < 0.001, respectively). EphA2 serum levels were not statistically significantly different from normals. In plasma, TIMP-1 levels in stage III ($p = <0.001$) and stage IV ($p = <0.001$) were significantly different from normal, but levels in stages I and II were not. CAIX plasma levels were significantly higher in all stages (I, $p=<0.05$; II, $p= <0.001$; III, $p=<0.001$; IV, $p=<0.01$), and EGFR plasma levels were significantly decreased in stages II ($p=<0.05$), III ($p=<0.001$) and IV ($p=<0.001$), but not in stage I. EphA2 plasma levels were significantly higher than normals in stages I/II ($p=<0.05$) and stages III/IV ($p=<0.001$).

Conclusions: EGFR, TIMP-1, CAIX and EphA2 levels in ovarian cancer patients are significantly different from the levels found in normal, healthy females. These markers can be measured in early as well as later stage cancer and changes involve both decreases and increases in levels. The fact that these biomarkers behave differently from one another may reflect different biological pathways which could contribute to ovarian tumor heterogeneity. Using these biomarkers as a panel may aid clinicians in selecting the most appropriate therapy and monitoring therapy efficacy.

D-160

Circulating EphA2 levels in samples from a variety of cancers

S. P. Bradley, P. J. Hamer, W. P. Carney. *Oncogene Science, Cambridge, MA,*

Introduction: The EphA2 receptor tyrosine kinase critically controls many aspects of cell behavior. It is expressed in a manner that is consistent with the regulation of cell growth, migration and invasion. Analysis by immunhistochemistry (IHC) has shown that elevated expression of EphA2 exhibits predictive value in lung cancer survival and recurrence. Current data suggests that therapies targeting EphA2 could be beneficial and is currently being evaluated in ovarian cancer patients. We have developed a prototype ELISA for circulating EphA2 receptor in order to investigate expression levels of soluble EphA2 in certain cancer types. Circulating EphA2 may benefit cancer patients by helping to guide EphA2 targeted therapies.

Materials and Methods: Microtiter plates were coated with an affinity-purified goat polyclonal antibody specific for the extracellular domain (ECD) of human EphA2. Human serum and plasma samples were diluted 5-fold in a sample diluent with blocking reagents. The standard was a mammalian cell-derived form of the ECD for the human EphA2 receptor. Standards and samples were incubated on the plates for 2 hours, 37C. The detector antibody was a biotinylated affinity-purified goat polyclonal antibody and was added to the plates for 1 hour, 37C. Plates were washed and a streptavidin-HRP conjugate was added to the plates for 30 minutes, 22C. A final wash was performed and a TMB substrate was added to the plates for color development. The reaction was stopped with sulfuric acid and plates were read at an absorbance of 450nm.

Results: Data using this prototype ELISA for circulating EphA2 indicates that increased levels of soluble EphA2 occurs in many cancers versus normal samples tested. The 95% confidence interval (CI) of total normal plasma and serum was 672-1306 pg/mL and 894-1429 pg/mL, respectively. Capture inhibition studies revealed specificity of the assay in human samples (100% inhibition). Parallelism studies revealed acceptable performance in serially diluted samples. Significant elevations of soluble-EphA2 was observed in benign lung cancers versus normals (100%, 10/10) while staged lung cancers exhibited

significantly lower levels of circulating EphA2 ($p<0.001$) compared to benign (96%, 148/154). Elevations of circulating EphA2 were found in glioblastoma (73%, 22/30), ovarian (48%, 28/59), pancreatic (46%, 19/41), and late stage breast (44%, 15/34) cancer patients. Immunoprecipitation experiments are ongoing to visualize soluble EphA2 by western blot, with initial data showing a specific band at ~60kD.
Conclusions: This study describes circulating levels EphA2 receptor in human samples using a prototype ELISA. Levels of soluble-EphA2 are increased in glioblastoma, ovarian, pancreatic, and late stage breast cancer samples. Decreased levels of the circulating form of EphA2 is observed in staged lung cancer versus benign. A test for circulating EphA2 may have value in selecting patients for EphA2 targeted therapies as well as monitoring the efficacy of EphA2 targeted therapies.

D-161

Plasma tumour M2-pyruvate kinase and carcionembryonic antigen are complementary in early detection of colorectal cancer

A. Fernandez-Suarez[1], J. J. Puente Gutiérrez[2], M. A. Marín Moreno[2], J. M. Aguilar Benítez[1], E. Bernal Blanco[2], D. Fatela Cantillo[3], J. M. Díaz Iglesias[1]. *[1]Hospital Alto Guadalquivir - Department of Biotechnology, Andújar (Jaen), Spain, [2]Hospital Alto Guadalquivir - Department of Digestive Diseases, Andújar (Jaen), Spain, [3]Hospital Sierra de Segura - Department of Biotechnology, (Jaen), Spain,*

Background. The tumour M2-pyruvate kinase (M2-PK), a pyruvate kinase isoenzyme, is found in proliferating tissues such as tumour cells. The investigation of M2-PK in EDTA-plasma samples of patients with gastrointestinal tumours revealed an up regulation of M2-PK in oesophageal, gastric, colonic and rectal carcinomas. Detection of M2-PK in colorectal cancer (CRC) indicated that it might have better performance characteristics than other markers like carcinoembryonic antigen (CEA), with low sensitivity for detecting early lesions.
Objective. The aim of this study is to determine whether the quantification of M2-PK in plasma samples for the early detection of CRC is effective and to perform a prospective comparison with CEA serum levels.
Materials and Methods. Consecutive patients from three different hospitals in Jaén region (Empresa Pública Hospital Alto Guadalquivir) were recruited between February 2008 and December 2009. The study includes patients with a high clinical suspicion of CRC undergoing diagnostic colonoscopy. All markers were measured before colonoscopy procedure. M2-PK plasma levels were analysed by enzyme-linked immunosorbent assay (ScheBo Biotech AG, Giessen, Germany) and CEA serum levels using a cobas® 6000 system (Roche Diagnostics).
Results. Overall, 164 participants [mean age 61.6 years (20-93), 55.3% females] were included in the analyses. Diagnostics colonoscopies found that 28 patients had CRC, 35 polyps (19 hyperplasic polyps and 16 adenomas), 61 other benign digestive diseases, 5 other non-CRC cancers and 35 patients did not demonstrate any remarkable pathology. According to TNM stage system, 17 CRC patients were classified as stage I, 5 as stage II, 3 as stage III, 2 as stage IV and 1 as stage 0. The median (percentile 25 - percentile 75) M2-PK concentration for CRC group was 23.05 U/mL (7.53-38.25), for polyps group 13.70 U/mL (5.80-18.90), for benign digestive diseases 11.85 U/mL (4.34-19.25), for other non-CRC cancers 24.95 U/mL (5.72-72.87) and for patients without evident pathology 7.5 U/mL (2.2-14.2). M2-PK levels correlate with TMN stage (Spearman 4.426, $p=0.024$), but not with CRC size, localization or degree of differentiation. The area under receiver-operating characteristic curves (95% CI) was 0.667 (0.551-0.784) for M2-PK and 0.671 (0.562-0.779) for CEA. Diagnostic cut-off value for CRC detection was 20 U/mL for M2-PK (sensitivity 55.2%, specificity 78.7%) and 5 ng/mL for CEA (sensitivity 27.6%, specificity 91.2%). Using a cut-off point for CEA of 3 ng/mL, combined sensitivity for both markers increased to 60.7%. Four cases with polyps (3 adenomas) were detected by CEA, while M2-PK was positive in seven patients with polyps (2 adenomas). Excluding cancer cases, the sensitivity of M2-PK in detecting polyps with a cut-off point of 15 U/mL was 40% (70% specificity).
Conclusions. Plasma M2-PK is commonly elevated in patients with CRC and other types of cancer. In combination with established tumour markers (like CEA), EDTA-plasma M2-PK is a potential biological marker in early diagnosis of colorectal cancer; both markers combination increase the sensitivity for CRC detection by 1.13-fold. Further clinical studies are needed to confirm these findings.

D-162

Circulating levels of HER-2/*neu* in patients with clinical suspicion of colorectal cancer

A. Fernandez-Suarez[1], J. J. Puente Gutiérrez[2], J. M. Aguilar Benítez[1], M. A. Marín Moreno[2], E. Bernal Blanco[2], J. L. Domínguez Jiménez[2], J. M. Díaz Iglesias[1]. *[1]Hospital Alto Guadalquivir - Department of Biotechnology, Andújar (Jaen), Spain, [2]Hospital Alto Guadalquivir - Department of Digestive Diseases, Andújar (Jaen), Spain,*

Background. The oncogen HER-2/*neu* (erbB-2) encodes a protein of 185.000 daltons. HER-2/*neu* oncogenic protein is over expressed in approximately 20% of human adenocarcinomas. HER-2/*neu* has also been studied in colorectal cancer (CRC); indeed, 30-50% of colon and rectal adenocarcinomas over express the HER-2/*neu* protein. Previous studies by other authors found elevated serum levels of this antigen in colorectal cancer patients.
Objective. The purpose of this study was to determine HER-2/*neu* in patients with clinical suspicion of colorectal cancer and to investigate its potential usefulness in CRC diagnosis and its probably correlation with the principal pathologic variables.
Materials and Methods. All patients with suspected diagnosis of colorectal cancer undergoing diagnostic colonoscopy were included in the study. HER-2/*neu* was measured using the ADVIA Centaur HER-2/*neu* assay (Siemens Medical Solutions Diagnostics) in serum at 158 patients prior diagnostic colonoscopy performed at our institution from February 2008 to December 2009. The cut-off used for HER-2/*neu* was 15 U/mL.
Results. After analyzing the results of colonoscopies we found three different groups of patients: 26 patient presented colorectal adenocarcinoma, in 36 patients the presence of polyps were detected (20 hyperplasic polyps and 16 adenomas), and in the 96 remaining showed a benign pathology or no relevant findings. Abnormal levels of this antigen were found in 7.7% (2 of 26) of the colorectal tumours. The median (percentile 25 - percentile 75) HER-2/*neu* concentration did not permit a significant discrimination of colorectal cancers [10.35 ng/mL (8.03-12.13)] from patients with polyps [11.15 ng/mL (9.45-13.25)] and patients with bening or irrelevant pathologies [10.9 ng/mL (9.23-12.88)]. No differences were found between hyperplasic polyps [11.25 ng/mL (9.35-13.25)] and adenomas [11.15 ng/mL (9.85-13.10)]. Her-2/*neu* serum levels did not correlate with any of the main prognostic variables in the group of colorectal adenocarcinomas (stage, tumour size, degree of differentiation), nor in the group of patients with polyps (number of polyps, size, morphology, presence of advanced adenoma, grade of dysplasia).
Conclusions. Serum HER-2/*neu* does not discriminate in the presence of colonic polyps, or in cases of colorectal adenocarcinoma. The results obtained in this study did not find differences between cancer pathology, the presence of adenomatous polyps and benign diseases. These findings indicate that serum HER-2/*neu* levels are not of clinical value in detection of colorectal cancer patients. Negative levels of HER-2/*neu* found in patients with tumours pathology may be because most cases are early stages of the disease. Her-2/*neu* is not a recommended marker for early diagnosis of CRC.

D-163

Mammalian Cardenolide Hormone (MCH-518): A Novel Biomarker in Breast Cancer Prevention

M. Al-Ghoul, W. Tucker, B. Bogdanov, R. Higashi, R. Valdes. *University of Louisville School of Medicine, Louisville, KY,*

Hypothesis: The endogenous compound (MCH-518) found in human serum kills breast cancer cells and not normal breast cells.
Background: Several recent reports have suggested that plant cardenolides are cytotoxic to several types of cancer cells, including breast cancer. Laboratories in the USA and Europe are trying to understand the mechanism-of-action of these compounds. However, the results of using plant cardenolides to treat cancer remain controversial. We posit that an endogenous mammalian counterpart, such as MCH-518, would have increased specificity in targeting cancer cells over normal cells. We previously showed that this compound induced apoptosis in lymphoma cells but not leukemia cells (Clin. Chem., 2007; 53: 1315 - 1322) and more recently showed that this compound extracted from human serum and characterized by FT-ICRMS has a m/z of 518.3221 (hence our nomenclature as MCH-518). In this study we provide evidence that MCH-518 has the elemental composition of C28H47O7 and that the activity of this compound is more specific than digoxin at inducing apoptosis in breast cancer cells in culture.
Methodology: We isolated the MCH-518 compound from human serum using solid phase extraction followed by HPLC purification. The HPLC peak of interest was analyzed using FT-ICRMS, tandem mass spectrometry and XCaliber software to determine mass and elemental composition. For biological activity we used breast cancer cell lines MCF7 and MDA-MB-231 and epithelial cells MCF 10A as a control. Cells were incubated with MCH-518 and separately with digoxin. Cell viability was measured by MTT assay. Apoptosis was confirmed by flow cytometry at the IC50 concentrations.

Results: The purified HPLC peak showed a peak with m/z = 518.3221 using FT-ICR-MS and a molecular composition consistent with C28H47O7. Digoxin at LC50 of 100±15 nM caused cell death (45±5% cell death) in both breast cancer cell lines and in normal cells (n=3). However, MCH-518 at an LC50 of 0.2 nM (digoxin immuno-equivalence) was cytotoxic to breast cancer cells (100% cell death) and not to normal cells (n=3).
Conclusion: We conclude that MCH-518 has a molecular composition of C28H47O7 and that it kills breast cancer cells and not epithelial cells. These findings suggest the possibility that subjects with appropriate blood concentrations of MCH-518 may be inherently protected from developing breast cancers compared to those who do not produce enough MCH-518. (NIEHS P30ES014443 and DoD BCRP BC075171).

D-164

A High Throughput Semi-automated RNA Extraction Method from Formalin-Fixed, Paraffin-Embedded Specimens for mRNA Quantitative Assays of Cancer Biomarkers

C. Santini[1], A. Iverson[1], C. Sigua[1], D. Tang[1], S. Y. Chang[1], J. Sninsky[1], S. Anderson[2], D. Eberhard[2], R. White[3], W. Vandermolen[3], T. Bunting[3], B. Calhoun[3]. *[1]Celera, Alameda, CA, [2]Laboratory Corporation of America, Research Triangle Park, NC, [3]Blumenthal Cancer Center, Carolinas Healthcare System, Charlotte, NC,*

Introduction. Formalin-fixed, paraffin-embedded (FFPE) sections are commonly used as the sample source for biomarker discovery and diagnostic assays in the field of cancer. RNA extraction from FFPE sections typically include a de-wax step with biohazardous xylene, a proteinase K digestion, and column purification for total RNA. The process is laborious and requires several hours of hands-on time.
Methods. We developed a protocol that uses the proteinase K digested lysate from non-dewaxed FFPE section to start the automated process of the RNeasy® FPPE kit (QIAGEN) on the QIAcube (QIAGEN). In addition, a DNase digestion step is incorporated into the automated protocol to eliminate genomic DNA completely.
Results. The entire extraction process, from scraping FFPE sections off of the slides to RNA, takes approximately 2½ hours for 12 FFPE specimens with 80-min hands-free time. We compared the RNA recovery from slides sectioned from the same FFPE block using manual and semi-automated protocols for eight specimens. RNA yield was slightly higher with the automated protocol for all eight specimens (R^2=0.976). We also compared the RNA recovery from one FFPE slide vs. two slides per column. The RNA yield per slide was slightly higher with two FFPE slides per column for all eight specimens (R^2=0.996). The established automated RNA extraction protocol was applied to a study of breast cancer tumor FFPE specimens. We completed the RNA extraction from two FFPE sections of each 124 breast tumor specimens using the automated protocol on QIAcube in three days, and determined the estrogen (ER), progesterone (PR) and growth factor HER2 receptor status using a single-tube multiplex quantitative RT-PCR assay (Iverson *et al. J. Mol. Diagn* 2009 **11**:117) in one day. The RT-PCR and immunohistochemistry (IHC) results were compared, and the concordances of RT-PCR assay for ER (n=124) and PR (n=123) were 98% and 91%, respectively. The HER2 fluorescent *in situ* hybridization assay classified 118 specimens as negative (no HER2 DNA amplification), 2 specimens as equivocal, and 1 specimen as positive while all 121 specimens were determined to have normal expression HER2 mRNA by RT-PCR.
Conclusions. We developed a semi-automated method to streamline the RNA extraction from FFPE sections, and demonstrated the concordance between mRNA levels and IHC results for hormonal and growth factor receptors for breast cancer tumor specimens.

D-165

Discovery of Stomach cancer specific biomarkers by the application of serum proteomics

J. S. Park[1], K. Y. Lee[2], Y. M. Yoon[3], M. W. Yoo[2], H. S. Han[4], K. P. Kim[1]. *[1]Department of Molecular Biotechnology and Institute of Biomedical Science and Technology,Konkuk University, Seoul, Korea, Republic of, [2]Department of surgery,Konkuk University school of medicine, Seoul, Korea, Republic of, [3]Department of clinical pathology,Konkuk University school of medicine, Seoul, Korea, Republic of, [4]Department of pathology, Konkuk University school of medicine, Seoul, Korea, Republic of,*

Background: Stomach cancer is one of the most leading cause of cancer related death in worldwide, especially Asia. In case of early diagnosis, the 5-year survival rate reached more than 90%. However, the current diagnostic marker proteins are not sufficiently specific and sensitive in clinical practice such as Carcinoembryonic antigen **(**CEA), CA 19-9, CA 125 to detect the cancer in early stage.
Recently an astonishing progression in proteomics, which can perform simultaneously high throughput analysis of proteins, is giving a great chance to mine the novel disease specific marker proteins.
Methods: Herein we analyzed serum samples from patients with various stages of stomach cancer using a proteomic method based on LC-MS/MS to discover the differentially expressed proteins in three groups, according to their progression of disease, advanced cancer, early cancer and healthy control. In spite of having a great advantage of proteomics, it's still difficult to handle the body fluid (e.g. serum) with this technology, because body fluid sample has a great dynamic range of protein concentration and complexity. To circumvent such difficulties, we introduced combined fractionation of serum proteins with depletion of high abundance proteins and an easy separation step, reverse phase column, C4.
Results: By successful introduction of the combined depletion method, we identified a total of 197 proteins with less than 1% False Discovery Rate(FDR) and multiple peptides from three groups of patients. Among them 167, 174 and 144 proteins are identified in sera of patients with advanced cancer, early cancer group and healthy control, respectively. In addition, quantitative analysis was performed by comparing spectral counts of the identified proteins from different groups to select marker candidates.
Conclusion: With choosing several candidate proteins which were shown more than 2 fold changes in the total number of spectra, we finally selected potential candidate proteins. Those proteins are differentially up and down regulated in the level of protein concentration according to cancer progression. To validate alteration of protein expression in serum, we analyzed their abundances through multiple reaction monitoring(MRM) method and immunoassay with statistical analysis.

D-166

GeneChip Analysis of Peripheral Blood in Hepatocellular Carcinoma

W. RUN, Y. TIAN, X. WEN. *PLA General Hospital, Beijing, China,*

Background: Hepatoma carcinoma (HCC) is a complicated and common cancer . Most patients diagnosed as HCC are at advanced stage, and do not survive long-term. Current diagnostic markers of HCC rely on the detection of an elevated level of serum alpha fetoprotein (AFP). However, AFP is not exclusive to HCC and has been observed in benign hepatic disease, such as cirrhosis and other cancers. The predominant molecular changes underlying HCC in most patients are far from clear. Recently, genome-wide analysis by microarray offers a systematic approach to uncover comprehensive information about gene expression profile of cancer.
Aims: To understand the process of HCC at the molecular level, and find HCC related genes in peripheral blood samples, we used Affymetrix Human Genome U133 plus 2.0 GeneChip to investigate the differentiated expression genes of peripheral blood monouclear cell (PBMC) in HCC .
Methods: We recruited 9 HCC patients and 6 control subjects, and analyze the differential gene expression with Affymetrix Human Genome U133 plus 2.0 GeneChip. With new HCC patients, liver cirrhosis ones, virus hepatitis ones and health control subjects blood samples by GeXP technology, we validated some significant differential expression genes.
Results: With Genespring GX 10.0 analysis software, we found that 1232 genes were differentially regulated in PBMC expression patterns from HCC patients and healthy control ($p<0.05$, fold change>2.00). Of these, 563 genes were down-regulated and 669 genes were up-regulated. Among the differentially regulated genes, 50 genes involved in immune system process. And 5 genes:CXCR4, IL8, PFDN5, CALR and GOS2 were significant differential expression. With GeXP technology, contrast to PBMC samples of healthy control, CXCR4, IL8, GOS2 and PFDN5are up-regulated in virus hepatitis patients ($P<0.05$), however, not significantly up-regulated in LC patients ($P>0.05$) and significantly up-regulated in HCC ($P<0.05$); CALR is up-regulated in virus hepatitis patients and LC patients ($P<0.05$) and down-regulated in HCC ($P<0.05$).**Conclusion**: The molecular signatures were clearly different between HCC and health control. It was indicated also that the five genes: CXCR4, IL8, PFDN5, CALR and GOS2 could be sensitive markers for the differential diagnosis of health control, HCC and other liver disease including virus hepatitis and liver cirrhosis.

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Diagnostic usefulness of serum TIMP-1 and IGFBP-2 for pancreatic adenocarcinoma

H. Park, B. Cho, M. Jung, E. Kang, J. Kim, S. Lee. *Samsung Medical Center, Seoul, Korea, Republic of,*

Background: Pancreatic cancer is hard to diagnose and the survival rate is not good. CA19-9 is a representative follow-up marker in pancreatic cancer patients under treatment, but there is a limit as to screen the patients. We selected TIMPs (Tissue inhibitors of metalloproteinases) and IGFBPs (Insulin-like growth factor binding proteins) through system biology.
Methods: We investigated serum expression of TIMP-1,2,3,4 and IGFBP-1,2,3,4,5 in 51 patients with pancreatic ductal adenocarcinoma by multiplexed fluorescent microplate analyzer (Bioplex, Bio-Rad, USA). Multiplex Kit was used to simultaneously assess the

levels of four TIMP molecules and five IGFBP molecules in a single sample, respectively. As control groups, 5 acute pancreatitis, 10 chronic pancreatitis, 5 healthy subjects and 5 other cancer patients were analyzed. Four stages of pancreatic cancer were included: 6 patients with stage I, 10 with stage II, 15 with stage III, and 20 with stage IV. We analyzed the serum expression level of 9 biomarkers in the 76 subjects. Mann-Whitney U test and Kruskall-Wallis test were used to evaluate statistical significance.

Results: TIMP-1, TIMP-2 and IGFBP-2 were highly expressed in pancreatic cancer patients compared to the healthy control or other cancer patients ($P<0.05$). There was no significant difference in TIMP or IGFBP expression according to the stage of pancreatic cancer. Cut-off, sensitivity, specificity and AUC are as follows: 2388.2 pg/mL, 0.882, 0.770, and 0.828 for TIMP-1, 1776.5 pg/mL, 0.667, 0.882, and 0.781 for TIMP-2, 1.0 ng/mL, 0.667, 0.800, and 0.655 for IGFBP-1, 11.0 ng/mL, 0.627, 0.900, and 0.736 for IGFBP-2.

Conclusions: We found that TIMP-1, TIMP-2, and IGFBP-2 are sensitive biomarkers for pancreatic cancer. To confirm the clinical usefulness, it is necessary to evaluate these markers in large-scale study in future.

Table 1. Diagnostic sensitivity of TIMP and IGFBP in patients with pancreatic cancer

	Pancreatic cancer (%)	Control (%)
TIMP1	88	60
TIMP2	67	60
IGFBP1	69	64
IGFBP2	63	32
CA19-9	57	28
TIMP1 + CA19-9	92	64
TIMP2 + CA19-9	82	72
IGFBP1 + CA19-9	88	76
IGFBP2 + CA19-9	86	48
TIMP1 + IGFBP1	98	88
TIMP1 + IGFBP2	96	64
TIMP1 + TIMP2	96	76

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Clinical relevance of analyte levels for commercial Tumor Marker Multi Constituent Controls

M. Olsson, O. Nilsson, C. Hall, C. Fermér, K. Majnesjö, M. Lundin. *Fujirebio Diagnostics AB, Gothenburg, Sweden,*

Background: Performance testing of laboratory methods requires access to control samples similar to patient samples, with analyte concentrations at medically relevant levels and stability over long periods. Several Multi Constituent Tumor Marker Control kits are available for this purpose. **Objective:** The objective of this study was to compare the performance of six commercially available Multi Constituent Tumor Marker Control kits with particular focus on clinically relevant analyte concentrations.

Material and Methods: AFP, CA15-3, CA19-9, CA125, CEA, Ferritin, FreePSA, PSA and the novel marker HE4 were determined for six Multi Constituent Tumor Marker Control kits: Bio-Rad Liquichek™ (REF 548X), Bio-Rad Lyphochek® (REF 368X), CliniQA Liquid QC™ (REF 91302), MAS® T-marker (REF TUM-S1), Fujirebio Diagnostics TM Control (REF 108-20) and Randox Quality Control Sera (REF IA2633). Abbott Architect values according to instructions for use (IFU) were compared. Controls lacking assigned values for FreePSA and PSA were analysed according to IFU in Architect FreePSA and Total PSA assays (REF: AFP 7K67, CA15-3® 2K44, CA19-9™XR 2K91, CA125II 2K45, CEA 7K68, Ferritin 6C11, FreePSA 6C07, Total PSA 6C06). A comparison was made of control matrix and stability claims for open use according to information provided in IFU.

Results: All six control kits contained at least one level of each marker at concentrations corresponding to healthy individuals (according to Architect IFU) and one elevated level. Exceptions were Liquichek and Lyphochek lacking CA19-9 within normal range and Randox lacking CA19-9 and CEA within normal range. The novel ovarian cancer biomarker HE4 was only included in the Fujirebio control. Clinically relevant %FreePSA levels were only noticed in the Fujirebio and CliniQA controls, with approximately 30% and 10% FreePSA, respectively. The other controls had >90% FreePSA. All six controls had open use stability for ≥7 days at 2-8°C. The analyte limiting prolonged stability at 2-8°C was FreePSA. For extended open use stability, storage at ≤-20°C was recommended for Lyphochek (≤30 days), Fujirebio (≤60 days) and Randox (≤28 days). Freezing and thawing was not recommended for Liquicheck, MAS and CliniQA, however they had open use stability for ≤30 days at 2-8°C. According to IFU Lyphochek, Fujirebio and Randox are lyophilized human serum based controls. The other three controls are provided in liquid form; Liquicheck is non serum based, MAS is based on human serum and CliniQA on human plasma. Liquicheck and Lyphocheck contain constituents of animal origin.

Conclusion: Clinically relevant analyte levels and an appropriate sample matrix are the most important parameters for any quality control material. The only multi constituent tumor marker controls with clinically relevant %FreePSA levels were the Fujirebio and CliniQA controls. The other controls contained >90% FreePSA and would therefore be less suitable for quality control of Total PSA assays. Fujirebio Diagnostics TM control is currently the only control that offers the novel marker HE4. The open use stability was acceptable for laboratory praxis for all six tumor marker controls.

Reference: Lilja H, Ulmert D, Vickers A. Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. Nature Reviews Cancer 8, 268-278 (2008).

D-169

Performance of ARCHITECT HE4 in Recognizing Recurrence or Progression of Epithelial Ovarian Cancer

T. Kettlety, R. Radwan, A. Vasko, B. Burkhart, C. Glover, L. Zhu, B. Acon, J. Young, J. Polkowski, K. Falcone, S. Raju, Z. Li, D. Dickson, R. Simamora, G. Barnes. *Fujirebio Diagnostics Inc., Malvern, PA,*

Objective: Human epididymis protein 4 (HE4) has been shown to be a promising biomarker for epithelial ovarian cancer (EOC). ARCHITECT HE4 assay was developed to measure the level of HE4 in human sera. This study was conducted to evaluate the clinical performance of an investigational ARCHITECT HE4 assay in recognizing the recurrence or progression of EOC via measurement of HE4 in longitudinal serum samples.

Methods: In the ARCHITECT HE4 assay, serum sample and anti-HE4 antibody 2H5-coated paramagnetic microparticles were combined and incubated first. Following a wash, the acridinium-labeled anti-HE4 antibody 3D8 conjugate was added to the mixture. Pre-trigger and Trigger solutions were then added to the reaction mixture. The generated chemiluminescence relative light units were used to measure the amount of HE4 antigen in the sample. Longitudinal serum samples (N = 506), including initial time point and follow-up visits (average follow-up visits = 5.7 per subject), were from subjects (N = 76) diagnosed with EOC. The samples were collected either during therapy or following the completion of therapy for the treatment of EOC. All of the samples were handled in accordance with an Institutional Review Board-approved and Good Clinical Practice-compliant protocol and tested with an investigational ARCHITECT HE4 assay reagent lot on the ARCHITECT i2000SR.

Results: In deciding the recurrence status of EOC, whether a subject is at the point of having no evidence of disease (NED) as opposed to a subject with clinical manifestations of recurrence, 98% (209/213 serum samples) of subjects with NED had HE4 levels below 140 pmol/L, as opposed to 51% (150/293 serum samples) of subjects with clinical manifestations of recurrence. In deciding the progression status of EOC, a 14% or more increase of the HE4 ratios of the successive HE4 readings was set as the cut-off to categorize the successive readings into those that were and were not elevated for the longitudinal samples. The sensitivity, specificity, positive predictive value and negative predictive value were 53.4% (95% CI: 44.1% - 62.5%), 78.8% (95% CI: 74.7% - 82.4%), 43.2% (95% CI: 32.9% - 54.1%) and 84.9% (95% CI: 78.5% - 89.6%) respectively. The total concordance between HE4 elevation and disease progression was 73.0% (95% CI: 68.9% - 76.7%). Receiver Operator Characteristic analysis showed an area under the curve of 0.685 (SE = 0.033) for the determination of EOC progression from the ratio of successive HE4 readings.

Conclusion: The increase of ARCHITECT HE4 values in longitudinal serum samples appeared to be of significance in recognizing the recurrence or progression of EOC.

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Development of a Chemiluminescent Microparticle CYFRA 21-1 Assay for ARCHITECT Instrument System

A. Vasko[1], B. Acon[1], K. Krocker[2], K. Hofmann[2], B. Burkhart[1], C. Glover[1], L. Zhu[1], Z. Li[1], P. Stieber[2], T. Kettlety[1]. *[1]Fujirebio Diagnostics Inc., Malvern, PA, [2]University of Munich, Munich, Germany,*

Objective: CYFRA 21-1 is the soluble fragments of cytokeratin 19 and has been shown to be a biomarker for lung cancer. This study was conducted to evaluate the analytical performance of the ARCHITECT CYFRA 21-1 assay in development.

Methods: In the ARCHITECT CYFRA 21-1 assay, serum or buffer-based sample and antibody KS19.1-coated paramagnetic microparticles were combined and incubated in the first step. Following a wash, an acridinium-labeled antibody BM19.21 conjugate was added to the mixture in the second step. Pre-trigger and Trigger solutions were then added to the reaction mixture. The chemiluminescence relative light units generated were used to determine the amount of CYFRA 21-1 antigen in the sample.

Results: The assay has a calibration range of 0-100 ng/mL. An imprecision study showed total CV ≤ 5.3% and within run CV ≤ 4.9% for buffer-based controls (5, 15 and 35 ng/mL) and serum-based panels (1, 3, 16 and 83 ng/mL) on two ARCHITECT analyzers (i1000SR and i2000SR). The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) of the assay were respectively 0.02, 0.07 and 0.07 ng/mL on two

ARCHITECT analyzers (i1000SR and i2000). When correlating the ARCHITECT assay (four ARCHITECT analyzers: i1000SR, i2000 and two i2000SRs) to Roche Elecsys using serum samples (N=106) with CYFRA 21-1 values ranging from 0.5 to 70 ng/mL, Passing & Bablok fit showed a slope ranging from 0.93 to 1.00 and an intercept ranging from -0.07 to -0.19.
Conclusion: The ARCHITECT CYFRA 21-1 assay is an accurate, sensitive and precise assay for the measurement of CYFRA 21-1 levels in human sera.

D-171

Exploring the Pathogenesis of Renal Cell Carcinoma Through Differential MicroRNA Expression
M. Diamandis[1], N. M. A. White[2], T. Chow[2], Y. Youssef[2], R. J. Honey[2], R. Stewart[2], K. T. Pace[2], M. Y. Gabril[3], G. M. Yousef[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]St. Michael's Hospital, Toronto, ON, Canada, [3]London Health Sciences Centre, London, ON, Canada,*

Background: MicroRNAs (miRNAs) are small stretches of non protein coding RNA that can control normal cell growth, differentiation and apoptosis. miRNAs are also known to be differentially expressed in many malignancies, including breast, lung, prostate and ovarian cancers. This study aimed to identify differentially expressed miRNAs in the clear cell subtype of renal cell carcinoma (ccRCC), the most common tumor of the adult kidney.
Methods: Histopathological assessment was used to make the diagnosis of ccRCC. Microarray analysis was then performed on 23 pairs of matched cancer and normal fresh frozen tissue samples from the same patients. Microarray expression results were validated in an independent cohort of 50 matched pairs using quantitative real-time PCR with miRNA-specific probes. Bioinformatics-based target prediction algorithms were used to determine potential targets of the identified dysregulated miRNAs.
Results: Microarray analysis identified a total of 166 differentially expressed miRNAs in ccRCC, including 21 significantly upregulated miRNAs. The top dysregulated miRNAs were validated by real-time PCR. Bioinformatic algorithms and literature searches showed that many of these miRNAs, including miR-21, miR-17, miR-17-3b and let-7, have previously been reported to be dysregulated in other malignancies. This suggests that they have a potential role in cancer pathogenesis by functioning as oncogenes or tumor-suppressors. The differentially expressed miRNAs showed a significant correlation with reported regions of chromosomal aberration, including regions of amplification or loss. Bioinformatic analysis also showed that many of these miRNAs tend to cluster in the same chromosomal region, indicating the possibility for a mechanism of co-expression. Phylogenetic analysis showed that ~50% of the dysregulated miRNAs are conserved among different species, indicating the existence of an important conserved biological function. Our target prediction analyses showed that many key molecules involved in the pathogenesis of RCC, including VHL, HIF, and VEGF, represent potential targets of the identified dysregulated miRNAs. Furthermore, our results show that the same dysregulated miRNAs can target multiple molecules, and that the same molecule can be targeted by multiple miRNAs. One of the identified dysregulated miRNAs, miR-210, is known to be induced in hypoxia.
Conclusion: Our study identified a number of dysregulated miRNAs in ccRCC that are predicted to target important molecules involved in the pathogenesis of this disease. Our finding that a known hypoxia-induced miRNA is dysregulated in ccRCC cells implies a direct role for this miRNA in kidney cancer, as hypoxia is documented to have a central role in RCC pathogenesis.

D-172

MethyLight assay for urine DNA based screening for colorectal cancer
B. P. Song[1], S. Jain[1], T. M. Block[1], D. E. Brenner[2], Y. Su[1]. *[1]Drexel University, Doylestwon, PA, [2]University of Michigan, Ann Arbor, MI,*

Background: We demonstrated previously that human urine contains circulation-derived low molecular weight (LMW) DNA (<300 base pairs) and that LMW urine DNA can be used to detect cancer-specific mutations. The objective of this study was to develop an assay using LMW urine DNA as a substrate for detecting a known colorectal cancer (CRC) methylated marker in the urine of patients with CRC that could be used for cancer screening.
Methods: We developed three quantitative MethyLight PCR-based assays, one 1-step PCR assay targeting a 63-bp template and two 2-step nested PCR assays targeting a 39-bp and a 40-bp template, respectively, of two previously suggested CRC-specific methylated regions in the vimentin gene (mVIM). The sensitivity of each assay was determined using reconstituted standard samples ranging from 1.25 to 1225 copies of positive control DNA per 50 ng of negative control Huh7 DNA. To determine the suitability of the assay for LMW urine DNA, archived LMW urine DNA samples from 10 normal subjects and 10 patients with CRC were tested.
Results: It was found that the 2-step nested PCR based assay with a target size of 40 bp could be used for detecting mVIM in low MW DNA. In contrast, the one-step PCR assay targeting a 63 bp template did not detect any mVIM in low MW urine DNA. A blinded concordance study was performed. mVIM was detected in 75% (15/20) of urine samples from patients diagnosed with CRC and 0% (0/10) of urine from healthy controls, and there was a 60% concordance in detecting mVIM between the CRC tissues and corresponding urine samples.
Conclusion: We conclue that a shorter amplicon could detect mVIM with a higher sensitivity and a targeting sequence as short as 40 nt might be needed to be sensitive enough for detecting the circulation derived methylation DNA markers in urine. To our knowledge, this is the first time that a circulation derived methylation marker is detected in the urine of cancer patients. This data demonstrates the promising potential of developing a urine test for colon cancer screening.

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Optimizing analytical assay performance for biomarker discovery and validation: A study of autoantibody biomarkers for the early detection of colorectal cancer.
J. A. Straseski[1], C. N. Rosenzweig[1], Y. Li[1], X. Sun[1], N. Ramachandran[2], D. W. Chan[1], Z. Zhang[1]. *[1]Johns Hopkins Medical Institutions, Baltimore, MD, [2]Life Technologies, Carlsbad, CA,*

Analytical assay performance plays an important role in biomarker research. It directly affects the ability to detect disease-associated changes and overall study sample size requirements. However, in practice, there is little focus on characterization and optimization of assays used for biomarker discovery and/or validation efforts. We have recently completed a biomarker discovery study for the early detection of colorectal cancer (CRC) and are initiating a large-scale independent validation study of these biomarkers. This study serves as an example of the efforts required to optimize assays for biomarker discovery and validation. Compared with other cancers, CRC exhibits a relatively long progression time with few clinical symptoms prior to diagnosis of late-stage disease. Non-invasive tests capable of detecting CRC in this early, and possibly lengthy, window may considerably aid detection rates. We hypothesized that patient autoimmune responses to changes in the bioenvironment during early cancer development may act as biomarkers for CRC, possibly before clinical symptoms arise. Plasma samples from 100 cases and 100 cohort-matched controls were randomly selected from a longitudinal cohort using nested case-control design and analyzed for biomarker discovery using the Invitrogen ProtoArray v.4 protein microarray (>8000 unique antigens). A unique advantage of this longitudinal sample set is that case specimens were collected prior to the diagnosis of CRC. Therefore, sample collection and processing were identical between cases and controls, eliminating bias toward disease status. Factorial study design was used to identify key processing factors and settings that maximized the signal-to-noise ratio (S/N) and minimized the within-chip coefficient of variation (CV). S/N was calculated from the lowest concentration of the positive control (human IgG) and the mean of all concentrations of the negative control (human GST). The resultant ratio emphasized the detection of changes in low abundance proteins, which is critical for biomarker discovery. ROC curves were constructed to assess cutoff values (average AUC 0.78) and false discovery rates were estimated with phenotype label permutation and bootstrap re-sampling (40%). Microarray protocol optimization reduced within-chip CV of QC spots by an average of 18% (p=0.01) and maximized S/N by almost 2 times the value obtained with the standard manufacturer's protocol. Using rigorous statistical criteria, a total of 42 potential biomarkers were selected for further validation. A novel array-based, multiplexed assay was designed for quantitative large-scale validation studies of newly discovered cancer biomarkers. Initial evaluation and optimization of this quantitative validation assay was performed with a pooled serum sample of known positive signal (n=12 arrays, triplicate analysis of 4 serum concentrations). The assay showed clear concentration-dependent responses among serum samples analyzed at 200, 400, 800, and 1600-fold dilutions with 1-6% CVs. Multiplexed arrays using the same quantitative design are being spotted for further validation of the 42 putative CRC autoantibody biomarkers. In conclusion, optimization of analytical assay performance should be a key step in biomarker research and requires deliberate and calculated design. Discovery assays require optimization of different parameters than assays for biomarker validation, and each should be focused on independently of each other.

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Circulating levels and serial monitoring of TIMP-1 and uPA in pancreatic cancer patients

R. Vargas[1], P. J. Hamer[2], V. Shrivastava[1], K. Leitzel[1], J. H. Marx[1], L. Demers[1], H. A. Harvey[1], S. M. Ali[1], S. Brown-Shimer[2], A. Lipton[1], W. P. Carney[2]. *[1]Penn State University/Hershey Medical Center, Hershey, PA, [2]Oncogene Science, Cambridge, MA,*

Background: Tissue inhibitor of metalloproteinase-1 (TIMP-1) has been shown to have diverse multifunctional roles in tumorigenesis such as inhibition of the catalytic activity of MMPs, growth promotion, inhibition of apoptosis and regulation of angiogenesis. Urokinase-type plasminogen activator (uPA) is a serine protease secreted by tumors, adjacent stromal cells, and fibroblasts and is associated with tumor cell migration, angiogenesis, metastasis, and invasion. Elevated circulating levels of these biomarkers of metastasis have been associated with a variety of cancers. In this study we evaluated circulating levels of TIMP-1 and uPA in patients with advanced pancreatic cancer.
Methods: This study measured the circulating levels of TIMP-1 and uPA in pretreatment samples of pancreatic cancer patients in a randomized, double-blind, placebo-controlled phase III clinical trial. Serum biomarker levels were then correlated with patient survival. Pretreatment serum TIMP-1 levels were measured in 157 patients whereas uPA levels were measured in 149 patients. Both the TIMP-1 and uPA tests were developed and manufactured by Oncogene Science (Cambridge, MA)
Results: Serum TIMP-1 levels in 157 pancreatic patients had a median of 409.9 ng/mL, and ranged from 144 to 1078 ng/mL. Patients with higher serum TIMP-1 had significantly shorter survival on a continuous basis ($p = 0.001$), on quartile analysis ($p = 0.004$), and on a dichotomous cutpoint analysis of upper 25 % vs lower 75 % (median survival 101 days vs. 197 days) ($p< 0.001$). In pancreatic cancer patients, serum uPA levels were elevated in 23 of 63 (36%) female patients, and 40 of 86 (46%) male patients. In addition, mean serum uPA levels were significantly higher in both female (1.85 ng/mL, $p<0.00001$) and male (1.96 ng/mL, $p<0.00001$) pancreatic cancer patients when compared to controls. Patients with elevated serum uPA trended toward a shortened survival (median 127 days) compared to patients with normal serum uPA levels (median 198 days) ($p=0.083$)
Conclusions: Evaluation of pretreatment serum TIMP-1 levels in 157 advanced pancreatic cancer patients showed that those with higher serum TIMP-1 levels had significantly shorter overall survival on a continuous basis, quartile basis, and on cutpoint analysis. In addition, serum uPA levels are significantly elevated in 41% of advanced pancreatic cancer patients, and these patients trended toward shortened survival. Additional studies are warranted to determine the predictive value and serial monitoring utility of these circulating biomarkers for patient outcome to novel uPA directed therapies.

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Pretreatment levels of soluble CAIX and TIMP-1 are associated with reduced progression-free survival and overall survival in trastuzumab-treated metastatic cancer patients

K. Leitzel[1], H. Y. Hou[1], U. Anyanwu[1], K. Pierce[2], P. J. Hamer[2], S. Brown-Shimer[2], V. Shrivastava[1], M. Evans[1], W. Kostler[3], E. Fuchs[3], L. Demers[1], S. M. Ali[1], A. Lipton[1], W. P. Carney[2]. *[1]Penn State University/Hershey Medical Center, Hershey, PA, [2]Oncogene Science, Cambridge, MA, [3]Medical University of Vienna, Vienna, Austria,*

Background: Approximately half of HER2-positive breast cancer patients will respond to first-line trastuzumab-containing therapy. However, in those patients with an initial trastuzumab response, most will progress within a year with acquired resistance. Since trastuzumab treatment is also now used in the HER2-positive adjuvant breast cancer setting, trastuzumab resistance will continue to be a vexing clinical problem, and better predictive and prognostic biomarkers are urgently needed.
Methods: Serum HER-2, tissue inhibitor of metalloproteinase-1 (TIMP-1), urokinase-type plasminogen activator (uPA), carbonic anhydrase IX, VEGF-165, and endoglin were determined using ELISA. The HER-2, TIMP-1, uPA, CAIX and VEGF-165 ELISAs were developed and manufactured by Oncogene Science, (Cambridge, MA). The endoglin ELISA was purchased from R&D Systems, Minneapolis, MN. Pretreatment serum samples from 66 metastatic breast cancer (MBC) patients were evaluated for circulating levels of these biomarkers before starting first-line trastuzumab-containing therapy. Progression-free survival (PFS) was then analyzed using the Kaplan-Meier method and Cox modeling with continuous pretreatment serum biomarker variables.
Results: Pretreatment serum levels were determined for HER-2, TIMP-1, uPA, endoglin, CAIX and VEGF-165. In multivariate analysis for PFS with all 6 biomarkers, only serum CAIX ($p = 0.002$) remained a significant independent covariate. In multivariate analysis for PFS using only 4 biomarkers, (HER-2, TIMP-1, uPA and endoglin) none were significant independent covariates. However, if serum HER-2 was omitted from the multivariate analysis (since patients were selected for tumor HER2 IHC-positivity), serum TIMP-1 ($p=.034$), became an independent predictive factor for PFS.
Conclusions: We have looked at several circulating biomarkers in MBC patients treated with first-line trastuzumab-containing therapy and found that elevated pretreatment serum CAIX and TIMP-1 levels predicted reduced PFS. These serum biomarkers deserve further study in larger trials of HER2-targeted breast cancer treatment. Supported in part by a grant from Komen for the Cure.

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Changes in the circulating levels of uPA predict progression-free survival in first-line trastuzumab-treated breast cancer

H. Y. Hou[1], W. Kostler[2], P. J. Hamer[3], U. Anyanwu[1], N. Jamshidi[1], K. Leitzel[1], S. M. Ali[1], E. Fuchs[2], A. Lipton[1], W. P. Carney[3]. *[1]Penn State University/ Hershey Medical Center, Hershey, PA, [2]Medical University of Vienna, Vienna, Austria, [3]Oncogene Science, Cambridge, MA,*

Background: About half of HER-2-positive breast cancer patients will respond to first-line trastuzumab-containing therapy, but of these most will progress within a year with acquired resistance. Since trastuzumab treatment is now used in the HER-2-positive adjuvant breast cancer setting, trastuzumab resistance will continue to be a recurring clinical problem, and better predictive and prognostic biomarkers are urgently needed.
Methods: Serum TIMP-1, uPA, and carbonic anhydrase IX (CAIX) were measured using ELISA in 60 metastatic breast cancer patients before starting first-line trastuzumab-containing therapy, and at the closest 1 month post-trastuzumab blood draw. The TIMP-1, uPA and CAIX ELISAs were developed and manufactured by Oncogene Science, Cambridge, MA. Progression-free (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method and Cox modeling.
Results: When serum biomarker change (pretreatment to 1 month post-trastuzumab treatment) was analyzed on a continuous basis, change in serum TIMP-1 and CA IX were not significant for PFS or OS. However the change in serum uPA was a significant predictive factor for PFS to first-line trastuzumab treatment (HR = 1.0176, $p= 0.006$) and for OS (HR = 1.0167, $p= 0.027$): declining serum uPA predicted for increased survival, and increasing serum uPA predicted for decreased survival. Using cutpoint analysis, patients who achieved a 25% decline in serum uPA levels (14/60, 23%) had a significantly longer PFS (median 1331 days) compared to those patients who did not (median 364 days) (HR 4.6, $p = 0.004$). In similar fashion, patients who achieved the 25% decline in serum uPA levels had a significantly longer OS (median 2130 days) compared to those patients who did not (median 1079 days) (HR 4.3, $p = 0.017$).
Conclusions: The change in serum uPA level from pretreatment to 1 month post-trastuzumab treatment predicted PFS and overall survival in metastatic breast cancer patients treated with first-line trastuzumab-containing therapy. Circulating biomarkers such as uPA can provide real-time information on patient status during therapy and provide an early warning signal for additional therapeutic interventions.
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Identification of a variant S100B (vS100B), a unique, single EF-hand, member of the S100 family of proteins

M. Hadi[1], F. Farzaneh[1], R. Sherwood[2]. *[1]King's College London, London, United Kingdom, [2]King's College Hospital NHS Trust, London, United Kingdom,*

S100 protein family represents a highly conserved group of EF-hand (helix-loop-helix) calcium binding proteins, with increased intracellular and serum concentrations of the protein in a range of human malignancies. One of the most extensively studied members of this family, S100B, is present at elevated intracellular levels in glioblastoma, malignant melanoma and S100B positive T-cell leukaemia. Recent studies have shown that S100B can bind to p53, suppressing p53-dependent transcription of target genes including *hdm*2, *p*21 and *bcl*2, thus affecting a range of functions including DNA repair, cell-cycle arrest and apoptosis. S100*B* gene contains p53 binding sites in its promoter and may therefore have its own transcription regulated by p53.
Previous studies on the genomic organisation and transcription of S100*B* have mapped the gene to chromosome 21q22, encoding a messenger RNA of 1096 bases, transcribed from 3 exons which can be translated into a 92 amino acid protein, present as homo- and hetero-dimers of approximately 21 KDa. In the present study the expression of S100B was examined in a range of leukaemic and other malignantly transformed cell lines. Detailed analysis of S100B transcripts in U373-MG human glioblastoma cell line has enabled us to identify a new S100B exon, present within the region originally labelled as intron 2. The new variant S100B (vS100B) transcript encodes a predicted 94 aa protein with a calculated molecular mass of 10.3 KDa. The presence of vS100B transcript was confirmed in human cancer lines including MALME-3M (malignant melanoma), JURKAT, SUP-T1, CEM-SS (T-cell leukaemia); U937 (monocytic leukaemia) and K562 (erythrolaeukemia).

The first N-terminal 46 amino acids of the vS100B are identical to the classical S100B (cS100B). However, in contrast to the cS100B, which contains two EF-hands (a characteristic of the S100 family members), the incorporation of the new exon in the vS100B causes a predicted frame-shift in the C-terminal 48 amino acids, making this half of vS100B entirely different from cS100B. This change in the C-terminal half of vS100B is predicted to result in loss of the C-terminal EF-hand, making it a unique member of the S100 family, as all other members, including cS100B, contain two EF-hands.
In conclusion, we have obtained experimental evidence for the presence of a vS100B, produced by differential splicing of the S100B transcript. The presence of a single EF-hand, is likely to bestow the predicted protein product with dramatically different protein-protein interaction properties, potentially both as a homodimer, and in heterodimerisation with other S100 family members, including cS100B.

D-178

Serum cytokines profiling in well Differentiated Thyroid Carcinoma

A. E. Stanciu[1], N. Stafidov[1], A. Hurduc[1], R. Fumarel[1], S. Cinca[1], M. Stanciu[2].
[1]"Prof.Dr.Al.Trestioreanu" Institute of Oncology, Bucharest, Romania,
[2]University "Politehnica" of Bucharest, Bucharest, Romania,

Background: Total or near-total thyroidectomy followed by radioiodine (^{131}I) ablation of residual thyroid tissue (remnant ablation) is considered the ideal treatment for differentiated thyroid carcinoma (DTC). Sensitive monitoring for thyroid cancer recurrence or persistence includes whole-body radioiodine scanning (WBS) and measurement of serum thyroglobulin (Tg) after endogenous or exogenous thyrotropin (TSH) stimulation. Our objective was to perform an immune system monitoring for thyroid cancer recurrence or persistence. This supplementary monitoring included measurement of cytokines serum levels (interleukins IL-1β, IL-4, Tumor Necrosis Factor - TNF-α). IL-1β and TNF-α are pro-inflammatory cytokines and IL-4, a pleiotropic cytokine mainly produced by activated helper T lymphocytes type 2 (Th2) is an anti-inflammatory one.
Methods and patients: 85 patients with DTC and post-surgical radioiodine therapy indication (11M/74F, aged 14-69 years, 14.62% papillary thyroid cancer and 85.38% follicular thyroid cancer) were enrolled in the study. In all patients, blood specimens were drawn from a peripheral vein before radioiodine thyroid remnant ablation. The serum levels of TSH, Tg, thyroid autoantibodies against thyroglobulin - antiTg, IL-1β, IL-4 and TNF-α were measured at the same time by an ELISA method. WBS was performed after therapeutic administration of radioiodine (post therapeutic at 3-5 days).
Results: 3 patients with a serum concentration of anti-Tg higher than 85 IU/ml were eliminated from the study, because a high level of anti-TG may interfere with correct determination of serum thyroglobulin and with immune system. Serum level of TSH for all patients was higher than 30 mIU/l because radioiodine treatment is done under TSH stimulation. We observed discordance between Tg and IL-4 serum concentrations. 48 patients with normal Tg values (lower than 10 ng/ml) had an IL-4 value higher than 8 pg/ml. These patients with increased serum levels of IL-4 (88.5 ± 82.3 pg/ml) had a good prognostic. It is important to indicate a significant positive correlation between serum level of Tg and TNF-α ($r = 0.45 \pm 0.41$, $p<0.05$), Tg and TNF-α/IL-4 ($r = 0.71 \pm 0.25$, $p < 0.05$). WBS and Tg are usually concordant but in some instances may be discordant. A positive WBS result, in the presence of an increased TNF-α serum level, maybe a false-positive result because the accumulation of radioiodine may result from the inflammatory reaction, after the trauma. Inflammatory reaction means vasodilation, increased capillary permeability and humoral and cellular mediators of inflammation (IL-1β, TNF-α) may together result in increased delivery of ^{131}I to the injured area.
Conclusions: As regards IL-4 measurement, the most patients with normal Tg values had increased IL-4 concentration levels and a decreased TNF-α/IL-4 ratio and patients with high serum levels of Tg had a low value of IL-4 concentration and an increased TNF-α/IL-4 ratio. Taken together, these findings suggest that thyroid cancer cells receive significant protection from apoptosis by IL-4.

Thursday AM, July 29

Poster Session: 9:00 am – 12:00 pm
Technology/Design Development

E-01

Evaluation of General Chemistry Assays on the cobas c 111 Analyzer

F. H. Yu[1], R. M. Robinson[2], N. Sidiropoulos[2], H. K. Lee[3]. *[1]Dartmouth College, Hanover, NH, [2]Dartmouth-Hitchcock Medical Center, Lebanon, NH, [3]Dartmouth Medical School & Dartmouth-Hitchcock Medical Center, Lebanon, NH,*

Background: The Roche cobas c 111 analyzer is designed for automated testing in small laboratories. It is compact and contains 27 slots (approximately 14 assays) per reagent disk, and 3 channels on the ISE module. It has a throughput of 65 tests/hour for photometry tests and 180 tests/hour for ISE tests. The analyzer is able to sample from primary tubes, sample cups, micro-cups or cups on tubes.
Objectives: This study evaluated the precision, accuracy, linearity, carryover potential, and interference cutoffs of 18 assays (15 general chemistry assays and 3 ISE assays) on the cobas c 111 analyzer, using the Roche Modular PPE and Cobas 6000 as the reference methods.
Methods: Intra-day precision was determined by running the high and low controls 20 times in one day, while inter-day precision was determined by running the controls daily for at least 20 days. Accuracy of the cobas c 111 was assessed through correlation of 40 to 57 patient samples between the cobas c 111 and each reference method. Linearity studies were accomplished by using linearity sets supplied by Maine Standards. Carryover studies were done with two consecutive runs of triplicate high and low controls. Interference studies for hemolysis, lipemia, and icterus were conducted using a $\pm$5% margin of variability and compared to manufacturer's claims.
Results: All the inter-day precisions had CVs below 8.1 %, and most assays had CVs ranging from 1% to 5.7%. Regression analysis for the correlation of patient samples between the cobas c 111 (y) and reference (x) methods showed maximum deviations of 0.1043 from a slope of 1, and 8.9118 from an intercept of zero. All the assays showed correlation coefficients $\geq$ 0.97, except direct bilirubin, and sodium, where the r^2 values were 0.94 and 0.92 respectively. All assays were linear within the analytical measuring ranges claimed by the manufacturer. No carryover was detected for all assays tested. For interference studies, the number of assays that achieved interference cutoffs equal to or higher than manufacturer's claims were 8/18 (44%) for hemolysis, 14/18 (78%) for lipemia, and 15/16 (94%) for icterus interference. All the interference cutoffs determined in-house were adopted in the testing procedures.
Conclusions: The performance characteristics of albumin, alkaline phosphatase, ALT, amylase, AST, bicarbonate, BUN, calcium, chloride, creatine kinase, creatinine, direct bilirubin, glucose, lipase, potassium, sodium, total bilirubin, and total protein on the cobas c 111 were comparable to Modular PPE and Cobas 6000. It can be concluded that the cobas c 111 is a suitable analyzer for small laboratories.

E-02

First system for fully automatic pre-analytical processing of faecal samples.

L. Hansson, A. M. Havelka. *Department of Molecular Medicine and Surgery, Stockholm, Sweden,*

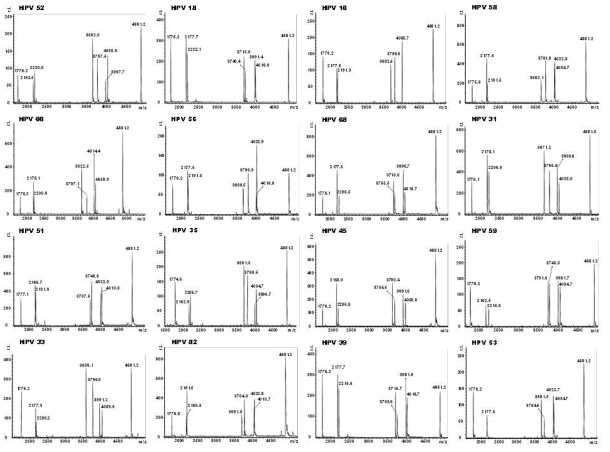
The discovery of new markers in stool has shown slow development compared to finding of markers in blood and urine. The main reasons for this are the low analytic throughput and the inconvenient hygienic working environment caused by the manual pre-analytical processing of faecal samples. In recent years faeces analyses have received high attention, resulting in the development of important diagnostic markers in faeces such as calprotectin for inflammatory bowel disease as well as various cancer markers. This has leads to increased number of faecales analyses samples which in turn requires more efficient system for faecal handling of faecal samples.
We have invented a fullyn automatic system for pre-analytical processing of faecal samples which increases extraction efficiency, improves quality and reproducibility of processed samples and significantly improves the working environment.
The system performs all steps that are required for efficient sample processing including, registration, weighing of the sample, addition of appropriate buffer volume, ultrasound sonification for efficient homogenization of the sample, followed by centrifugation and transferring of the supernatant to a properly labeled secondary tube(s) for subsequent analysis.
The system has been verified and compared to the manual routine for sample processing at the Karolinska University Laboratory.
The new automatic system offers following improvements compared to the manual method:
• Specially designed sample collection container that yields the right amount of faecal sample during at sample collection.
• Sonification of samples results in faster extraction process and improved extraction efficiency.
• Automatic preparation results in higher and more consistent quality of the extracted samples.
• An automatic process increases the throughput capacity and the sample traceability and reduces the need of personnel resources.
• The method eliminates manual handling of the samples resulting in a more hygienic and safer working environment.
• The fully automatic system for pre-analytical processing of faecal samples will efficiently create possibilities to detect new diagnostic both molecular and protein biomarkers in stool samples e.g. various new cancer and inflammatory markers.

E-03

A Broad spectrum Genotyping of Human Papillomavirus by Restriction Fragment Mass Polymorphism in Cervical Samples

S. Hong[1], H. Lee[1], E. Lee[2], S. Hwang[1], S. Shin[1], T. Hwang[3], S. Kim[1], H. Lee[3]. *[1]GeneMatrix, Yongin, Korea, Republic of, [2]Green Cross Reference Laboratory, Yongin, Korea, Republic of, [3]KonKuk University School of Medicine, Seoul, Korea, Republic of,*

Infection with human papillomavirus (HPV) is the main cause of cervical cancer, and accurate and broad-spectrum HPV genotyping is increasing importance for determining clinical course and management of the disease based on HPV genotypes. A MALDI-TOF mass spectrometry (MS)-based Restriction Fragment Mass Polymorphism (RFMP) assay has proven to accurately and reliably genotype a wide variety of HPV. We evaluated the clinical utility of the RFMP assay in HPV genotyping by testing a total of 426 specimens taken from liquid-based cytology, which was composed of normal cytology, atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL) and invasive squamous cervical cancer (SCC). The RFMP assay reproducibly detected 10-100 genomic equivalents of various HPV types calibrated against WHO international standard HPV DNA. The HPV positive rates were increased as increasing severity level of cervical lesion. The high-risk HPV positivity according to cytologic diagnosis were 7.9% (8/101), 31.7% (38/120), 50.0% (55/110), 86.0% (37/43), 96.2% (50/52) in normal, ASCUS, LSIL, HSIL and SCC subgroups, respectively. Diagnostic sensitivities/specificities for the cervical lesion of HSIL and SCC were found to be 91.6%/92.1%, 96.2%/92.1%, respectively. The results demonstrated that RFMP assay should be clinically suitable for HPV genotyping in laboratories.



E-04

Evaluation of hepatitis virus marker assays on Sysmex HISCL-2000i automated chemiluminescent enzyme immunoassay system

M. Maekawa, T. Kondo, E. Hamada, K. Iwahara, S. Uchiyama, Y. Kobayashi. *Hamamatsu University School of Medicine, Hamamatsu, Japan,*

Objective: Serological diagnosis of viral hepatitis has been applied to screening, assessment of the effectiveness of vaccination, and evaluation of treatments with interferon, nucleic acid analogs, etc. In the present study, we undertook analytical and clinical investigations using HISCL-2000i, an analyzer based on chemiluminescent enzyme immunoassay (CLEIA), and corresponding reagents; HISCL HBs antigen (HBsAg), HISCL HBs antibody (HBsAb) and HISCL HCV antibody (HCVAb).

Methods: The imprecision, linearity, lower limit of detection, and correlation with other immunoassay were examined using the totally automated immunoassay system HISCL-2000i, and the reagents HISCL HBsAg, HISCL HBsAb and HISCL HCVAb (Sysmex Corp., Kobe, Japan). Correlation was performed by comparing with fully automated chemiluminescent enzyme immunoassay system LUMIPULSE f and its corresponding reagents (FUJIREBIO Inc.)

Results: The coefficient of variance was calculated for measurements run on the reagents HBsAg, HBsAb and HCVAb, using control blood serum at two different concentrations, and very good results were obtained. Good linearity was confirmed for samples of three different concentrations (high, medium, and low) tested with HBsAg. When 158 samples with negative HBs antigen by a different method were tested using HBsAg reagent on HISCL-2000i, all the samples indicated 0.00 IU/ml. This means a clear-cut distribution of negative values on HISCL-2000i. Lower limit of detection of the HBs antigen calculated by the ± 2 SD method was 0.015 IU/ml. Concordance with the results obtained using LUMIPULSE f (FUJIREBIO INC.) was generally good, being 99.4% for HBsAg, 97.4% for HBsAb, and 96.8% for HCVAb. Four patients of chronic hepatitis B (positive for HBe antigen or HBe antibody) treated with nucleic acid analogs were measured for HBV-DNA and HBsAg values. The HBe antibody positive patients showed a more prominent reduction in HBsAg than the HBe antigen positive patients did. When a drug resistant strain appeared, HBsAg level elevated even though HBV-DNA was below the detection limit.

Conclusion: Analytical performance of the reagents HISCL HBsAg, HISCL HBsAb and HISCL HCVAb were acceptable. The lower limit of detection was particularly good with the HBsAg reagent, and the negative values of HBsAg were clearly distinguished. The eminent sensitivity of the HBsAg testing by HISCL would be effective for monitoring HBV positive patients.

E-05

Rapid Molecular Detection of Sexually Transmitted Infections using a fully automated, microfluidic CARD™

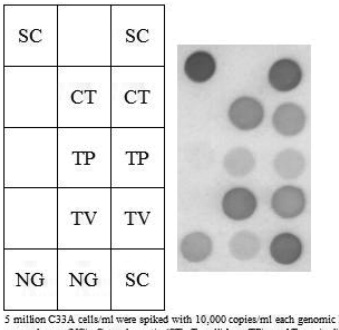
G. Spizz[1], R. Yasmin[1], W. Honey[1], T. Roswech[1], T. Graham[1], S. Rink[1], P. Zhou[1], R. A. Montagna[2]. *[1]Rheonix, Inc., Ithaca, NY, [2]Rheonix, Inc., Grand Island, NY,*

Objective: To develop a fully integrated and automated molecular diagnostic CARD (Chemical and Reagent Device) to simultaneously detect four sexually transmitted infections (N. gonorrhoeae, C. trachomatis, T. pallidum and T. vaginalis) via multiplex PCR followed by reverse dot blot (RDB) assay.

Relevance: Sexually transmitted infections (STIs) represent a considerable global health burden with an estimated 340 million new cases occurring each year. Although modern antibiotic therapy could cure most of these cases, the early stages of infection often go unnoticed and can proceed to disabling pelvic inflammatory disease in women, which in turn can lead to infertility, infant mortality and infant blindness. Nucleic acid diagnostics are currently available for some of these infectious agents, but test complexity and need for sophisticated equipment make then unsuitable for many nontraditional health care settings.

Methodology: Once applied to the CARD the samples are subjected to the following automatic steps, without further user intervention: cell lysis, nucleic acid purification, PCR amplification and RDB. PCR is performed using biotinylated primer pairs designed to amplify genes specific to the individual organisms. The denatured amplicons can then hybridize to membrane bound capture probes and detected by automatic addition of streptavidin-conjugated HRP and substrate. Validation: Human C33A cervical epithelial cells were spiked with different levels of genomic DNA from the four species of interest, singly or in combination. The figure shows a representative RDB resulting from STI CARD assay of C33A cells containing 10 copies of each of the 4 pathogens.

Conclusions: The CARD STI assay provides a "turn key" solution to automatically perform rapid, point-of-care molecular testing for four sexually transmitted infections. In addition, the CARD assay is fully adaptable to an increased target set in order to include other known STIs. Ease-of-performance of the assay makes it well suited for use in nontraditional healthcare settings.



5 million C33A cells/ml were spiked with 10,000 copies/ml each genomic DNA from *N. gonorrhoeae (NG), C. trachomatis (CT), T. palidum (TP), and T. vaginalis (TV),* followed by analysis of 1 µL (i.e., 10 copies) on STI CARD™. Left panel: Filter key; SC: Spoting Control- biotin labeled poly-A. Right panel: Results from STI CARD demonstrate the presence of all 4 STIs as anticipated.

E-06

Improved Accuracy and Precision of Methotrexate Reagent Packs on the Siemens Dimension RXL and Vista Analyzers

D. Gallagher, V. Bush. *Bassett Medical Center, Cooperstown, NY,*

Background. The user-applied capability of Dimension instruments allows laboratories to incorporate assays into the menu without a pre-manufactured reagent flex. The Siemens/Syva® EMIT homogeneous enzyme immunoassay for methotrexate is one such kit where off-board prepared reagents can be added to wells of empty flexes with user defined parameters. When following the manufacturer's instructions for reagent flex preparation, we experienced calibration failures, poor precision and poor reagent stability. Calibration failures and QC drifts were noted after 24 hours for on-board opened wells, rather than the claimed 15 days.

Objective. Describe a method for improving the open analyzer stability (OAS) and precision of the Siemens/Syva EMIT methotrexate assay on the Dimension RXL and VISTA.

Methodology. Reagents were prepared according to Dimension's instructions with modifications made to filling and storing flexes. Center holes were made in the well for both venting and reagent filling to facilitate sealing. After the addition of reagents, holes were sealed with tape to reduce evaporation during storage on and off the analyzer. The OAS was reduced to 24 hours. Ten or 20 test flexes were prepared and stored off-board the analyzer for up to 4 weeks. Sufficient reagent was added to the wells for the expected test volume to minimize waste.

Results. Two-way ANOVA showed statistical differences in %CV among level of QC/Cal and flex method used (table below). Additionally, calibration standards met expected targets on initial calibration.

Discussion. With the modified preparation technique and reduced OAS, fewer calibrations required repeating. Sealing the prepared flex after filling and limiting the well openings to one hole improves precision. The intra- run CVs and functional sensitivity (0.2 µmol/L) of this preparation, coupled with quick turn-around time, is acceptable for monitoring patients on methotrexate therapy. A similar technique could be applied to any third party assay on the Dimension instruments.

Precision Data

QC Level (peer group mean)	%CV Non-sealed Flexes on RXL	%CV Sealed Flexes on RXL	%CV Sealed Flexes on VISTA	Level/Flex Method p-values
Inter-run Precision				
QC 1 (0.5 umol/L)	32	27	18	0.006/0.04
QC 2 (1.5 umol/L)	13.8	11.7	10.7	
QC 3 (9 umol/L)	18.3	14	10	
Intra-run Precision				
QC 1 (0.5 umol/L)	28	11.9	6.4	0.073/0.016
QC 2 (1.5 umol/L)	14	2.8	4.1	
QC 3 (9 umol/L)	14	3.7	5.0	
Calibrator Precision				
Cal 1 (0.2 umol/L)	55.5	25.7	8.6	0.097/0.343
Cal 2 (0.5 umol/L)	12.8	7.1	6.5	
Cal 3 (1.0 umol/L)	8.7	4.8	6.2	
Cal 4 (1.5 umol/L)	5.1	4.4	8.0	
Cal 5 (2.0 umol/L)	5.6	5.4	9.1	

E-07

Preliminary biological evaluation of dextran-mannose derivates labelled with ^{99m}Tc for receptor targeted lymphoscintigraphy

D. Niculae[1], M. Purice[2], I. Pirmettis[3], M. Iliescu[1], I. Patrascu[1], C. Mustaciosu[1], G. Voicu[2], I. Ursu[1], C. Tuta[1]. *[1]"Horia Hulubei" National Institute of Physics and Nuclear Energy, Bucharest, Romania, [2]"C.I.Parhon" National Institute of Endocrinology Bucharest, Bucharest, Romania, [3]NCSR Demokritos Institute of Radioisotope-Radiodiagnostic Products, Athens, Greece,*

Different microcolloids of 99mTc-labelled albumin are presently utilized for sentinel node detection but none of these agents have ideal properties regarding selective accumulation, being uptaked by distal lymph nodes also. The lymphoscintigraphy agent requires a high density of receptor substrate sites to achieve a receptor affinity required for proper sentinel node detection. The solution for this problem could be given by receptor-binding radiopharmaceuticals that can be synthesized with high specific activities, compatible with typical target tissue receptor densities. The aims of this study were to radiolabel mannosyl-cysteine-dextran macromolecules with 99mTc to obtain a high purity and stable radiolabelled conjugate suitable for sentinel node detection with distal lymph accumulation and in vivo biological evaluation.

Methods Different probes containing dextran-cysteine, dextran-cysteine-mannose, dextran-cystein-amine-mannose, synthesised at NCSR Demokritos, Greece were tested. Different radiolabelling strategies, including novel ^{99m}Tc-tricarbonyl cores, were evaluated in order to select and optimize the most efficient and specific one. The quality control of radiolabelled conjugates using ITLC (acetonitrile, physiologic saline (0.9% NaCl) or acidified methanol (0.1% TFA) as the mobile phase) and HPLC with radiation detection (Nucleosil C-18 reversed-phase column, 5µm, 250 mm x 4.6 mm) was performed. The biological behaviour of the compounds was tested in vivo (gamma imaging lymphoscintigraphy) on Wistar rats. The specific uptake and accumulation in the sentinel lymph node were evaluated. All the experiments were made also with NANOCIS as gold standard, for comparative evaluation.

For lymphoscintigraphy 0.1 mL of the radiopharmaceutical (9.25-18.5 MBq) was injected intradermal into both rear footpads of the rats. The acquisitions were done in dual detector e.cam Signature Series gamma camera (SIEMENS Madical Solutions Inc., USA), using LEHR collimators, anterior detector only, matrix size 256x256, zoom 2 (1 min/frame; 60 frames) for dynamic studies and matrix size 512x512, zoom 2 for static studies.

Results Both static and dynamic acquisitions were done for imaging studies of ^{99m}Tc radiolabelled dextran derived compounds. We explored their uptake, clearance and in vivo stability from the injection in experimental model up to 3 h post injection. The time-activity accumulation curves in SLN and in whole body were determined for each compound. Analyzing the images, an optimal in vivo behaviour was observed for 3 tested compounds: a fast accumulation in sentinel (popliteal) lymph node of 15-30 min, low accumulation in critical organs and a very good in vivo stability, up to 3 h post injecton.

Conclusions The 99mTc labelled dextran-mannose derivatives show specific accumulation in the sentinel lymph node. We propose further evaluation of 3 selected compounds for which the dynamic studies confirmed the rapid uptake of and in vivo stability, up to 3 h p.i. as potential agents for targeted lymphoscintigraphy.

E-08

ice-COLD-PCR enriches all low-prevalence unknown mutations efficiently using a wild-type-blocking reference sequence during COLD-PCR

C. A. Milbury[1], J. Li[1], G. M. Makrigiorgos[2]. *[1]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, [2]Dana-Farber/Brigham and Women's Cancer Center, Harvard Medical School, Boston, MA,*

Objectives: Molecular profiling of somatic mutations in cancer often requires the identification of low-prevalence DNA mutations in an excess of wild-type (WT) DNA; however, the method selectivity and sensitivity are often limiting factors. COLD-PCR (CO-amplification at Lower Denaturation Temperature) resolves several limitations in low-level mutation detection by using critical denaturation temperatures to enrich mutant-containing amplicons during PCR. However, certain mutation types are not enriched by some COLD-PCR formats. We report a novel enhancement in COLD-PCR that enables Improved and Complete Enrichment (ice-COLD-PCR) for all mutation types in an efficient and robust manner.

Methodology: A Reference Sequence (RS) that preferably forms double-stranded structures with WT sequences, but not with mutant sequences, was added to COLD-PCR. A RS was designed to bind to one WT-sequence strand, avoid primer binding and prevent polymerase extension. To validate the use of a RS in COLD-PCR, we evaluated segments of TP53 exon 8. Serial dilutions of mutant cell-line DNA, or human lung tumor DNA, in WT DNA were analyzed. Mutations that increase, decrease, or retain the amplicon melting temperature were tested. Following conventional-PCR, COLD-PCR (full or fast COLD-PCR formats), and ice-COLD-PCR methods amplicons were sequenced and the degree of mutation enrichment was compared. Several clinical lung adenocarcinoma samples with known low-level mutations were also analyzed with ice-COLD-PCR.

Validation: ice-COLD-PCR yielded ~13-fold enrichment for Tm-increasing and Tm-equivalent mutations, and ~15-fold enrichment for Tm-reducing mutations. In contrast, Full-COLD-PCR demonstrated ~5-fold enrichment for all mutations. Further, fast-COLD-PCR, which can only enrich Tm-reducing mutations, exhibited ~17-fold enrichment for these types of mutations, while the Tm-increasing and Tm-equivalent mutations remained undetectable. Regardless of mutant type and position, after ice-COLD-PCR amplification, all mutation types are strongly enriched and can be reliably sequenced down to a level of 1-3%. Ice-COLD-PCR duration is ~1hour, compared to several hours for full-COLD-PCR.

Conclusions: The inclusion of an appropriately designed RS within COLD-PCR selectively inhibits WT amplification throughout PCR, while preferentially enriching mutants and reducing time-intensive hybridization times. Ice-COLD-PCR combines high sensitivity, speed, and low-cost, and facilitates direct sequencing for all types of unknown low-prevalence mutations in clinical cancer samples.

E-09

Assay Validation for Quantitative Immuno-PCR (qIPCR) in the Ultrasensitive Detection of Ricin Toxin in Human and Mouse Sera

J. Barletta, A. Bartolome, N. Constantine. *University of Maryland, Baltimore, MD,*

Ricin is a Category B agent of biological terrorism which is easily mass produced and disseminated. The limit of detection for immunological methods is at nanogram/mL concentrations. In mice, the LD_{50} of Ricin ranges from 3-24 g/kg by inhalation or injection. Ricin must be detected at femtogram/mL concentrations for accurate diagnosis of specific intoxication. When using highly sensitive assays such as qIPCR, it is important to develop qualified validation protocols for accurate quantification of molecules (such as Ricin) which are highly toxic at ultra-low levels (fg/mL). In this report, we outline the validation protocol for the real-time immuno-polymerase chain reaction (qIPCR), a combination of the ELISA and PCR, and show the method is more sensitive for Ricin detection than other currently reported methods.

qIPCR displays improved statistical validation of accuracy over IPCR. Inter-assay precision of qIPCR is typically 5-10% vs 15-20% for IPCR. Using qIPCR, immediate interpretation and quantification of positive data is possible and has great importance for specific emergency situations when pathologic proteins may be present in body tissues at extremely low concentrations and rapid, early diagnosis is important for immediate palliative treatment.

We present a qIPCR validation protocol suitable for FDA approval in the quantification accuracy for the ultrasensitive detection of Ricin in human and mouse sera. Using qIPCR, the limit of detection ranged from 1-10 fg/mL Ricin in normal human and mouse plasma with standard curves displaying a linear correlation coefficient of 0.967-0.997. This limit of detection is 10-100X more sensitive than the highest LOD achieved using the ELISA method (10 pg/mL) for Ricin detection.

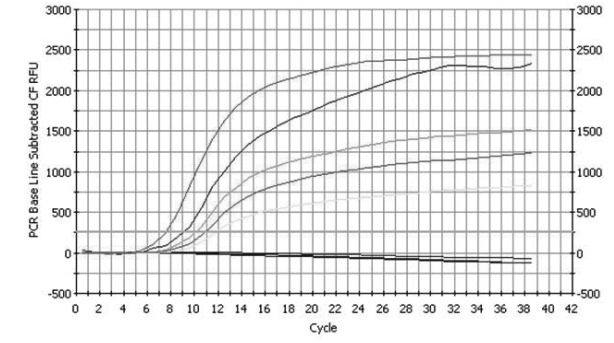


Fig. 1. Validation of Ricin Limit of Quantification. A standard calibration curve for Ricin spiked into normal human plasma (NHP) and normal mouse plasm (NMP) was tested using the qIPCR method. The limit of quantification was 100 fg/mL Ricin A in NHP, 1 fg/mL Ricin B in NHP, and 100 fg/mL Ricin A in NMS.

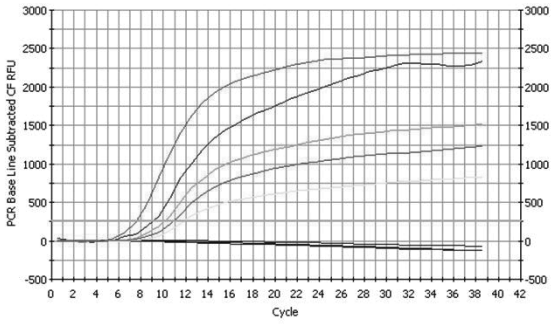


Fig. 1. Validation of Ricin Limit of Quantification. A standard calibration curve for Ricin spiked into normal human plasma (NHP) and normal mouse plasm (NMP) was tested using the qIPCR method. The limit of quantification was 100 fg/mL Ricin A in NHP, 1 fg/mL Ricin B in NHP, and 100 fg/mL Ricin A in NMS.

E-10

Development of Microfluidic Chip for Diagnosis of Influenza A to Improve Discomfort in Nasopharyngeal Specimen Sampling

H. Aoki, Y. Yamagata. *RIKEN, Wako, Japan,*

BACKGROUND: Diagnosis of influenza virus (Flu) widely uses immunochromatography (IC) that detects Flu in nasopharyngeal specimen (NPS). The NPS are collected with swab or aspiration; however, these methods are uncomfortable, because a swab tip is still large, for example, 3 by 15 mm and 75 μL-absorption. One approach to reduce this discomfort is to miniaturize this swab, which will lead to the reduction of collected NPS and sensitivity. We have developed a multiplex microfluidic chip (16 channels, 1 μL/channel) using chemiluminescence immunoassay and electrospray deposition (ESD) methods that spots proteins into nanoporous structures on the microchannels. We examined to detect a small volume of Flu A with this microfluidic chip and evaluated its sensitivity.

METHODS: Anti-Flu A nucleoprotein antibody (Anti-Flu A, HyTest, 1.8 ng/spot) were spotted on the microchannels with the ESD method and set into an automatic pumping apparatus. Immunoassays (n = 3) were done as following: wash with 0.6 mL of 2% ECL Advance Blocking Agent-PBS (EABA, GE Healthcare) for 10 s; immunoreaction with 10 μL/channel of Flu A (0-8 μg/mL in EABA, HyTest); wash; immunoreaction with 0.6 mL of HRP-Anti-Flu A (HyTest) for 10 min; wash; detection with ECL Advance (GE Healthcare) and a CCD camera for 1 min. The same Flu A (300 μL/assay) was subjected to IC (QuickVue Influenza A+B Test, Quidel).

RESULT: The microfluidic chip shows a good linearity ($R^2 = 0.984$) in the range of 0.125-2 μg/mL of Flu A (Fig. 1). Sensitivities of the microfluidic chip and the IC were 0.192 and 2 μg/mL, respectively. The microfluidic chip can reduce a volume of swab NPS to 2.6 μL with 10-fold higher sensitivity than that of the IC. Therefore the microfluidic chip will be able to diagnose Flu using the miniaturized swab stick with high sensitivity and improve the discomfort in NPS sampling.

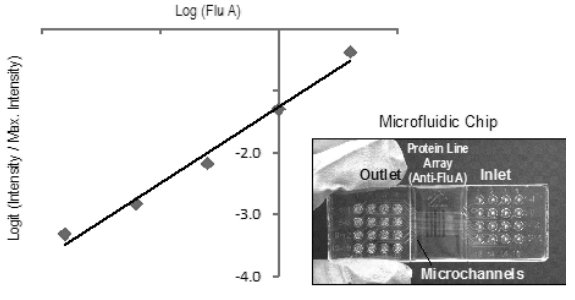


Fig.1 Standard Curve of Immunoassay of Flu A using the Microfluidic Chip.

E-11

First Precise Measurement Of Turnaround Time (Tat) Of Laboratory Processes Using Radiofrequency Identification (Rfid) Technology

H. Mayer[1], J. Brümmer[1], T. Brinkmann[2]. *[1]1Zentrum für Labormedizin, Mikrobiologie und Transfusionsmedizin, Städtisches Klinikum Karlsruhe, Karlsruhe, Germany, [2]Beckman Coulter, Nyon, Switzerland,*

Background. Turnaround time (TAT) is traditionally measured as time from receipt to time of test reporting, e.g. analytical or process TAT. Traditional measurements of mean TAT are useful largely for assessing intra-laboratory processes by extraction of TAT data from LIS. However, a precise TAT measurement requires additional data analysis tools.

Methods. We provided the emergency department of our hospital with 1,300 serum tubes (Sarstedt, Germany) labelled with RFID tags (Magellan, Australia) for common STAT clinical chemistry and troponin analysis. The following time stamps were measured with different RFID receivers in the laboratory: 1. pneumatic tube system reception, 2. centrifuge, 3. sample distributor AutoMate 1200 (Beckman Coulter), 4. analyser entry. In addition time stamps of LIS registration and analyser report were used for calculation.

Results. In total n=889 RFID-tagged serum tubes were used for TAT calculation. The following time periods were measured in h:min:sec: Pneumatic tube system → centrifuge (0:00:27-0:29:44, mean 0:01:55); centrifuge → automatic sample distributor (0:08:36-1:00:12, mean 0:15:49); automatic sample distributor → analyser entry (0:00:00-1:45:39, mean 0:04:48); analyser to result (0:00:25-16:12:20, mean 0:15:16). Approx. 40% of TAT was related to the centrifuge.[this would present nicely in a table]

Conclusion. TAT reduction was attributed to elimination of manual entry of laboratory orders into LIS. With this unique study design we were able to measure precisely the TAT in a medical laboratory. 40% of TAT is related to manual processes around the centrifugation . Laboratory personnel are often not aware of these processing time factors.

E-12

Quantitation of Ketosteroids in Human Serum Using Derivatization Chemistry and LC/MS/MS

M. Weinstock, B. Williamson, B. Purkayastha, S. Dey, S. Pillai. *AB SCIEX, Framingham, MA,*

Background: LC-MS/MS is becoming more widely used in the field of ketosteroids analysis due to its specificity and accuracy. However, the ionization efficiency for many neutral steroids may not be sufficient to reliably quantify trace concentrations in human serum. Derivatization with ionizable moiety could significantly improve the ionization efficiency of neutral steroids.

The objective of this study was to develop a highly sensitive electrospray (ESI) LC/MS/MS method to analyze ketosteroids in human serum by utilizing a novel derivatization chemistry.

Methods: The ketosteroids (e.g.Testosterone, Pregnenolone, Progesterone, etc.) are enriched from 200uL of serum samples by solid phase extraction. The samples are dried and the derivatization reaction is performed in acidic conditions (MeOH+acetic acid) for 15min at room temperature. LC-MS/MS separation and analysis is performed using a reversed phase column, H_2O/organic solvent/Formic acid gradient and a standard MS/MS instrument with ESI source, operating in MRM mode. Quantitation of the derivatized ketosteroids is enabled by using an isotopically enriched reagent as an Internal Standard (IS). A known amount of IS is spiked into the sample before analysis and the unknown concentration is extrapolated from the analyte/IS peak area ratio of a known standard curve.

Results: Product ion spectra of the derivatized ketosteroid (e.g.Testosterone, Progesterone, Pregnenolone, etc.) produced signature ions which are predominant and specific to the product. The resultant signal enhancement factor relatively to the underivatized ketosteroid is ~30-100 folds. The LODs for the derivatized ketosteroids in double charcoal stripped serum are 2.5-15pg/mL.The geometrical isomers of the ketosteroids which are formed upon derivatization can be chromatographically separated, or co-eluted as a single peak using a fast LC gradient. The ketosteroids derivatization method produced linear and reproducible data (<10%CV at LOQ). The Derivatization efficiency is >95% and the whole workflow (sample extraction plus derivatization) is amenable to automation. The sensitivity enhancement upon derivatization enables the detection of trace amounts of ketosteroids in biological samples.

Conclusions: A novel derivatization reagent for ketosteroids significantly improves the limits of detection by ESI/MS/MS and enables the detection of very low concentrations in serum samples.

E-13

Development of a dried blood collection, transport and test system for C-Reactive Protein for use in assessing inflammatory status in a dental setting

C. N. Warner, J. A. Maggiore. *Healthy Life Laboratories, Bannockburn, IL,*

Background: Much press has been given to the "oral-systemic connection", and the importance of good oral health to maintaining good overall health. One of the evident markers of oral inflammation is the acute phase reactant, C-Reactive Protein (CRP), which becomes elevated in the bloodstream during periodontal maladies. CRP may thus be used as both a marker of oral inflammation, and a tool to assess the effectiveness of periodontal therapies. Dental professionals have looked to the clinical laboratory community to provide a simple, accurate, and sensitive means to determine CRP in a dental office setting. Since dental offices are not staffed to perform moderately complex laboratory testing, and venipuncture is not practical in dental offices, our objective was to develop a CRP laboratory method for a fingernick blood sample which could be collected chairside in a dental setting.
Methods: The Roche Integra hs-CRP method was adapted for the Roche Diagnostics Integra 400 System to measure CRP in extracted capillary DBS samples collected onto Whatman 903 filter paper. Blood extractions were made by punching four 3-millimeter spots from a dried blood sample and eluting into a buffered saline solution. Assay modifications included increasing the analytical sample volume, decreasing the antibody reagent volume, adapting the calibration from a spline curve to a linear model, and decreasing the lowest measurable set point of the calibration curve. Test method validation included assessment of precision, analytical accuracy, sensitivity, linearity, and hemoglobin interference. The accuracy of the system was assessed by comparing CRP results from DBS samples as determined by the modified Roche Integra method, to CRP in paired plasma samples as determined using the standard Roche Integra hs-CRP assay.
Results: Within-run precision coefficients of variation (CV's) (n=20) at CRP levels of 1.0 and 3.9 mg/L were 7.7% and 5.8%, respectively. Between-run CV's (n=20) at the same CRP levels were 11.8% and 9.5%, respectively. The minimum detectable concentration of DBS CRP was determined to be 0.40 mg/L, (n = 20). Assay linearity was demonstrated between 0.6 and 20.0 mg/L). Least-squares regression analysis comparing plasma and DBS levels of CRP (n=55, range 0.4 - 19.8 mg/L) yielded a correlation coefficient (R^2) of 0.98, $y = 0.9995x + 0.008$. No influence from any degree of hemolysis was apparent across the analytical range for CRP.
Conclusions: The Roche Integra hs-CRP assay modified by Healthy Life Laboratories for DBS analysis was shown to provide a strong correlation with the Roche plasma method, with suitable precision and sensitivity for use in a dental setting to assess oral inflammation. Dental professionals have begun incorporating CRP testing as part of their periodontal assessment and treatment protocols, and have successfully demonstrated that the collection of a fingernick sample using the provided single-use, self retracting, and minimally invasive lancet is within their accepted scope of patient care services.

E-14

Enhanced melting curve analysis for mutation detection with self-quenched probes

Q. Huang, Y. Liao, Y. Zhang, Q. LI. *XMU, Xiamen, China,*

Backgrond and objective: Despite the wide use of melting curve analysis (MCA) in mutation detection, the existing chemistry has limited multiplexing ability and is restricted to detecting only a small number of known mutations in one reaction. Our aim was to identify alternative chemistries for MCA with improved multiplexity that could detect increased number of mutations, differentiate mutation types, and even identify unknown mutations.
Methods: We evaluated 10 chemistries currently used for real-time PCR in their ability of MCA. With the newly chosen chemistries, we established three enhanced multi-color MCA systems, which were however impossible for current MCA method. They included a 5-color MCA for genotyping of multiple mutations, a 3-color MCA for identification of mutations and discrimination them from polymorphic sites, and 4-color MCA for detecting unknown mutations within amplicons. All the systems were evaluated with blinded clinical samples and compared with sequencing analysis. The detection ranges were also investigated.
Results: We found that all probes that could elicit fluorescence change upon hybridization could be used for MCA. Among these probes, the stem-shared molecular beacons and nonfluorescent quencher-labeled TaqMan probes were advantaged by their high signal-to-noise ratio and were particularly suitable for multi-color MCA. With these probes, we established a new MCA system with both mutiplexity and flexibility improved. In one example, genotyping of up to 16 mutations in β-thalassemia was achieved with a 5-color MCA system. In another example, 6 mutation types in hepatitis B virus causing resistance to lamivudine and adefovir dipivoxil plus a polymorphic site were all correctly differentiated with a 3-color MCA system. Finally, mutations randomly occurred in an 81-bp region of *rpoB* gene causing resistance to rifampin were also successfully identified with a 4-color MCA system. The results with blinded samples were completely concordant with the sequencing analysis. All the three MCA could detect samples of concentrations ranging from 3 to 10^6 copies or CFU per reaction.
Conclusion: The increased multiplexity and flexibility of this new MCA system together with the wide availability of the chosen chemistries and their compatibility with regular real-time PCR thermocycler should boost MCA up to wider applications.

E-15

Development of ultra-sensitive immunoassay for HCV core antigen using antibody-dextran polymer conjugate labeled with bioluminescent enzyme, firefly luciferase

Y. Ohiro[1], H. Shimizu[1], N. Shimizu[1], S. Takayasu[1], H. Tsuge[1], Y. Sugamata[2], N. Maki[2], H. Arakawa[3]. *[1]Eiken Chemical Co., Ltd, Tochigi, Japan, [2]Advanced Life Science Institute, Inc., Saitama, Japan, [3]Showa University, Tokyo, Japan,*

The worldwide prevalence of Hepatitis C virus (HCV) infection affects estimated 200 million people, and approximately 70 % of HCV infected patients progress to chronic hepatitis. Since the c DNA of HCV was cloned in 1989, the study of HCV has been greatly advanced with the development of the diagnostic technologies, such as HCV antibody test and HCV RNA test. Furthermore, the immunoassay for HCV core antigen was recently put to practical use and it has been widely used in the diagnosis of HCV or the monitoring of HCV viral load of patients. Although the immunoassay is a more economical and simple method to detect HCV than HCV RNA test, the sensitivity is not enough to diagnose the early stage of HCV infection or to determine the disappearance of the HCV through therapy. Therefore, aiming to attain the equivalent sensitivity to the quantitative HCV RNA test, we constructed an ultra-sensitive immunoassay for HCV core antigen using bioluminescent enzyme with high quantum yield.
This method is based on the pretreatment of the specimen and two-step enzyme immunoassay. The objectives of pretreatment are inactivation of HCV antibody and extraction of the HCV core antigen. This method consists of antibody-coated magnetic particles as a solid phase and thermo-stable biotinylated firefly luciferase as a label. Firefly luciferase is suitable for high sensitive detection due to its high quantum yield. In this study, we succeeded in development of more sensitive method than conventional immunoassay by constructing antibody-dextran polymer conjugates labeled with the firefly luciferase. The assay protocol is as follows. Sample is mixed with pretreatment solution in a test tube. The mixture is incubated at 37°C for pretreatment. Then, reaction buffer and anti-HCV core antigen antibody immobilized on magnetic particles are added to the pretreated sample and incubated at 37°C as the first reaction. After washing the magnetic particles, they are suspended in designed antibody polymer conjugate solution. The suspended solution is incubated at 37°C as the second reaction. After another washing, the substrate (luciferin) solution is added to the test tube and the luminescent intensity is measured.
The measurable range of HCV core antigen was 1 to 10,000 fmol/L. The detection limit was 0.5 fmol/L (as the zero standard + 2.6SD). Also, all of 212 negative samples were quantified under the range. The intra-assay precisions (CV) at 1.1, 20.9 and 1271.1 fmol/L samples were 4.9%, 8.6% and 5.4% respectively, indicating good precision. In the dilution test using specimens, a good recovery rate was obtained until sixteen-fold dilution. The correlation between this method and a commercial chemiluminescent immunoassay "Lumispot 'Eiken' HCV antigen" was excellent. (y=0.955x+361.95, r=0.932, n=51)In the measurement of five series of seroconversion panels, this method indicated equivalent sensitivity to HCV RNA test.
This method was 40-fold more sensitive than conventional chemiluminescent immunoassay. It is possible to apply this method to a fully automated immunoassay system because it employs mild pretreatment of sample.

E-16

Laboratory Validation of Diagnostic Utilities of the Nucleic Acid-based Human Papillomavirus Typing Kit Clinichip® HPV

Y. Takahashi, N. Kondo, Y. Hiraishi, S. Oguchi, N. Shimizu, T. Fujii, M. Murata, M. Wakui. *Keio University Hospital, Shinjuku-ku,Tokyo, Japan,*

Background:Human papillomavirus (HPV) is detected in 99% of patients with invasive cervical cancer, and, to date, at least 100 types of HPV have been identified. Of these, 13 HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) are known to be associated with a high risk of cervical cancer. It is therefore clinically important to perform efficient HPV genotyping. Conventionally, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or PCR sequencing has been employed to detect HPV. However, these procedures are complicated and the long assay times often preclude their introduction as laboratory tests. In order to provide simpler procedures and shorter assay times, a new assay method, Clinichip HPV, has been developed by combining the loop-

mediated isothermal amplification (LAMP) method with a current-detecting DNA chip. In this study, to evaluate the performance of this new assay method, we determined the reproducibility of HPV genotyping using synthesized DNA in a preliminary test, and then assessed the rate of concordance with the results obtained by PCR sequencing.
Methods:In these studies, we used a Genelyzer DNA detection system (Toshiba Hokuto Electronics Corporation). 1) Within-day reproducibility: Clinichip HPV was used for assays performed in triplicate using PCR products of 2 different types (A, a mixture of HPV types 18 and 31; B, a mixture of HPV types 35 and 51) obtained from synthesized DNA. 2) Day-to-day reproducibility: Measurements were performed on 3 different days using the same samples as those used in the test of within-day reproducibility. 3) Concordance rate: Using QIAamp, HPV DNA was extracted from the cervical smear samples of patients whose HPV types had previously been identified by PCR sequencing. Subsequent assays were performed using Clinichip HPV. We then evaluated the consistency between the results obtained and the established data.
Results: Data for both within-day reproducibility and day-to-day reproducibility were obtained for all the PCR products of the 2 different HPV type mixtures obtained from synthesized DNA. Assessment of the concordance rate showed that the results of the initial assay were consistent for 16 of the 18 clinical samples and were inconsistent for the remaining 2 samples. After re-examination, the concordance rate was 100%. The 2 samples that produced inconsistent results during the initial assay are being further examined.
Conclusion:Clinichip HPV was able to detect 13 different high-risk HPV types rapidly and easily in both PCR products and clinical samples. These results indicate the feasibility of using this kit as a laboratory test.

E-17

One Step Detection of Pathogenic Bacteria with Sexually Transmitted Disease (STD) by Single-Tube Multiplex PCR

L. Jiaming, L. Jinnan, W. Mingtian. *West China Hospital of Sichuan University, Chengdu, China,*

Background: We developed and evaluated a single-tube multiplex PCR (mPCR) assay with silver stain to simultaneously detect Gonorrhoeae(NG),Chlamydia trachomatis(CT) and Ureaplasma urealyticum(UU)bacteria in reproductive tract secretion.
Method: This method employed a single tube and multiple specific primers which yielded 171,207,313bp PCR products, respectively. Three sets of special primers directed to the Pjd1 plasmid gene of NG(674bp-844bp), Plgv440 hidden plasmid gene of CT(63bp-269bp) and 16S rRNA coding region of V5 and V7 regions of UU(432bp-732bp) were designed respectively using Primer Priemer 5.0,Oligo 6.0 and Blast in Genebank in order to check their specificity and guarantee similar melting temperatures. The primers were as follows:NG:5'CCTATCGGGCTGTTATCTGATTATGG3';5'AAGAAGAGGTCGGGTTGGGTGTAT3';CT:5'CTTCCCCAGAACAATAAGAACACA3';5'TAGGGATTCCTGTAACAACAAGTCA3';UU:5'TAAATGTCGGCTCGAACGAG3';5'GCAGTATCGCTAGAAAAGCAAC3'.Standard strains were collected from clinical microorganism Division, West China Hospital of Sichuan University following routine processing, and were subjected to the mPCR assay.PCR system contained reaction buffer(10*)7.5ul;DNTP(10mmol):1.5ul;Taq enzyme (5U/ul):1.5ul;Mg2+(25mmol/ul):4.5ul;Primers (20P)6ul;water:440ul;template:each bacteria 2ul. Thermal cycling parameters were as follows: 94°C 4min,94°C 30s,62°C 30s,70°C30s,36 cycles, finally 72 °C10min.We used silver stain after amplification. Interference test was done to evaluate its validity using E. coli and S. aureus which were provided by laboratory medicine department, West China following routine processing. Serial dilutions in the range from 10-10 fg/µl to 10-5 fg/µl were done to evaluate sensitivity. To evaluate clinical match rate, we performed clinical test on the detection of 50 residual discard urogenital secretion specimen collected between June and August in 2009 and stored at -20°C by laboratory medicine department, West China with fluorescence-quantitative PCR in conjunction with another detection technique as the golden standard.
Result: It could detect NG,CT and UU at one time. The resulting amplicons were cloned and sequenced to match the expected sequences exactly. The 30 cases of STD positives (including 22cases of mixed infections) and 20 cases of STD negatives detected by fluorescence-quantitative PCR in conjunction with another detection technique matched the results detected by single-tube multiplex PCR technique. The sensitivity, specificity and match rate were 10-9fg/µl,100% and 98.0% respectively. There was no significant difference between mPCR specimen and controlled specimen(P=0.09) using Chi-square test. There was no interaction or strong inhibition as well. The repeatability of 7 continuous days of 50 clinical specimens was good.
Conclusion: This mPCR assay with silver stain features good specificity, the lowest sensitivity which is similar to simple PCR test and fluorescence quantitative PCR and good repeatability as well. Meanwhile, it is cheap, simple, fast, accurate and reliable. Thus, this mPCR assay may be useful for the rapid diagnosis of single and multiple STD infections.

E-18

Multicenter evaluation of a new HbA1c Gen. 3 assay and liquid HbA1c quality controls on Roche/Hitachi, COBAS INTEGRA® and cobas c systems

R. Roeddiger[1], Y. Schimpf[2], G. Hafner[3], N. von Ahsen[4]. *[1]Roche Diagnostics GmbH, Clinical Trials, Mannheim, Germany, [2]Synlab Clinical Trial GmbH, Berlin, Germany, [3]Zentrum fuer Labormedizin und Mikrobiologie GmbH, Essen, Germany, [4]Georg-August-Universitaet Goetingen, Institut fuer Klinische Chemie, Goettingen, Germany,*

Background/Objective: The present abstract describes the analytical performance of a new HbA1c assay (Tina-quant® [a] HbA1c Gen. 3, Roche Diagnostics GmbH, Mannheim, Germany) with applications on RD/MODULAR P, COBAS INTEGRA® 800 and **cobas c** 501 systems.
Methods: Total Hb is measured bichromatically during the pre-incubation phase of the immunological HbA1c determination without a separate colorimetric reagent. The method is based on the turbidimetric inhibition immunoassay principle. The assay is standardized against the IFCC reference method and converted to results traceable to DCCT/NGSP by calculation. All experiments are performed following a protocol based on the concepts of CLSI documents and in compliance with the Standard Operation Procedures (SOP) of Roche Diagnostics.
Results: Recovery of assigned IFCC and CAP samples (n=10) was found within a range of 100 ± 9.8% HbA1c. Repeatability (%CV) ranged from 0.5 - 2.5 (Median 1.1) for concentrations between 5.1 - 10.9 % HbA1c using new liquid ready to use quality control materials PreciControl HbA1c norm and path. CV's calculated from daily routine simulation experiments (cobas c 501, n=20 days) using 2000 aliquots without weekly cleaning solution and 4000 aliquots with weekly cleaning solution gave comparable results (0.9 - 3.0 % and 1.1 - 1.7 % respectively). The primary measuring range, which was defined by linearity testing, was confirmed from 2.48 to 24.8 mmol/L Hb and 0.186 - 1.55 mmol/L HbA1c (recovery rate: 100 ± 10%). Statistical Passing/Bablok analysis of method comparison against Tina-quant® [a] HbA1c Gen. 2 and Biorad HbA1c yielded correlation coefficients > 0.96, slopes between 0.94 - 1.10 and intercepts from - 0.64 to + 0.48 % HbA1c using ≥ 122 whole blood samples.
Conclusions: In this study, the new HbA1c Gen. 3 assay demonstrated highly reliable analytical performance, good correlation with existing HbA1c methods and convenient reagent handling during routine use.
Disclaimer: The Roche HbA1c Gen. 3 assay is not yet cleared for use in the U.S.

E-19

Evaluation of Lipoprotein Particle Sizes Using Dynamic Light Scattering

T. Sakurai[1], S. Trirongjitmoah[2], Y. Nishibata[1], T. Namita[2], S. Jin[1], S. Hui[3], M. Tsuji[4], K. Shimizu[2], H. Chiba[1]. *[1]Faculty of Health Sciences, Hokkaido University, Sapporo, Japan, [2]Graduate School of Information Science and Technology, Hokkaido University, Sapporo, Japan, [3]Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Japan, [4]Division of Internal Medicine, Health Sciences University of Hokkaido Hospital, Sapporo, Japan,*

Background: Many studies have shown significant role of small dense LDL (sd LDL) in the development of atherosclerosis, and simple method for LDL particle-size determination is needed in clinical laboratories. In this study, dynamic light scattering (DLS) was tested for lipoprotein-size measurement, with special reference to sd LDL.
Methods: Calibration of DLS was done using polystyrene beads of 21 nm and 28 nm. Human serum lipoproteins were isolated by a combination of ultracentrifugation and gel chromatography or by sequential ultracentrifugation. All DLS measurements were done on Model FDLS-3000 (Otsuka Electronics Co., Ltd.), and a CONTIN algorithm was used for data analysis. The DLS measures of sample sera and the isolated lipoproteins were compared by nonparametric Kruskal-Wallis test. The DLS measures of the large LDL and sd LDL fractions were compared by Wilcoxon signed-rank test. A statistically significant difference was defined as $p < 0.05$.
Results: The polystyrene beads of 21 nm and 28 nm were determined by DLS as 19.1±0.9 nm (n=17, mean±SD) and 25.8±1.3 nm (n=17), respectively. The coefficients of within-run variation were 4.7% (21 nm) and 4.9% (28 nm). The DLS measures for the serum samples, the four isolated lipoprotein fractions (CM+VLDL+IDL, large LDL, sd LDL and HDL), and the non-lipoprotein fraction (the bottom fraction) were 13.1±7.5 nm, 37.0±5.2 nm, 21.5±0.8 nm, 20.3±1.1 nm, 8.6±1.5 nm, and 8.8±2.0 nm, respectively, with statistically significant differences among the fractions (n=11, p=0.00000051). The particle sizes for the sd LDL fraction were significantly lower than those for the large LDL fraction (p=0.016).
Conclusions: The proposed DLS method can differentiate the sizes of isolated lipoprotein particles, including large LDL and sd LDL.

E-20

Simultaneous quantitative endocrine immunoassays with Evidence biochip arrays.

F. M. Kelly, A. Beattie, P. Lowry, R. I. McConnell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background: The endocrine system is involved in the integration of the developmental system and co-ordination of metabolism, respiration, reproduction and sensory perception. It therefore impacts heavily on global health and well being. It functions through the actions of multiple hormones affecting cellular function. As such, it is of benefit to study the action of multiple hormones for any given disorder. A multiplexed measurement of hormones with a single sample using biochip array technology, offers more information than single analyte determination as it provides a patient's profile and increases the number of test results for each sample, thereby reducing cost and sample/reagent consumption.
Relevance: We report the performance evaluation of a biochip array for the simultaneous measurement of cortisol, dehydroepiandrosterone-sulphate, 17α-hydroxyprogesterone and leptin. This is of value as analytical tool for applications in clinical settings.
Methodology: The core of the biochip array technology is the biochip (9mm x 9mm) and represents not only the platform in which the capture ligands are immobilized and stabilised defining microarrays of discrete test sites, but is also the vessel where the simultaneous immunoassays take place. Simultaneous chemiluminescent combined sandwich and competitive immunoassays are employed in this array on the semi-automated bench top analyser Evidence Investigator.™ The system incorporates dedicated software to process and archive the multiple data generated.
Results: Performance evaluation of the biochip array shows specific recognition of the dehydroepiandrosterone-sulphate assay for the target (no cross-reactivity was observed with dehydroepiandrosterone or androstenedione). No interference with the leptin receptor (within normal expected values) was observed in the leptin assay. For the calibration ranges 0-800ng/ml (cortisol), 0-10µg/ml (dehydroepiandrosterone-sulphate), 0-20ng/ml (17 α-hydroxyprogesterone) and 0-100 ng/ml (leptin), the sensitivity values of the assays were <6.0 ng/ml, <0.003µg/ml, 0.1ng/ml and 0.64ng/ml respectively. Intra-assay and inter-assay precision, expressed as %CV were typically <12% and <15%.
Conclusion: Data indicates that biochip array technology is applicable to the simultaneous measurement of hormones with a single sample. The use of this multi-analytical methodology can allow for more accurate investigation in disorders of the endocrine system and the effect of environmental factors such as stress on its normal functioning.

E-21

Development of a liquid clinical chemistry control stable for the whole range of the analytical parameters.

A. Shanks, P. Armstrong, J. Campbell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background The use of liquid clinical chemistry controls is convenient and removes errors associated with the reconstitution required if lyophilised controls were to be employed. Some analytes in these multi-analyte liquid control materials, such as triglycerides, bilirubin, and enzymes are unstable at +2-+8^{0}C leading to implications in the stability and usefulness of the whole product. Liquid multi-analyte controls stable for the whole range of parameters are useful for quality control applications in clinical settings.
Relevance: We report the development of a liquid stable clinical chemistry control containing multiple analytes, including triglycerides, bilirubin and enzymes, which presents excellent stability for the whole range of the analytes. This is of value to assess accuracy and precision and to monitor performance in clinical settings.
Methodology: Multi-analyte liquid clinical chemistry human sera, containing 92 analytes**,** were generated at two levels covering normal and pathological ranges. Each level of control material was dispensed in 5ml vials and stored below -20^{0}C. Serum homogeneity was determined by measuring 125 vials for alanine amino transferase, aspartate amino transferase, cholesterol, creatine kinase, sodium, total bilirubin and triglycerides at two different concentration levels, results were expressed as %CV. The stability of the multi-analyte liquid control material was determined as the percentage recovery of each level stored at +2-+8^{0}C related to the same material stored at -80^{0}C at the time points 7 and 14 days. The shelf life for was determined as the percentage recovery of each level of the control material stored at -20^{0}C related to the same material stored at -80^{0}C after 24 months. Measurements were performed on various automated analysers.
Results: The evaluation of the developed liquid multi-analyte control showed for the seven analytes tested at two different concentration levels to assess homogeneity, a %CV (n=125) ≤ 3. The stability for all the analytes at each concentration level as percentage recovery of control material stored at +2-+8^{0}C compared to -80^{0}C was ≤ 10% after 14 days. The shelf life data showed a percentage recovery of control material stored at -20^{0}C compared to -80^{0}C typically within 10% after 24 months for the analytes at each concentration level.
Conclusion: Data indicate that the developed multi-analyte liquid control for clinical chemistry exhibits homogeneity. The whole range of the parameters present in this control material is stable for the two different concentration levels with a shelf life of 24 months. This is of value as a convenient control material to use for analytical applications in clinical settings.

E-22

A Dendrimeric Approach to Short Aptamers with High-Avidity

S. W. Cotten. *University of North Carolina at Chapel Hill, Chapel Hill, NC,*

Aptamers that bind to therapeutically relevant biomarkers are of great importance in numerous biomedical applications. Unfortunately, such applications are hindered because of the difficulty in synthesizing high-affinity and specific aptamers that are typically 70-80 nucleotides in length. Short aptamers of 40 nucleotides, while generally easier to synthesize, pose a different set of problems in that they may not elicit or maintain the desired target-binding properties. The objective of the research was to develop a general method to synthesize clinically useful, diagnostic aptamers with high-binding affinity from short apatmers (<40 nucleotides). Overcoming this synthetic barrier would allow for faster translation of aptamers designed to recognize new biomarkers into clinical laboratory medicine. Using the thrombin DNA aptamer (ARC183), we examined structurally well-defined trimeric and hexameric aptamers generated through a dendrimeric approach using the doubler and trebler phosphoramidites. CD spectroscopy was used to confirm the secondary structure of the thrombin apatmers. All the aptamers showed characteristic positive absorption at 295nm indicative of antiparallel guanine-quadroplex formation. Trimeric and hexameric aptamers showed improved nuclease resistance to exonuclease I and T7 exonuclease relative to the monomer in vitro. In the presence of human plasma, 70% of the trimeric apatmer could be recovered after 30 hours while only 35% of the monomer could be recovered. Surface plasmon resosance illustrated the binding affinity of the trimeric aptamer to thrombin increased 250 fold (K_D ~0.175 nM) compared to the monomer (K_D 45 nM). This increase was a result of a 50 fold faster on-rate and 5 fold slower off-rate. The hexameric thrombin aptamer had a similar off-rate to the trimer but a 10 fold faster on-rate (K_D ~20 pM). Anticoagulant activity of the thrombin apatmers was compared in vitro using a human plasma clotting assay. Time-dependent change in absorbance at 410 nm was monitored after addition of 1 NIH unit of thrombin in the presence or absence of the thrombin aptamers. At concentrations of 100 nM, the time required to achieve half-maximal absorbance change was 90 seconds and 210 seconds for the monomer and trimer respectively. Interestingly, the activity of the hexameric aptamer was generally equal of less effective than the trimer. Even when 1/3 the concentration of the trimeric apatmer was used relative to the monomer, the trimer continued to demonstrate superior anticoagulant activity. At a concentration of 300 nM, the trimer showed only a 3% increase above the baseline signal, while the monomer reached half maximal absorbance after 125 seconds at the same concentration. The results illustrate that the dendrimeric thrombin atpamers showed increased stability against nuclease digestion, at least a 200-fold increase in affinity towards thrombin, and more potent anticoagulant activity. Since short dendrimeric aptamers can be readily synthesized with high yield, the strategy we report here could be generally applied to improve the binding strength and stability of newly developed short aptamers that recognize therapeutic biomarkers for use in clinical diagnostics.

E-23

To apply PCR-Reverse Dot Blot for rapid detection of deep seated Candida

J. Li, C. Li, Y. Li. *Renmin Hospital of Wuhan University, Wuhan, China,*

Objective: To establish rapid methods to detect and identify clinical deep seated candida by using PCR- Reverse Dot Blot.
Methods: Six species-specific oligonucleotide probes (Candida albicans, Candida tropicalis, Candida parapsilosis, Candida dubliniensis, Candida glabrata, Candida krusei) were designed based on ITS1 and added multimer T tails so as to inoculated on hybond membrane. The DNA of the Candida spp were amplified with biotin labeled universal primers based on rDNA. The PCR products were detected and identified by hybridization with the probes on the membrane. To detect clinical specimens , and assess its clinical application.
Results: 218bp of PCR products were amplified in all the 6 species of candida . Each species- specific probe only hybridizes with its target molecules among the 6 species candidas. Amplification and hybridization results were negative in Normal human genome DNA and several common clinical bacterial. There were 19 cases of which the conventional culture method and reverse hybridization membrane positive, there were 2 cases of which the conventional conventional culture-positive and negative reverse hybridization membrane. There were 6 cases of which conventional culture-negative and positive reverse hybridization membrane. The sensitivity of reverse hybridization

membrane was 90.48% (19/21), the specificity was 93.94% (93/99), positive predictive value was 76% (19/25), negative predictive value was 97.89% (93/95), the accuracy was 93.33% (112/120). The positive rate of hybridization membrane and conventional culture were 20.83% (25/120), 17.5% (21/120) respectly, Mc Nemar's test P value = 0.289> 0.05, two detection methods showed no significant differences. values> 0.7, indicating that the results of the two detection methods heaved a strong degree.
Conclusion: The PCR reverse hybridization membrane is proved to be a fast, sensitive and specific technology, the sensitivity was 90.48% , the specificity was 93.94% , it only needed 3 or 4 hours and was potential for clinical application.

E-24

Evidence biochip arrays for the multiplexed determination of analytes related to metabolic dysfunctions.
P. Lowry, J. Porter, R. Duncan, R. I. McConnell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background: Biochip array technology provides a multi-analytical platform for the simultaneous determination of multiple analytes with a single sample. It uses miniaturized assay procedures with implications in the reduction of sample/reagent consumption and cost-effectiveness of the tests. Analytes such as adiponectin, C-peptide, CRP, cystatin C, ferritin, IL-1α, IL-6, insulin, leptin, PAI-1, resistin, TNFα, visfatin take part in physiological processes such as insulin secretion, glucose regulation, fatty acid metabolism, inflammation, iron homeostasis, energy homeostasis/endocrine regulation, fibrin regulation. Variations in the levels of these analytes have been associated to metabolic dysfunctions such as insulin resistance, diabetes, obesity. A multiplexed measurement of these molecules using biochip arrays offers more information than single analyte determination as it provides a patient's profile, which could be of value in the investigation of their involvement in physiologic and pathological processes.
Relevance: We report the development of two biochip arrays for the multiplexed determination of analytes related to metabolic disorders. One biochip array enables the simultaneous measurement of adiponectin, cystatin C and CRP. With the other biochip, C-peptide, ferritin, IL-1α, IL-6, insulin, leptin, PAI-1, resistin, TNF and visfatin are simultaneously determined per sample. This is of value as analytical tool for research applications in clinical settings.
Methodology: The core of the technology is the biochip (9mm x 9mm) and represents not only the platform in which the capture ligands are immobilized and stabilised defining microarrays of discrete test sites, but is also the vessel where the simultaneous immunoreactions are performed. Simultaneous chemiluminescent immunoassays are employed in these arrays and applied to the semi-automated analyser Evidence Investigator.™ The system incorporates dedicated software to process and archive the multiple data generated.
Results: Initial evaluation of the developed biochip arrays shows: with one biochip array for the calibration ranges 0-110ng/ml (adiponectin), 0-50ng/ml (cystatin C) and 0-12mg/l (CRP), the sensitivity values were 1ng/ml, 0.3ng/ml and 0.67mg/l respectively. With the other biochip the sensitivity values ranged from 0.8pg/ml (IL-1α, calibration range 0-500pg/ml) to 1ng/ml (ferritin and resistin calibration ranges 0-1000ng/ml and 0-50ng/ml respectively). For all the immunoassays, the intra-assay precision (n=6) for different concentration levels and expressed as %CV was typically ≤15%
Conclusion: Data show that this multi-analytical biochip array-based approach is applicable to the determination of analytes related to metabolic disorders enhancing the scope of tests per single patient sample with beneficial implications in research settings.

E-25

Development of a tri-level liquid multi-analyte immunoassay control stable for the whole range of the analytical parameters.
A. Shanks, P. Armstrong, J. Campbell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background Lyophilised multi-analyte controls are used due to their stability. However, liquid controls are more advantageous as they are easy to use and minimise errors associated with reconstitution. In these multi-analyte liquid control materials, some analytes such as folate, PSA, vitamin B12 are ustable at +2-+8^{0}C and this has implications in the stability and usefulness of the whole product. Stable liquid multi-analyte controls for the whole range of assayed parameters are useful for quality control applications in clinical settings.
Relevance: We report the development of a tri-level liquid stable immunoassay control containing multiple analytes, including folate, PSA and vitamin B12 and presenting excellent stability for the whole range of the analytes which is of value to assess accuracy, precision and to monitor performance in clinical settings.
Methodology: Multi-analyte human liquid immunoassay sera, containing 51 analytes**,** were generated at three levels covering normal and pathological ranges. Each level of control material was dispensed in 5ml vials and stored below -20^{0}C. Serum homogeneity was determined by measuring 20 vials for luteinising hormone and sodium at three different concentrations, results were expressed as %CV. The stability of the multi-analyte liquid control material was determined as the percentage recovery of each level stored at +2-+8^{0}C related to the same material stored at -80^{0}C at the time points 7 and 14 days. The shelf life was determined as the percentage recovery of each level of the control material stored at -20^{0}C related to the same material stored at -80^{0}C for 24 months. Measurements were performed using automated immunoassay analysers.
Results: The evaluation of the developed tri-level liquid multi-analyte control showed, regarding homogeneity, %CV (n=20) ≤ 3 and ≤ 0.9 for three different levels of luteinising hormone and sodium respectively. Considering the stability, the percentage recovery of control material stored at +2-+8^{0}C compared to -80^{0}C was ≤ 10% after 14 days for all the analytes at each concentration level. The shelf life data showed a percentage recovery of control material stored at -20^{0}C compared to -80^{0}C typically within 10% after 24 months for all the analytes at each concentration level.
Conclusion: Data indicate that the developed tri-level multi-analyte liquid immunoassay control exhibits homogeneity and is stable for the whole range of the included parameters. This is of value as a convenient control material to use for analytical applications in clinical settings.

E-26

Development of a biochip array for the multiplexed detection of sexually transmitted infections.
M. Crockard, C. Deane, J. V. Lamont, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background: Sexually transmitted infections (STI) present an increasing challenge to world public health. Many infections are asymptomatic and remain undiagnosed, leading to significant health problems including infertility. Undiagnosed STI's also increase the risk of unhindered spread. It is vital, therefore, that to avoid preventable health complications, rapid and robust STI screening devices become available, to encourage compliance and reduce the burden on the healthcare system. In addition, simultaneous screening of multiple STI pathogens could identify secondary infections and allow more specific treatments to be administered. This has implications through more appropriate use of antibiotics, which will reduce the potential for antibiotic resistance. We report the development of a multiplex PCR coupled to a biochip array allowing rapid and specific amplification of ten STI's, followed by spatially discrete detection on biochips.
Relevance: The use of the reported multiplex methodology for the detection of STIs offers greater specificity than conventional detection methods and enables the simultaneous detection of multiple infections as well as the identification of primary infection requiring most immediate treatment, which is of value in clinical applications.
Methodology: Two innovative technologies were incorporated: Dual Priming Oligonucleotide Technology (DPO) and Biochip Array Technology (BAT). DPO technology confers high amplification specificity and resultant amplicons are further challenged through hybridisation to a biochip array onto which only the correct targets will couple, quantitatively, to spatially discrete pathogen probes. The 10 pathogens simultaneously detected from urine or swab samples are; *Chlamydia trachomatis, Neisseria gonorrhea, Trichomonas vaginalis, Treponema pallidum, Herpes simplex 1, Herpes simplex 2, Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum* and *Haemophilus ducreyi*. Trials of the biochip array were performed on QC-MD (Q-nostics) urine or swab sample sets containing different levels of particular pathogens, allowing sensitivities and specificities to be defined for the assay. Comparator assays on the same samples were also provided, so performance can be compared.
Results: The Chlamydia set contained 10 samples at differing bacterial loads which the biochip assayed with 100% specificity and 70% sensitivity (due to 3, 4 and 37 CFU samples). Ten samples from the gonorrhea QCMD set gave 100% sensitivity and 100% specificity, while the Herpes 1 and 2 QCMD samples produced 100% specificity and 80% sensitivity. In all cases, multiplex PCR's were used, despite only looking for single pathogens. No cross-reactivity was detected in any of the samples tested. Biochips were also tested against ATCC reference samples for all 10 pathogens, showing 100% specificity and sensitivity. As a multiplex assay, the STI biochip array compares very favourably with commercially available single or duplex tests in respect of sensitivity and specificity.
Conclusion: The data indicate applicability of the biochip array technology to the simultaneous detection of ten STIs with the capability of identification of primary infection requiring most immediate treatment. The sensitivity and speed of the test ensures that only true infections are detected and patient anxiety is reduced by reporting results within a few hours of sample collection. This is of value in clinical settings and has implications in the reduction of effort and costs.

E-27

Validation of an Improved Unconjugated Estriol Assay on the IMMULITE® 2000 System

J. Martel[1], D. Thomas[2], P. Wyatt[2], Y. Luna[3], M. Mahue[3], R. Ludewig[1], J. Meenan[4]. *[1]Siemens Healthcare Diagnostics, Tarrytown, NY, [2]Genzyme Genetics, Santa Fe, NM, [3]Siemens Healthcare Diagnostics, Los Angeles, CA, [4]Siemens Healthcare Diagnostics, Glasgow, DE,*

Estriol is almost exclusively produced during pregnancy, originating from a precursor synthesized by fetal adrenal glands and then converted to estriol by fetal liver and placenta. In the maternal liver, estriol is conjugated to form sulfates and glucoronides, which are then excreted in the urine with a half-life of approximately 20 minutes. In the maternal circulation unconjugated estriol accounts for approximately 10% of total estriol. The unconjugated estriol concentration rises during pregnancy, being two to three orders of magnitude higher in the final trimester. During this period, a rapid decrease in estriol levels is indicative of fetal distress.

Siemens has improved its fully automated unconjugated estriol (uE3) immunoassay for testing serum samples on the IMMULITE 2000 platform. The objective of this study was to evaluate the analytical performance of the new uE3 assay on this platform.

The IMMULITE 2000 uE3 is a solid-phase, competitive chemiluminescent enzyme immunoassay. The assay range is 0.07-12 ng/mL, and the assay is standardized against GC/MS.

The performance of the uE3 assay was assessed at US clinical sites. Imprecision was evaluated on repeated measurements using control materials and prepared serum pools. On samples with uE3 concentrations of 0.14-10.20 ng/mL, the within-run CVs ranged from 5.4% to 16.0% and total CVs from 6.1% to 19.1% for lot A, and within-run CVs ranged from 5.9% to 15.0% and total CVs from 5.9% to 16.4% for lot B.

A method comparison against the Beckman Coulter ACCESS 2 uE3 assay was performed at one site on 275 serum samples, covering the range of the assay. Two lots of IMMULITE 2000 uE3 reagent were tested; the correlation coefficients were 0.96 and 0.98.

In conclusion, the results of this clinical evaluation indicate that the IMMULITE 2000 uE3 immunoassay is a precise method for measuring uE3, especially at the low end and across a wide range of clinically relevant concentrations. The method comparison study also showed equivalent performance to the Beckman Coulter ACCESS 2 uE3 assay.

E-28

SPARCL™: a novel technology for sensitive homogeneous immunoassays

H. Akhavan-Tafti[1], M. Salvati[2], B. H. Odegaard[2], R. de Silva[1], W. Xie[1], J. C. Todtleben[2], M. L. Bertelson[2], N. Shapir[2]. *[1]Beckman Coulter, Southfield, MI, [2]Beckman Coulter, Chaska, MN,*

Objective: To develop novel sensitive immunoassays utilizing SPARCL™ technology.

Relevance: SPARCL is a proximity-dependent chemiluminescent detection method under development that eliminates the need for washing and separation. In SPARCL, the chemiluminescent substrate (acridan) is brought into proximity to an oxidative enzyme (horseradish peroxidase, HRP) through an analyte-specific interaction.

Methodology: SPARCL technology was successfully demonstrated in both the presence and absence of a solid capture phase. Using a solid capture phase, both acridan and a specific capture antibody were coupled to microparticles, while a complementary antibody (sandwich assay) or analyte (competitive assay) was conjugated to HRP. In the absence of solid phase, the capture antibody was labeled with acridan, while the complementary moiety was labeled with HRP. In both cases, specific analyte/antibody interactions bring the two components (acridan and HRP) into proximity. Without the need to remove excess reactants, a flash of light can be generated by the addition of a trigger solution. Furthermore, the addition of a background-reducing agent enables modulation of signal-to-noise ratios.

Validation: Table lists analytical parameters for a variety of assays utilizing SPARCL (particle-based and solution-phase formats)**.

Analyte	Assay Format	SPARCL Format	Analytical Sensitivity	Dynamic Range	Access Analytical Sensitivity*
PSA	Sandwich	Solid phase	0.004 ng/mL	0 - 100 ng/mL	0.008 ng/mL
PSA	Sandwich	Solution phase	0.003 ng/mL	0 - 68 ng/mL	0.008 ng/mL
Estradiol	Competitive	Solid phase	0.04 ng/mL	0 - 32 ng/mL	0.02 ng/mL
cAMP	Competitive	Solution phase	1.2 nM	0 - 33 nM	NA
βhCG	Sandwich	Solid phase	0.07 mIU/mL	0 - 836 mIU/mL	0.5 mIU/mL
CK-MB	Sandwich	Solid phase	0.028 ng/mL	0 - 1500 ng/mL	0.1 ng/mL
CK-MB	Sandwich	Solution phase	0.1 ng/mL	0 - 500 ng/mL	0.1 ng/mL

*Analytical sensitivity of current commercially available assay

Conclusions: SPARCL technology offers unique characteristics that make it an attractive technology for deployments such as point of care and combined immunoassay/chemistry work cells: elimination of the need for washing or separation, and immediate flash generation of signal. The solution-phase format eliminates challenges of solid-phase suspension and mixing coupled with improved kinetics and a more native biological environment. Thus, SPARCL technology should enable improved immunodiagnostic devices.

**Internal feasibility assays, not for commercial distribution of diagnostic products.

E-29

Colorectal Cancer Gene Expression Profiling Using a Novel Microarray Technology

B. Moody[1], B. Burgett[1], D. Englert[2], D. Wilson[2], R. J. Molinaro[1]. *[1]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, [2]Axela Inc., Toronto, ON, Canada,*

Background: Understanding the molecular mechanisms involved in colon cancer progression can lead to enhanced methods of control through novel forms of risk assessment, diagnostics, prognostics and therapeutics. In this study we focused on microarray gene expression analysis as a method for validation of molecular biomarkers in colon cancer. The major objective of this study was to evaluate the effectiveness of TipChip arrays (Ziplex® System, Axela Inc.) for differential gene expression analysis of colon cancer-related genes. Two TipChip arrays were produced with a total of 243 oligonucleotide probes (plus standard controls) printed in duplicate. The probes are considered specific representations of the genes differently expressed in colon tumors and were designed to quantify transcripts of genes that: 1) were expected to be differentially expressed between colorectal cancer and normal tissues based on publicly available expression databases, or 2) were expected to be stably expressed and potentially be useful as normalization controls.

Methods: The TipChip arrays were evaluated by hybridizing cRNA samples prepared from fresh frozen samples of colorectal cancer tissue and samples of matched normal tissues from benign areas of the colon. Total RNA was isolated from tissue samples, amplified, labeled and then processed in the robotic Ziplex® Automated Workstation.

Results: Genes on both of the TipChips were ranked according to their expression stability values About 20 normalization control probes were identified on each TipChip. In nearly all cases both probes for the normalization of candidate genes were relatively invariant across the samples. Approximately half of the genes identified were ribosomal proteins which are expected to be "housekeeping" genes. To assess repeatability, eight of the samples were run twice on different days, and the results of the runs were correlated. The R^2 values of the correlations ranged from 0.971 to 0.991, indicating good repeatability. Forty-seven probes (33 genes) were apparently differentially expressed between normal and tumor samples. Probes were considered differentially expressed if the p value from a t test was less than 0.05, and the measured expression difference was greater than 50%. For 40% of the differentially expressed genes, both probes for the genes met these criteria, and in all but two cases both probes indicated differential expression. The tumor and normal samples were distinguished using normalized data.

Conclusions: In this study, we applied a well-controlled stepwise approach to obtain a gene expression profile for colorectal cancer from two different TipChip arrays. Probes for genes relatively invariant and differentially expressed between colorectal tumors and normal tissues were identified in this pilot experiment. These probes, with further validation, may be useful for the advancement colorectal cancer research.

E-30

Rapid Analysis Of Dried Blood Spots For Polyfluoroalkyl By Online Solid-Phase Extraction Lc\Ms\Ms

S. Beaudet, S. Ramagiri, F. Zhong, M. Sibum. *AB Sciex, Concord, ON, Canada,*

Background Perfluoroalkyl chemicals (PFCs) are widely distributed products. Their persistence and toxicity in the environment and in biological systems is leading to an increase number of studies and concerns. A rapid and sensitive online solid-phase extraction/Liquid Chromatography/Tandem mass spectrometry (SPE/LC/MS/MS) method is proposed to analyze PFC's in dried blood spots (DBS). This online method enables, concentration and separation of plasma PFC's in a very short analysis time. It also reduces potential for human error associated with labor intensive steps usually required in offline solid-phase extraction while maintaining sample quality and throughput.

Method We used an online solid-phase extraction system coupled to a hybrid triple quadrupole/linear ion trap mass spectrometer. The following PFCs were studied: PFOA, PFOS, PFHS (perfluorohexane sulphonate) and PFNA (perfluorononanoate), PFHxS. Calibration curve (0.01 ng/mL - 1000 ng/mL) and quality control were prepared in whole blood. A 20 µL of calibrators and QC's were deposited on filter cards and allow to dry for several hours. We manually punched a 6 mm piece out of the samples in a polypropylene tube. Simple extraction was then performed with 200 µL methanol, internal standard was added and tubes were sonicated for about 10 min. Plasma samples were picked up by the autosampler and injected onto the SPE C18 cartridge.

Results The use of SPE led to significant cleaner samples and was highly effective in decreasing interferences present in the matrix that can cause problems with many HPLC-based methods. Several SPE materials were tested and C18 cartridge was selected based on the recovery, reproducibility, and peak shape. The eluted sample was then transferred onto an analytical column Phenomenex Kinetex C18 (2.1 x 50 mm) 2,6 µm for chromatographic separation and subsequent MS/MS detection. The acquisition was performed in multiple reaction monitoring (MRM) mode using negative electrospray ionization (ESI) and structural confirmation (MS/MS)obtained with MRM triggered Enhanced Product Ion (EPI). Integrating Information Dependant Acquisition (IDA) into the automated LC/MS/MS workflow provides increased information versus the MRM-only PFC's quantitation. The ability of the system to automatically trigger full scan MS/MS and MS^3 spectra from the MRM transitions can be used to distinguish the target compounds from interferences. The assay was checked for linearity, imprecision, limit of detection, matrix effects and process efficiency. The analytical LODs and LOQs for each analytes were determined as $3S_0$ and $10S_0$ from five replicates respectively. Detection limits were 0.03-0.1 ng/mL (ppb) and the intra-day and inter-day precisions were 2.3-9.6% and 10.6-15.5%, respectively.

Conclusion The combination of the online solid phase extraction with the hybrid -triple quadrupole/Linear Ion Trap mass spectral detection provides an automated, sensitive and selective method to identify and quantitate PFC's in blood samples.

E-31

Next Generation Uric Acid Assay Application for the Abbott ARCHITECT® cSystems™

S. Colberg, C. Kasal, B. Combs, G. Marseille. *Abbott Laboratories, Irving, TX,*

Objective: The Next Generation Uric Acid (UA) reagent application was evaluated on the ARCHITECT cSystems. This new assay demonstrates extended on-board stability and is not affected by coexisting substances such as ascorbic acid or bilirubin and will be traceable to NIST SRM 913.

Background: Uric acid is a metabolite of purines, nucleic acids, and nucleoproteins. Determination of uric acid is an important clinical test in diagnosis of hyperuricemia which can be observed in renal dysfunction, gout, leukemia, polycythemia, atherosclerosis, diabetes, hypothyroidism, or some genetic disorders.

Method: The Next Generation UA Assay utilizes a two part liquid format based on the uricase method, employing N-(3-sulfopropyl)-3-methoxy-5-methlaniline (HMMPS) and 4-aminoantipyrine to undergo a quantitative oxidative condensation catalyzed by peroxidase, producing a blue pigment.

Study	Protocol	Results* (mg/dL)	
20-Day Precision	NCCLS EP5-A2 (N=80)	Serum Mean: 4.1 mg/dL	Serum Mean: 8.9 mg/dL
		Total	Total
		% CV 1.3	% CV 0.8
		Urine Mean: 9.5 mg/dL	Urine Mean: 17.9 mg/dL
		Total	Total
		% CV 2.2	% CV 1.4
Linearity	NCCLS EP6A	Serum: up to 50 mg/dL	
		Urine: up to 250 mg/dL	
Sensitivity	NCCLS EP17-A	Serum LOD: 0.2 mg/dL	
		Urine LOD 1.0 mg/dL	
On-Board and Calibration Stability	% Recovery to Day 0	Minimum of 30 days	
Ascorbic Acid Interference	Dose Response Method	2.7 mg/dL uric acid	
		Recovery	
		97% with 30 mg/dL AA	
		32.1 mg/dL uric acid	
		Recovery	
		98% with 200 mg/dL AA	
Bilirubin Interference	Dose Response Method	3.3 mg/dL uric acid	
		Recovery	
		94% with 60 mg/dL Bilirubin	
		(30 mg/dL Conj + 30 mg/dL Unconj)	
Hemolysis Interference	Dose Response Method	2.7 mg/dL uric acid	
		Recovery	
		100% recovery with 2000 mg/dL Hemolysis	
Lipemia Interference	Dose Response Method	2.8 mg/dL uric acid	
		Recovery	
		95% recovery with 1000 mg/dL Intralipid	

* In development

Conclusion: The Next Generation Uric Acid reagent application on the Abbott ARCHITECT cSystems provides a methodology with excellent precision, linearity and interference performance. This assay will also provide customers with an extended on-board stability, minimum of 30 days without the need for calibration. It meets the requirement of the European In Vitro Diagnostics Directive by demonstrating metrological traceability to recognized reference methods.

E-32

Multicolor Combinatorial Probe Coding for Real-Time PCR

Q. Huang[1], L. Zheng[1], Y. Zhu[1], J. Zhang[1], W. Li[2], Y. Zhou[3], Y. Jiang[3], J. Huang[4], X. Xu[4], X. Zhao[5], Q. LI[1]. *[1]XMU, Xiamen, China, [2]Department of Medical Genetics, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China, [3]Prenatal Diagnostic Center, Xiamen Maternal & Child Health Care Hospital, Xiamen, China, [4]Department of Microbiology, Xiamen Centre for Disease Control and Prevention, Xiamen, China, [5]Public Health Research Institute, New Jersey Medical School, UMDNJ, Newark, NJ,*

Background: The throughput of conventional multiplex, real-time PCR assays is limited by the number of fluorescent dyes available and the number of fluorescence acquisition channels present in the PCR instrument.

Method: We hereby explored a probe labeling strategy that significantly increased the throughput of real-time PCR detection in one reaction. The labeling paradigm, termed "Multicolor Combinatorial Probe Coding" (MCPC), uses a limited number (n) of differently colored fluorophors in various combination to label each probe, enabling the number of genetic targets to be detected in a one reaction to as many as 2^n-1.

Results: The proof-of-principle of MCPC was established by identification of 15 human papillomavirus subtypes, which is the maximum target number theoretically detectable by MCPC with a 4-color channel instrument, in one reaction. MCPC was then improved from a one-primer-pair setting to a multiple-primer-pair format through Homo-Tag Assisted Non-Dimer (HAND) system. The improvement was demonstrated via the identification of foodborne pathogens by targeting 10 species-specific genes with detection limits equivalent to the uniplex PCR. MCPC was further evaluated when multiple targets were co-amplified for detecting five β-globin gene mutations.

Conclusions: By breaking the throughput bottleneck of traditional real-time PCR, MCPC strategy should expand the scope of PCR assays into many challenging fields.

E-33

Fluorescent Silica Nanoparticles-Based Lateral Flow Immunoassay for the Detection of *Salmonella typhimurium*

X. Li, Y. Xu, Q. LI. *XMU, Xiamen, China,*

Introduction: *Typhimurium* is one of the main serotypes of *Salmonella enterica*. It is the most common agent of food poisoning related with *Salmonella* species. According to the WHO, salmonellosis caused by *Salmonella* spp. is one of the most frequently reported foodborne diseases world wide. The economic implications of salmonellosis are significant. These facts emphasize the importance of early detection of *S. typhimurium* in food in order to prevent Salmonella-related illnesses. Available methods developed so far are, however, laborious, time-consuming or insensitive. **Objective:** We aim to develope a rapid, simple, and sensitive lateral flow immunoassay to detect *S. typhimurium* using a novel, lanthanide chelates-labeled fluorescent silica nanoparticle as reporter.
Methods: Conjugation of *S. typhimurium* monoclonal antibody (mAb) to Eu(III):BHHCT-coated silica nanoparticles was bridged through an oxidized Dextran linker. After blocking and washings, the conjugates were lyophilized on the conjugate pad before assembling. Nitrocellulose membrane was spotted with *S. typhimurium* mAb (2 mg/mL) and goat anti-mouse IgG (1 mg/mL) in PBS buffer at a rate of 0.75 μL/cm as test and control lines, respectively, leaving a 0.5 cm space between the two lines. The test line was used to detect the analyte. The control line was for quality control of the assay. Only labeled functional *S. typhimurium* mAb could be detected at the control line. All of the bacterial samples were sterilized by placing in a thermostat at 99°C for 20 min, except for some special tests. 30 μL of pure culture was mixed with the same volume of assist Chromatography buffer, and then added to the sample pad. After 30 min reaction in darkness, the test strips were then placed onto the base plate inside the signal acquisition device for imaging, or submitted to the ESE-Quant Scanner for signal reading.
Results: The limit of detection was 7.3×10^2 CFU/mL for heat-killed *S. typhimurium*. The coefficients of variations (CVs) along the entire range were between 1.0%-19.4% (n=6). According to the calibration curve, the dynamic range of our LFIA system is 3 orders of magnitude, spanning *S.typhimurium* concentration of 7.5×10^2 CFU/mL through 6.0×10^5 CFU/mL (R=0.999). Fourteen non-*Salmonella* strains and nine *Salmonella* strains at ~10^7 CFU/mL were used to evaluate the specificity. The results did not show any cross-reactivity with non-*Salmonella* strains. Three *Salmonella* strains, the same subgroup (Group B) as *S.typhimurium*, gave the cross-reactive results. All of 30 real samples, including milk, meat ball and peanut butter, were negatively detected by the immunostrips or by the China National Standard of Microbiological Examination of Food Hygienic Determination. These results indicate that these two methods have good consistency.
Conclusion: Our method has the advantages of rapidness, ease of use and much improved sensitivity. It is a potential alternative method for field screening of contaminated food samples.

E-34

Rapid Detection of Unamplified HCV RNA in Clinical Specimens Using Unmodified Gold Nanoparticles

S. M. Shawky, H. M. E. Azzazy. *American University In cairo, Cairo, Egypt,*

Background: HCV RNA detection is currently limited to RT-PCR, bDNA, and TMA. Despite the high sensitivity and specificity of these methods, they are time consuming, labor intensive and expensive. The unique photophysical properties of gold nanoparticles (AuNPs) made them superior tools for developing RNA detection assays with comparable performance but with more simplicity and lower cost. AuNPs exhibit a unique phenomenon known as surface Plasmon resonance which is responsible for their intense red color. This color changes to blue upon aggregation of AuNPs. Short single stranded oligonucleotides uncoil and adsorb effectively onto AuNPs and thus prevent their salt-induced aggregation. When AuNPs are added to a saline solution containing the target RNA and its complementary primer, AuNPs aggregate (since the primers are not free to adsorb on the AuNPs) and the solution color changes to blue. However, in the absence of the target, the primers adsorb onto the AuNPs thus preventing their aggregation and the solution color remains red.
Objective: This work aims to develop a rapid, simple and cheap assay for direct detection of unamplified HCV RNA extracted from clinical samples using unmodified AuNPs.
Methods: A colloidal solution of AuNPs with a diameter of 15 nm ± 2 was prepared by citrate reduction of hydrogen tetrachloroaurate (III). The size and extinction spectra of the prepared AuNPs were characterized using field emission scanning electron microscopy and spectrophotometry, respectively. Serum samples were collected from healthy volunteers (n = 20) and chronic HCV patients (n = 25; positive by antibody and PCR tests). HCV RNA was extracted using SV total RNA isolation system with a modified protocol. The assay was performed by mixing 7 μL of the extracted RNA and 3 μL of the hybridization buffer containing 10 mM PBS and a primer targeting a sequence in the 5'UTR of HCV specific to all genotypes and subtypes. The mixture was denatured at 95°C for 30s, annealed at 59°C for 30s, and then cooled to room temperature for 10 min. 10 μL of colloidal AuNPs were then added to the mixture and the color was observed. Serial dilutions of HCV positive samples with known viral load were used to determine the detection limit of the assay.
Results: The extinction spectra of the prepared AuNPs were around 518-520 nm. Salt, primer, AuNPs concentrations and annealing temperature and time were optimized. In HCV positive specimens, the color of the solution changed from red to blue within 1 min. Also, a red-shift and broadening of the absorbance peak of AuNPs were observed. The assay has a sensitivity of 92% and a specificity of 90%. The assay detection limit was 50 copies/reaction.
Conclusions: To our knowledge, this is the first assay that allows the detection of unamplified HCV RNA in clinical specimens using unmodified AuNPs. The developed assay is highly sensitive, has a turnaround time of 30 min, and eliminates the need for thermal cycling and detection instruments. This assay could be further developed for HCV viral load monitoring and genotyping.

E-35

Methodology for Measuring Colloidal Immunogold Conjugate Release from Lateral Flow Conjugate Release Matrixes

N. Lukinova, G. Fomovska, A. Dubitsky. *Pall Corporation, Port Washington, NY,*

Introduction: In most lateral flow point of care (LF POC) assays, a conjugate release pad houses a dry detection reagent. Conjugate contains detection particles coupled with antibodies specific to a target analyte. To ensure consistent performance of LF assays, the conjugate pad should be able to hold, resolubilize and quickly release the conjugate when a sample flows into the pad. Industry expectation is over 90% release of conjugate in 180 sec, however, there are no published methods for monitoring conjugate release kinetics or evaluation of conjugate pads for POC assays.
Objectives: Establish a methodology for the evaluation of colloidal immunogold release from a conjugate pad matrix as a tool for lateral flow assay development and optimization. Compare different commonly used conjugate pad materials; cellulose-based media as well as treated and untreated borosilicate glass in terms of kinetics and percentage of colloidal immunogold release. Correlate total conjugate release with performance in a model immunoassay.
Method: Ten 12 mm discs of each pad type were used for applying hCG-specific immunogold at a volume of 10 μL. Discs were dried at 37°C for 2 h, and the color of each disc was measured with a handheld densitometer. Color intensity of the media before applying conjugate immunogold was used as a control. Using 12 mm holder, chase buffer and a syringe pump set for a flow rate 0.05 mL/min, the release of colloidal gold conjugate as measured. Fractions were collected every 15 sec, and OD at 530 nm was measured in 96-well plate. After collecting 12 fractions (180 sec of conjugate release) discs were placed on a strip of Vivid™ lateral flow nitrocellulose membrane to dry. Color intensity was measured again to calculate total conjugate release.
Results: A rapid and effective method has been established to evaluate the release of conjugate reagent during first 180 sec after initiation of an immunoassay as well as measure the total amount of the released conjugate at an endpoint. It was shown that under tested conditions glass fiber-based conjugate pads resulted in 99-100% release of conjugate immunogold in less than 120 sec. Cellulose-based media demonstrated similar early kinetics of conjugate release, with slightly lower amount of released gold for each time point and 92% total conjugate immunogold release. A model hCG immunoassay demonstrated that cellulose-based conjugate pad with average 92% conjugate release resulted in slightly lower test line intensity compared to glass fiber pad providing 99-100% conjugate release.
Conclusion: An effective methodology has been developed for the evaluation of conjugate pad performance in lateral flow POC assays. The procedure allows for monitoring the release kinetics of conjugate reagent from the conjugate pad matrix. In addition, the methodology can be used for the optimization of conjugate dilution buffers and drying conditions for the conjugate. This method results in a robust tool for lateral flow assay development.

E-36

Detection of prostate specific antigen (PSA) in the serum of radical prostatectomy patients at femtogram per milliliter levels using digital ELISA based on single molecule arrays (SiMoA)

D. C. Duffy[1], D. M. Rissin[1], C. W. Kan[1], T. G. Campbell[1], S. C. Howes[1], D. R. Fournier[1], L. Song[1], T. Piech[1], P. P. Patel[1], L. Chang[1], A. J. Rivnak[1], E. P. Ferrell[1], J. D. Randall[1], G. K. Provuncher[1], D. R. Walt[2]. [1]*Quanterix Corporation, Cambridge, MA,* [2]*Tufts University, Medford, MA,*

Objective: The aim of this study was to detect prostate specific antigen (PSA) in the serum of patients who had undergone radical prostatectomy (RP). To achieve this objective, an ultra-sensitive ELISA for PSA based on single molecule detection was developed and evaluated.

Relevance: The ability to detect PSA in RP patients 4-6 weeks after surgery (nadir PSA levels) has potential prognostic value, and monitoring of PSA at very low concentrations could also enable earlier detection of biochemical relapse. Using existing immunoanalyzers, however, PSA is often not detectable in the serum of RP patients for several years post-surgery until levels exceed a threshold determined by the analytical sensitivity of commercially available assays. New, ultra-sensitive assays are needed to measure PSA at femtogram per milliliter concentrations in order to detect PSA in all of these patients shortly after surgery. The single molecule detection approach described here fulfills this requirement.

Methodology: We have developed a method for detecting single immunocomplexes formed in the enzyme-linked immunosorbent assay (ELISA) using single molecule arrays (SiMoA). This digital ELISA method is based on isolating single immunocomplexes labeled with an enzyme in arrays of femtoliter wells, sealing the arrays in the presence of the enzyme substrate, and fluorescently imaging the array. Fluorescent product molecules of the enzyme-substrate reaction are confined in the femtoliter volume, giving rise to a local high concentration that can be easily detected using a standard fluorescent microscope. By using high density arrays of femtoliter wells, hundreds to thousands of single immunocomplexes can be detected simultaneously. Isolation of single immunocomplexes using SiMoA gives rise to a dramatic increase in sensitivity over bulk, ensemble detection methods. An ultra-sensitive digital ELISA for detecting PSA was developed that has a limit of detection (LOD) of 6 fg/mL (200 aM) in serum. This assay was used to measure PSA in the sera of thirty RP patients.

Validation: The digital ELISA for PSA was validated by using the assay to measure PSA in the serum of healthy males and in Bio-Rad PSA controls, and comparing the concentrations determined by a commercial immunoanalyzer.

Results & Conclusions: PSA was successfully detected by digital ELISA in sera from all thirty RP patients. No previously reported assay for PSA has successfully detected this protein in all RP patients tested; the most sensitive assays reported previously (LODs in the range 0.3 to 1 pg/mL) would have failed to detect PSA in 30-40% of the samples tested in this study. The lowest concentration detected by digital ELISA in the serum of an RP patient was 14 fg/mL (420 aM), and the PSA concentrations ranged from 14 fg/mL to 9.4 pg/mL. The mean concentration of PSA in the sera of these patients was 1.5 pg/mL. These results suggest that digital ELISA using SiMoA has the potential to provide a more favorable prognosis for men with the lowest measurable nadir values, and to detect biochemical recurrence months or years earlier than conventional test methods.

E-37

An Automated Approach to the Development of a Rapid and Robust SPE Method

S. Countryman, S. Huq, T. Matthews. *Phenomenex, Torrance, CA,*

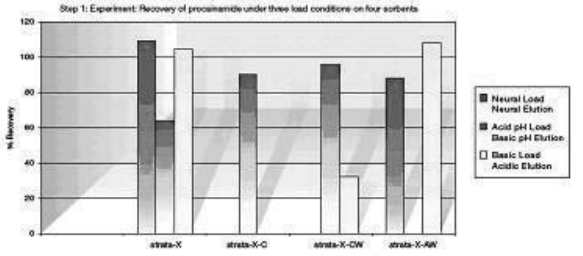
The purpose of this work was to develop and automated approach to selecting the appropriate solid phase extraction (SPE) media for a new clinical assay. SPE is widely recognized as the most effective way to reduce matrix interferences. However, the development of a suitable solid phase extraction techniques remains a lengthy time consuming process that can take several weeks to finalize.

This work describes a three step process utilizing a robotic liquid handler program to first select the optimum SPE sorbent by screening four different SPE sorbents to determine the strongest retention mechanism. Each sorbent's ability to retain the target compound is evaluated under three load, wash, and elution conditions. The eluent from each sorbent is then analyzed by LC/MS/MS to determine recovery and precision at ULOQ, sensitivity at LLOQ, matrix effect at BLK and extraction linearity.

Once the optimum sorbent has been selected, the load and wash conditions are optimized for that sorbent. Various wash procedures are screened and the elutents are again evaluated for recovery & precision at ULOQ, sensitivity at LLOQ, matrix effect at BLK and extraction linearity.

The final step is to perform a pre-validation with the selected method to verify the actual precision & accuracy, linearity, limit of detection, recovery, and matrix effect over multiple donors and disease states.

The performance of this procedure was evaluated for three pharmaceutical compounds: Procainamide, Indomethacin, and Cabamazepine from human plasma followed by analysis by LC/MS/MS. The whole SPE method development process was completed within one day for each compound by using this three step protocol.



E-38

Flash Sensor Tech: A novel electrochemical immunoassay for determination of serum antibodies

B. Clare, E. Boniface, E. Etherington, J. King. *Flash Sensor Tech, Inc., Tigard, OR,*

Objective: The objective of this study is to develop a rapid, electrochemical point-of-care test for the determination of serum antibodies and study its performance against a standard ELISA assay.

Relevance: The quantitative measure of serum IgE is used by clinicians in the diagnosis of food and environmental allergies. In one aspect of our development program we are designing and testing technologies for the point-of-care diagnosis of allergies. Here we describe the performance of the Flash Sensor Tech immunoassay in the context of a mouse model system for determining antibodies produced against gliadin, a common food allergen derived from wheat gluten.

Method: The basis of our analytical method is an electrochemical immunoassay. Flash Sensor Tech has developed an integrated diagnostic platform comprised of modified metal electrode sensors, a potentiostat reader and data analysis tools for recording and interpreting the sensor response. Here we validate our assay in a mouse model system. First, we identified a peptide epitope of gliadin having affinity to (ms) mAb anti-gliadin. Sensor surfaces were prepared using this peptide sequence. Next, a dose-response assay was performed using samples of a 1:100 dilution of mouse serum spiked with the target analyte. Performance was compared against a standard ELISA protocol.

Data: In this validation study, we find a logarithmic sensor response to analyte concentrations over 5 orders of magnitude (1000 ng/mL to 0.1 ng/mL). The limit of detection is approximately 1-10 ng/mL - comparable to an ELISA when performed using identical reagents. Storage stability is 6 weeks and on-going.

Conclusion: We validated the sensor platform and find that the Flash Sensor Tech immunoassay is comparable in sensitivity to standard ELISA protocols for detection of serum antibodies using a mouse model system. Future studies will evaluate this platform for determining serum IgE in clinical samples.

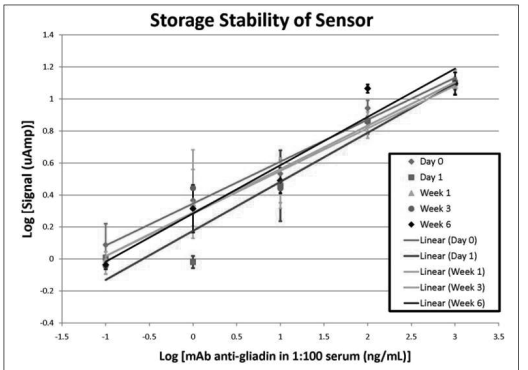


Figure 1: Logarithmic sensor response for analyte concentration spanning 5 orders of magnitude. Sensor storage stability measured by evaluating performance at timepoints over 6 weeks (on-going). Day 0: log y = 0.2622 (log x) + 0.3461, R^2 = 0.9832, Day 1: log y = 0.3066 (log x) + 0.1757, R^2 = 0.9364, Week 1: log y = 0.2665 (log x) + 0.2836, R^2 = 0.9889, Week 3: log y = 0.2717 (log x) + 0.2895, R^2 = 0.9457, Week 6: log y = 0.3016 (log x) + 0.2845, R^2 = 0.9475

E-39

Multi-Laboratory Precision Evaluation of a Microfluidic Lab-on-Chip Electrophoresis System for HDL2b% Measurement

A. F. Wong, A. H. Wu. *SFGH, San Francisco, CA,*

Background: High concentrations of high-density lipoprotein (HDL) is a negative risk factor for coronary heart disease (CHD). The HDL2b subclass is hypothesized to exhibit greater cardioprotective properties compare to other subclasses. Currently there is no standard method of measuring HDL subfractions, the most common practice being ultracentrifugation which requires accurate density solutions and slight variation in preparation can leas to inconsistent results. Agilent Technologies has developed a microfluidic Lab-on-Chip electrophoresis system for serum HDL subfraction measurement (HDL Profile System). Nine samples and two controls can be analyzed on one chip in about 30 minutes with HDL2b% of HDL cholesterol reported. The HDL Profile System uses micro-machined chips filled with linear polymer matrix as a sieving buffer. HDL particles are moved by voltage gradient and are visualized with a lipophilic dye to produce an electrophoretic trace that plots signal intensity against migration plot. HDL2b% is calculated based on the calibrators and alignment markers.

Methods: In a multi-center study, assay performance and precision of the HDL Profile System by both internal and external testing using serum samples from 99 subjects were evaluated. Serum samples with different HDL cholesterol concentrations from 0.259 to 2.072 mmol/L were pooled to prepare the low, middle and high targeted HDL2b% levels of 12, 30 and 35. The internal precision test was conducted at Agilent Technologies for 20 days with each sample assayed in triplicate on one chip twice a day by two operators. The external precision was held at three different laboratories with different lots of reagents and chips. Four chips were run each day for five days and each sample was also run in triplicate on each chip.

Results: Measurable range for serum samples with HDL cholesterol of 0.259 mmol/L to 2.072 mmol/L (10mg/dL to 80mg/dL) could be measured for HDL2b% subfractions with a %CV below 8%. The internal precision results had high precision with the intra-chip %CV, the inter-chip %CV and the total %CV being in the low single digit range except for the samples with low HDL2b%, the highest total %CV being 8%. The external precision also had high reproducibility with the intra-chip %CV, inter-chip %CV and the total %CV being in the low single digit range except for the low HDL2b%, the highest total %CV being 7%. Total precision was below 1.5 HDL2b% and linearity was observed to be between 8 and 32 HDL2b%.Correlation coefficients (R) of comparisons between one laboratory evaluated against any of the two other laboratories were all above 0.95. The absolute bias between any two laboratories was less than 2.5 SD (3.35% HDL2b).

Conclusions: Consistent performances were obtained from different laboratories with different manufactured reagent and lot chips. The Agilent HDL Profile System demonstrated high assay reproducibility by both internal and external precision tests with a lower %CV compared to other methods of HDL subfraction measurement.

E-40

Development and Validation of an LC/MS/MS Assay for Pain Management Drugs

S. Countryman, C. Sanchez, M. Campognone. *Phenomenex, Torrance, CA,*

The number of prescriptions written to support patients living with chronic pain has dramatically increased over the past 10 years. An unfortunate side affect of the drugs used to treat chronic pain is dependency, which has lead to abuse and created a large black market for unused medications. Additionally, chronic pain patients often resort to several drugs to treat their condition, further complicating the treatment plan for the physician. These developments have increased the need for a robust and comprehensive test to monitor patient compliance.

The purpose of this work was to develop and validate a high throughput assay for 13 commonly prescribed pain management drugs. The upper limit of quantitation (ULOQ) and lower limit of quantitation (LLOQ) were set at ten times above and below the therapeutically relevant levels, respectfully. A bioanalytical validation protocol using five different lots of urine samples was designed and tested. For each analyte stable-labeled internal standards were used, where available. Acceptable values for accuracy, precision, linearity, lot to lot variability, and matrix effects were demonstrated for each analyte. In order to decrease potential matrix interferences, and to prolong instrument up-time sample clean-up using solid phase extraction (SPE) was performed prior to analysis.

Fast turn around time is critical in the clinical environment. Fast LC technologies allowed this comprehensive assay to be completed in under 2 minutes while significantly improving sensitivity.

Analyte	ISTD	Linear Range (ng/mL)	Accuracy @ LLOQ (n=4)	Precision @ LLOQ (n=4)
morphine	D3-morphine	**2.5**-2500	81.20%	26.40%
oxymorphone	D6-hydromorphone	**2.5**-1000	**101.4%**	**10.1%**
hydromorphone	D6-hydromorphone	**2.5**-1000	**105.3%**	**9.4%**
codeine	D6-codeine	**2.5**-1000	**97.6%**	**14.5%**
naloxone	D6-oxycodone	**2.5**-2500	**86.2%**	**14.0%**
oxycodone	D6-oxycodone	**2.5**-1000	**97.4%**	**10.2%**
naltrexone	D6-oxycodone	**2.5**-1000	83.7%	**6.8%**
hydrocodone*	D6-oxycodone	**2.5**-1000	**110.1%**	**20.6%**
cis-tramadol*	C13-D3-tramadol	**2.5**-1000	**95.6%**	**19.9%**
meperidine*	D4-meperidine	**2.5**-1000	81.4%	**11.2%**
fentanyl	D4-buprenorphine	**0.05**-20	117.46%	**12.5%**
buprenorphine	D4-buprenorphine	**2.5**-1000	**105.78%**	**13.2%**
methadone*	D3-methadone	**10**-1000	N/A	N/A

E-41

Evaluation of the BGM Galectin-3™ Assay On The Dynex Technologies DSX™ 4-Plate System, An Automated Microplate Processing System.

S. Wang[1], K. Shepherd[2], N. Gordon[1]. [1]*BG Medicine, Inc., Waltham, MA,* [2]*Corgenix Medical Corporation, Broomfield, CO,*

Objective: This study served to evaluate the feasibility of automating the BGM Galectin-3™ assay on the DSX™ 4-plate automated microplate processing system by comparing the assay performance on the DSX system with those of the manual assay.

Relevance: Galectin-3 is a cardiac biomarker that is implicated in adverse ventricular remodeling and the progression of heart failure. The BGM Galectin-3™ assay is a microtiter plate based ELISA that quantitatively measures galectin-3 in serum and plasma and is intended to be used in conjunction with clinical evaluation as an aid in the stratification of patients diagnosed with heart failure. Having recently achieved CE Mark status, the assay is available for clinical use in Europe and is pending clearance by the FDA. As clinical laboratories adopt this new test, many may choose to automate the ELISA procedures in order to increase sample throughput to reduce labor costs and minimize variability associated with manual operation. This study evaluated the assay performance on the DSX 4-plate automated microplate processing system relative to that of the manual assay.

Methodology: We evaluated the analytical performance of the BGM Galectin-3 assay on the DSX 4-plate system from Dynex Technologies by assessing the precision and linearity of the assay. In addition, performance of the BGM Galectin-3 assay run on the DSX system relative to the manual method was compared by evaluating clinical samples with galectin-3 levels spanning the measurement range of the assay (1.32 ng/mL to 96.6 ng/mL).

Results: Precision for the BGM Galectin-3 assay on the DSX system was 6.27% and 2.73% at galectin-3 levels of 15.81 ng/mL and 72.03 ng/mL, respectively. The assay showed linearity between expected and measured galectin-3 levels with a slope of 0.986 an intercept of -0.032, and a correlation coefficient of 0.9979. The galectin-3 levels measured on the DSX system across a set of clinical samples (n=37) showed excellent correlation (r^2 = 0.9908) with those obtained using the manual protocol with a proportional bias of 0.92 (95% confidence interval: 0.83 to 1.101) and a constant bias of -0.76 (95% confidence interval: -2.15ng/mL to 0.63 ng/mL) based upon the Deming regression analysis.

Conclusions: In conclusion, this study confirms that the BGM Galectin-3 assay is readily amenable to automation on the DSX 4-plate system.

E-42

Thyroid Function Testing and Tumor marker by LOCI Technology on Dimension Vista

E. Hamada, K. Iwahara, S. Uchiyama, T. Kondo, M. Maekawa. *Hamamatsu University School of Medicine, Hamamatsu, Japan,*

Introduction: The full automated immunochemistry measurement system, Dimension VISTA contains detection modules for colorimetric, nephelometric, and immunoassays. Luminescent Oxygen Channeling Immunoassay (LOCI) is a homogenous, chemiluminescent technology used for detection of antigen-antibody binding employing two particles, one a photosensitizer that produces singlet oxygen when exposed to light and a second that produces chemiluminescence when reacted with the oxygen. When the

particles are in close proximity (eg, antigen-antibody complex) the light is produced. Here we evaluated analytical performance of the LOCI measurement system of thyroid function testing and tumor markers.
Methods: We examined analytical performances of the full-automated immunochemistry measurement system, Dimension VISTA 1500 and the dedicated reagents (TSH, FT3, FT4, CEA, AFP and ferritin) (*Siemens Medical Solutions Diagnostics K.K.*).
Result: The within-run and between-run precision of thyroid function testing and tumor marker was respectively 1.0 to 3.9% and 0.6 to 5.8%. A good linearity in the measurement range and no influence of coexistence material were observed. Any influences of coexistence material using Interference Check A Plus and Interference Check RF Plus (Sysmex Co., Japan) were not found. The detection limit of TSH was 0.007μIU/mL. Correlation with ECLIA assay system (Roche Diagnostics) was followings: TSH was Y=1.10X-0.11 and r=0.9968 (N=62). FT4 was Y=0.76X+0.13 and r=0.9808 (N=100). FT3 was Y=9.96X-0.18 and r=0.9954 (N=99). CEA was Y=1.09X+7.94 and r=0.995 (N=100). AFP was Y=1.28X-522 and r=0.996 (N=75). Excluding the diluted re-testing samples, it was Y=1.00X+2.68 and r=0.993 (N=63). Ferritin was Y=0.83X-2.42 and r=0.991 (N=95). One serum specimen dissociated from the correlation. In the specimen FT3 showed a relatively lower value and TSH showed a higher value. There were no non-specific reactions to measure sera with M protein or polyclonal gammopathy, chylous sera, and patients' sera with dialysis, and pregnant women. However, one of 11 specimens with high RF titer showed the dissociation in thyroid function testing. The reason of the dissociation should be elucidated.
Conclusions: Analytical performances of thyroid hormone testing and tumor markers by LOCI system were acceptable. Regarding the measurement of thyroid function testing and tumor markers, quick return of the results is important. Additionally, Dimension Vista can simultaneously measure the chemistry testing. The integrated system, Dimension Vista with LOCI is expected to be able to improve patients service and laboratory operations.

E-43

Statistical Test for Equivalence and Non-Inferiority in Analysis of Method Validation and Comparison Experiments: Application in Assessment of Carry-over

T. Keller[1], T. Brinkmann[2]. *[1]Acomed Statistik, Leipzig, Germany, [2]Beckman Coulter, Inc., Nyon, Switzerland,*

Introduction. One component of method validation experiments assesses performance under various operating conditions (e.g., carry-over, storage). Usually, the traditional null hypothesis of no difference in concentrations is tested in statistical analysis. Then, failing to reject the null hypothesis ($p>0.05$) leads to the conclusion of evidence in favor of safety. Because "absence of evidence is not evidence of absence", this approach is not appropriate.
Methods. Statistical testing of equivalence (two-sided) or non-inferiority (one-sided problem) is applied. Reversed null and alternative hypotheses have to be formulated. They incorporate user defined thresholds for maximal allowed relevant values measured under special conditions. Carry-over experiments were performed on UniCel DxC 880i (Beckman Coulter) using different ßhCG samples (2 high, 5 low concentrations (L1-L5)), 10 repetitive experiments). Hereby, non-inferiority of L1 in relation to other L-samples was assessed by proving the null hypothesis: upper 95% confidence limit of mean of L1 is larger than mean + standard deviation of concentrations of other samples L3-L5.
Results. For all 4 DxC 880i pipettors, the null hypotheses were rejected. Non-inferiority of concentrations and therefore absence of carry-over effect is concluded.
Conclusion. Statistical equivalence testing known from pharmaceutical research (bioequivalence) is an appropriate method if equivalence or non-inferiority have to be shown. Furthermore, changing from non-appropriate "conventional" statistical testing to equivalence testing is connected with a change of paradigm from proof of hazard to proof of safety. The methodology is applicable to analysis of a wide range of experiments (carry-over, robustness, commutability).

Results of analysis. p-values refer to significance of equivalence.

	P1	P2	P3	P4
N (L1)	10	10	10	10
Mean (1)	0.108	1.19	1.18	1.23
95% CL of mean (1)	0.155 p<0.05	1.25 p<0.05	1.25 p<0.05	1.29 p<0.05
N (L3-L5)	30	30	30	27
Mean (3-5)	0.110	1.22	1.20	1.24
Mean (3-5) + SD	0.191	1.31	1.32	1.32

E-44

cobas® 8000 Modular Analyzer Series at 7 Sites in Australia, Europe and the US, Evaluated Under Routine Like Conditions

M. McGovern[1], M. Conti[2], A. von Eckardstein[3], A. Hubbuch[1], R. Imdahl[4], G. Jones[5], S. Preston[6], H. J. Roth[7], T. Szekeres[8]. *[1]Roche Diagnostics GmbH, Mannheim, Germany, [2]CHU De Bicêtre, Le Kremlin – Bicêtre, France, [3]University Hospital Zurich, Zurich, Switzerland, [4]Labor Schottdorf, Augsburg, Germany, [5]St. Vincent's Hospital, Sydney, Australia, [6]Huntsville Hospital, Huntsville, AL, [7]Labor Dr. Limbach und Kollegen, Heidelberg, Germany, [8]Medizinische Universität Wien, Vienna, Austria,*

Objectives: The **cobas** 8000 modular analyzer series is the newest member* of the Roche **cobas** modular platform family. The new Serum Work Area solution is designed for high volume clinical chemistry and immunoassay testing. This multicentre study was designed to verify, that the analytical and practical performance of the new system meets the laboratory requirements under various use conditions. Here we report on the outcome of the routine like testing experiments with the new system applied in dedicated use for general chemistry and specific protein analysis.
Methods: The seven study sites representing large commercial and centralized hospital laboratories used 3 different configurations of the **cobas c** 701 and **cobas c** 502 photometric modules, combined with either one of the two available ISE modules, the core unit and the Module Sample Buffer. Most experiments were designed to allow focus on data generated, using request patterns and samples from the daily routine. Within laboratory precision on 21 days was calculated from daily QC processed throughout the evaluation period. System functionality was tested using simulated routine workloads with >4000 requests for up to 45 methods combined with numerous provocations (low sample, product missing, operator interactions, etc) to check proper system behavior. Test results and sampling patterns from the routine laboratory analyzers were electronically captured and up to ~1500 tubes were then reprocessed on the **cobas** 8000 platform for up to ~45 methods per experiment. Results were checked for clinically relevant deviations and evaluated using the Bablok/Passing regression procedure. Practicability was assessed using a Roche standard questionnaire with ~200 questions covering all important attributes of an analytical system.
Results: In total ~ 864,000 results were generated for 60 different methods in ~ 96,000 serum, plasma, urine and whole blood samples. Within laboratory CVs were below 3% for the general chemistry analytes and below 5% for the specific proteins and urine analytes with very few exceptions. Of the 180 method comparisons generated from randomly selected routine samples, ~80% actually yielded slopes and intercepts within the ranges (slope 0.95 - 1.05, relative intercept 95 - 105% for the general chemistry methods and 0.9 - 1.10 and 90 - 110% for the other methods) expected for comparisons performed using standard batch testing protocols and samples with analyte concentrations evenly distributed over the measuring range. Analysis of workflow showed average sample processing times of ~15 minutes during peak workloads on the high volume module configurations. The various workloads were handled efficiently and the optimized rack routing supported introduction of STAT samples with resulting processing times of ~11 minutes. 96 % of the 1470 practicability assessment gradings indicate that the new analyzer series met or exceeded laboratory expectations
Conclusion: The results of this study demonstrate that the cobas 8000 modular analyzer series met or exceeded laboratory requirements under simulated routine laboratory conditions. It showed excellent analytical performance, high reliability, speed of analysis and ease of use.
*This analyzer is currently under development and has not cleared for use in the US by FDA. (status: February 2010)

Thursday AM, July 29

Poster Session: 9:00 am – 12:00 pm
TDM/Toxicology/DAU

E-45

Evaluation of Tacrolimus, Sirolimus and Squamous Cell Carcinoma on the Abbott Architect i1000 Platform

C. W. Lam, D. Tham, W. Lin. *Singapore General Hospital, Singapore, Singapore,*

Introduction The Abbott Architect i1000 analyser was identified as a possible replacement analyser for the Abbott IMx analyser for the testing of tacrolimus, sirolimus and squamous cell carcinoma antigen (SCC) currently performed by the laboratory.
Method Within-run and total imprecision was evaluated according to the protocol of CLSI document EP15-A2. QC materials (Biorad-Abbott Immunosuppressant MCC Levels 1, 2 and 3) are tested by running three replicates over five days. The limit of detection (LoD) was evaluated using the recommended diluent (Calibrator A) from the manufacturer. The diluent was measured 20 times in a single assay run and is defined as the value 2SD above the mean measured value. Linearity was determined by the serial dilution of calibrators in triplicates and was demonstrated by the recovery of expected value within +/- 15% in all 5 serial dilutions. Carryover was evaluated by analysing 2 samples with low and high concentrations in the following sequence: $L_1\ L_2\ L_3\ H_1\ H_2\ L_4\ L_5$. Carryover is statistically significant if L_4 - L_3 > (2.8 x Within-Run SD). Method comparison was done against the Abbott IMx analyzer using samples spanning the analytical measuring range.
Results

	Tacrolimus	Sirolimus	SCC
Mean (µg/L)	4.9, 11.1, 23.0	5.3, 10.6, 20.3	2.0, 10.0, 49.6
Within-Run Imprecision (CV%)	3.5, 1.2, 1.9	1.4, 1.9, 2.4	2.6, 3.1, 2.9
Total Imprecision (CV%)	3.9, 2.9, 3.3	4.4, 6.2, 2.6	4.3, 4.3, 5.0
Dilution Recovery	96 - 107 %	89 - 105 %	94 - 104 %
LoD	0.4	1.1	0.1
Carryover	Not significant	Not significant	Not significant
Deming	Y = 1.13x - 0.95 (n=47)	Y = 1.06x + 1.9 (n=57)	Y = 1.02x + 1.2 (n=57)
R^2	0.98	0.94	0.98

Conclusion The Abbott Architect i1000 analyser is a fast, user friendly and low maintenance analyser that has good precision in the tests evaluated. The transfer of tacrolimus, sirolimus and SCC testing to this platform would improve the workflow process in our laboratory. However, one limitation identified was the necessary manual pre-treatment of the samples (tacrolimus and sirolimus) prior to analysis.

E-46

To assess the therapeutic effect of anti-fungus drug by the mRNA quantitation of the capsule associated protein 10 gene

Q. Ou, N. Lin, L. Jiang, Y. Chen, Z. Chen. *The department of medical laboratory science of the first affiliated hospital of Fujian medical university, Fuzhou, Fujian Province, China,*

Abstract:Objective To establish the fluorescent-quantitative PCR method to detect the mRNA expression of the capsule associated protein 10 of cryptococcus neoformans and assess the therapeutic effect of anti-fungus drug.
Methods The CAP10 gene was amplified from standard strain of cryptococcus neoformans by reverse-transcription PCR and to construct the standard substance named pGEMT-Easy-CAP10,the standard curve was set up using the serially diluted standard substance. We established the fluorescent-quantitative PCR and appraise it stability, specificity, and linear range. The copy number of CAP10 mRNA of 20 cases were detected by the FQ-PCR method before and after drug treatment.
Results We successfully constructed the plasmid standard substance and set up standard curve. The intra- and interassay variation were 0.31% and 2.73% respectively. The FQ-PCR system would specificly amplify the CAP10 gene of cryptococcus neoformans. The linear range was from 10^1copies/µl to 10^8 copies/µl. The copies of mRNA of CAP10 gene of Cryptococcus neoformans treated by Amphotericin B plus 5-Fluorocytosine dropped from 3.43±0.84 to 2.35±0.85, those treated by Fluconazole plus 5-Fluorocytosine dropped from 2.65±1.24 to 1.84±1.07(P<0.05).
Conclusion The FQ-PCR method for the detection of the capsule associated protein 10 gene of cryptococcus neoformans has been successfully established, it is specific and stable, its linear range is wide, it would be useful for assess the therapeutic effect of anti-fungus drug in patients infected by cryptococcus neoformans.

E-47

Simultaneous determination of Lovastatin and its metabolite (ß-hydroxy acid) in serum/plasma by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry.

D. R. Mendu, S. J. Soldin. *Georgetown University, Washington, DC, [1]Department of Medicine and Georgetown Clinical Research Center, Washington, DC, [2]Department of Pharmacology, Georgetown University and Georgetown Clinical Research Center, Washington, DC.*

Lovastatin, a competitive inhibitor of the enzyme 3-hydroxy-3-methylgultaryl-CoA reductase, is a highly effective cholesterol lowering agent, which is widely used in the treatment of hypercholesterolemia. The drug is administered as a lactone form and is rapidly converted in the liver into its ß-hydroxy acid (Lov-OH), the active metabolite of lovastatin. A specific, precise, sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of lovastatin and its ß-hydroxy acid metabolite in human plasma/serum was developed and validated.
An API-4000 triple-quadrupole mass spectrometer (Sciex, Concord, Canada) coupled with the IonSpray source and Shimadzu HPLC system (Shimadzu Scientific Instruments, Columbia, MD) was used employing tacrolimus as an internal standard (IS) for lovastatin and its metabolite. 100 µL of serum (plasma) was deproteinized by adding 150 µL of acetonitrile containing 1 ng/mL internal standard, tacrolimus. After centrifugation for 10 min at 10,000 rpm, 200 µL of supernatant was diluted with 500 µL of water and 400 µL of the sample was injected into a HPLC. The calibration curves for lovastatin and metabolite ranged from 0-10 ng/mL and 0-5 ng/mL, respectively. Chromatographic separation was achieved in a run time of 10 min on a Supelco C18 column (3.3 cm x 3mm, 3\m, Sigma-Aldrich, MO, USA). The sample injection was followed by washing the C18 column (Supelco) with 2% methanol containing 0.1% formic acid for 2.1 minutes. For the period of 2.1 to 9.0 minutes a 60-100% methanol gradient (containing 0.1% formic acid) was used. The retention times for lovastatin, beta hydroxy acid and tacrolimus were 4.82, 5.06 and 4.63 minutes, respectively.
Detection of analytes and IS was done in positive ion and multiple reaction monitoring (MRM) acquisition mode. The transition ions monitored for lovastatin, ß- hydroxy acid metabolite and internal standard were m/z 427.2-->325.2, 423.2-->303.4 and 821.8-->768.5, respectively.
Calibration curves (R^2>0.999) in spiked plasma were linear over the concentration range of 0-10 ng/mL for lovastatin and 0-5 ng/mL for lovastatin ß- hydroxy acid. The overall accuracy of this method was 98-104% for both the analytes. A within- and between-day imprecision value was below 7% at all QC concentrations tested. The assay has a lower limit of quantification of 0.05 ng/mL for lovastatin and 0.15 ng/mL for lovastatin ß- hydroxy acid in human plasma/serum samples. We have evaluated the ratio of lovastatin ß-hydroxy acid/ lovastatin in 60 plasma samples. The ratio varied from 0.5-2.9. Lovastatin is rapidly metabolized forming lovastatin ß-hydroxy acid.
The method was found to be both specific and sensitive for the rapid and accurate measurement of lovastatin and its metabolite in the human matrices studied (urine, plasma/serum).

E-48

Quantification of serum busulphan by liquid chromatography-tandem mass spectrometry using turbulent flow online extraction

C. Heideloff[1], D. R. Bunch[1], J. Ritchie[2], S. Wang[1]. *[1]Cleveland Clinic, Cleveland, OH, [2]Emory University Hospital Laboratory, Atlanta, GA,*

BACKGROUND Busulfan (BU) is a cell cycle non-specific alkylating agent which slows the growth of cancer cells. It is used in bone marrow transplantation especially in chronic myelogenous leukemia. Monitoring blood level after the initial dose of BU is essential in successfully adjusting subsequent dosages for optimal therapy. Due to the intraindividual and interindividual variability of the pharmacokinetics, therapeutic drug monitoring is a necessity.
OBJECTIVE To develop and validate a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to quantify BU in serum.
METHODS 100 µL serum was mixed with 300 µL internal standard precipitation solution (Busulfan-d_8 in methanol) and centrifuged at 15,500 g for 10 minutes. 50 µL of supernatant was loaded onto a Cyclone-P TurboFlow column (0.5 x 50mm, Thermo Fisher Scientific, Waltham, MA) for purification followed by a Hypersil Gold C18 analytical column (3.0 x 50 mm, Thermo Fisher Scientific) for chromatography on a TLX2 coupled with Quantum

Access mass spectrometer (Thermo Fisher Scientific). The mass spectrometer was set at positive electrospray ionization and multiple reaction monitoring mode. Quantification was based on peak area ratios of BU (m/z 264.2→151.2) to internal standard (m/z 272.2→159.1).
RESULTS The analytical time was 4 minutes per injection. The method was linear over the range of 35.65 to 4732.12 ng/mL with analytical recovery ranging from 87.9-97.3%. Precision was evaluated either by running 20 replicates or based on EP10-A2 protocol. For spiked serum samples with mean concentrations of 141.40 ng/mL, 1113.14 ng/mL, 2408.38 ng/mL, and 3767.70 ng/mL, the intra-assay coefficients of variation (CV) were 7.70%, 5.33%, 2.06%, and 3.36%, respectively. The inter-assay CV was 7.80%, 5.14%, 4.88%, and 3.97%, respectively. No significant carryover was observed from samples with concentrations up to 11408.28 ng/mL. Commercial controls (MAS, Camarillo, CA; Lyphochek, Irvine, CA; Monitrol, Fremont, CA) containing >100 therapeutic drugs and common endogenous substances were tested and showed no interference with this method. The correlation using 45 de-identified patient samples ranging from 231.53 to 4253.370 ng/mL showed a slope of 1.017 (95%CI: 0.969-1.064), an intercept of -3.8677 (95%CI: -85.5457-77.8103) and an r of 0.9883 in comparison with a commercial LC-MS/MS method at Emory University Hospital Laboratory (Atlanta, GA).
CONCLUSIONS Simple sample preparation, short analytical time, wide linear range, and high sensitivity and accuracy make this LC-MSMS method suitable for clinical monitoring of BU.

E-49

Urinary erythropoietin excretion rate depends on renal tubular function

J. R. Delanghe, E. Lecocq, M. Speeckaert. *University Hospital Ghent, B 9000 Gent, Belgium,*

Background: Recombinant human Epo (rhEpo) is illicitly used to improve performance in sports. Doping is discouraged by the screening of athletes for rhEPO in urine. This test can occasionally lead to false - positive results in post - exercise, protein - rich urine, because the adopted monoclonal anti - Epo antibodies are not monospecific. On the other hand, an important number of specimens do not show epo banding in the urinary epo test. We therefore have investigated what factors determine the urinary excretion rate of Epo.
Methods: Epo was analyzed in serum and urine using an immunoassay (Beckman) on a Beckman Acces analyser. Serum and urine samples from 21 patients were collected following surgery. Urine samples were stored at -20 °C until analysis. Alpha 1 microglobulin (A1M) was assayed in urine on a BN II nephelometer (Dade - Behring).
Results: Urinary Epo levels were detectable in all cases. The dynamic range of the Epo assay is 0.6 - 750 mIU/mL with an analytical sensitivity of 0.6 mIU/mL (median: 20 mIU/mL, interquartile range: 4 - 78 mIU/mL). Urinary Epo concentration did only moderately correlate with serum Epo values: log (urinary Epo, mIU/mL) = 0,380 + 0,576 log (serum Epo) ($r^2 = 0.159$). In contrast, urinary Epo concentration strongly correlated with urinary values for A1M (Y (Epo, mIU/mL) = 0.11 X (A1M, mg/L) + 37; $p < 0.0001$).
Conclusion: Urinary Epo excretion is strongly determined by the presence of tubular proteinuria. These findings are important since following streneous physical exercise, transient exercise - induced glomerular - tubular proteinuria with a half - time decay of ± 1 hour may occur. These findings highlight the importance of the pre - analytical phase in Epo - testing, in particular in athletes that present with exercise - induced proteinuria.

E-50

Immunosuppressant target therapeutic levels - from immunoassay to tandem mass spectrometry

W. Y. Ng, P. H. Lee, T. Y. S. Kee, C. P. Yeo, E. Jacob. *Singapore General Hospital, Singapore, Singapore,*

Background: Four immunosuppressants (cyclosporine CSA, tacrolimus TAC, sirolimus SRL and everolimus ERL) routinely monitored in solid organ transplantation, subscribe to therapeutic levels mostly monitored using immunoassays or HPLC. With tandem mass spectrometry (TMS) measurements, expected therapeutic levels require re-appraisal as it is generally acknowledged that TMS being more specific, determines lower values than immunoassays. A comparative study of immunosuppressant levels determined by TMS and immunoassay was taken to establish if adjustment of therapeutic levels is required.
Methods: Patients (n=99) on routine drug monitoring after renal transplants were studied - split-sampling for routine immunoassays (Abbott TDx/IMx - CSA, TAC, SRL; Seradyn TDx - ERL) and by TMS. Cyclosporine monitorings were typically at 0-hr and 2-hr, depending on the patient's management. Comparison of immunosuppressant levels by the two methods was evaluated by Deming regression and Bland-Altman bias plot analysis.
Results: For cyclosporine monitoring, the overall average bias by TMS was -60.8 µg/L across the range 25-1166 µg/L. Deming regression showed close correlation with immunoassay: y=1.00x - 62.8, Sy|x 143 µg/L, Spearman r^2=0.92. On subanalysis, those on 0-hr monitoring presented with -25.2 µg/L (n=60) and those on 2-hr monitoring gave -96.1 µg/L (n=75). In view of the larger TMS bias, adjustment of expected therapeutic levels for the 2-hr monitoring would be prudent. The current target 800-1300 µg/L could be adjusted to 700-1200 µg/L. For tacrolimus (n=53) and sirolimus (n=29) measurements by TMS, the average biases were -0.07 µg/L and -0.87 µg/L across the range 2-16 µg/L. Deming regressions obtained were: y=1.10x - 0.63, Sy|x 1.5 µg/L (TAC); y=1.19x - 2.41, Sy|x 2.8 µg/L (SRL). No adjustments would appear necessary. For everolimus (n=41, with supplementary data from heart transplants), a -2.8 µg/L bias (range 2.0-25.0 µg/L) was obtained, indicating a likely greater metabolite cross-reactivity of the Certican immunoassay reagents. Adjustment to 2 - 7 µg/L for trough everolimus levels (current 3-8 µg/L) would be needed.
Conclusions: Therapeutic levels for cyclosporine and everolimus measured by tandem mass spectrometry were adjusted slightly lower than that measured by immunoassay. No adjustments to target levels are necessary for tacrolimus and sirolimus. Caregivers should be cognizant of the lower levels determined by tandem mass spectrometry and follow through with closer monitoring of patient's clinical and other laboratory findings.

E-51

Frequent Inappropriate Use of Tacrolimus Levels in Clinical Care

N. I. Lindeman, D. W. Bates, P. M. Szumita, E. L. Milford, E. P. Alyea, M. J. Tanasijevic. *Brigham and Women's Hospital, Harvard Medical School, Boston, MA,*

Background: The utilization of laboratory testing for tacrolimus trough concentrations in a tertiary care center with an active transplant program was reviewed, to determine if samples were ordered, collected, and interpreted properly and, if not, if errors were associated with adverse patient outcome.
Methods: Medical charts were reviewed from all patients who had at least one sample tested for tacrolimus during July, 2007. Analyzed data included the test result, tacrolimus dose, transplant type, test indication, response to result, time of drug administration, time the sample was accessioned in the laboratory, and outcome of hospital stay.
Results: 489 samples were tested, from 74 patients. 82% of tests were for appropriate indications, such as GVHD, rejection, or toxicity. Of 352 samples with documented time of oral drug administration, 73 (21%) were collected as proper troughs, while 49% were drawn too soon after the preceding dose. 79% of the samples were followed by an appropriate dose adjustment. Overall, 52% of the samples were associated with at least one error.
Conclusions: Tacrolimus testing was associated with frequent errors. Nearly half of all samples were drawn too soon after the preceding dose, which would lead to an overestimated concentration. Less commonly, tests were inappropriately ordered for unrelated medical problems, or abnormal test results were not followed with dose adjustment. Caregiver education is indicated to improve the utilization of these tests. In particular, the importance of proper timing of phlebotomy relative to drug administration should be stressed.

E-52

Detection of metallothionein in biological specimens under heavy metal and toxic substance exposure

K. Nakajima, H. Kikuchi, K. Nakazato, H. Sekine, Y. Tokita, K. Suzuki, T. Nagamine. *Gunma University School of Heath Science, Maebashi, Japan,*

Background; We have developed an easy and specific enzyme-linked immunoassay (ELISA) for the determination of metallothinein-1 (MT-1) and 2 (MT-2) simultaneously in serum and other biological specimens in humans and experimental animals. MT assay is specifically useful for the detection of heavy metal toxicity and other poisonous substances because of its significant increase after the exposure of these toxic substances..
Methods: To develop a competitive ELISA, a specific polyclonal antibody was generated against rat MT-2. The epitope mapping of the antibody was investigated using MTs in mouse, rat, rabbit, human and the fragment peptides of human MT-2. MT1/2 knock mouse and cadmium treated mice were used for the evaluation of the ELISA. Pretreatment method of serum was examined to deplete blocking factors for this assay.
Results: The antibody used for this ELISA had the same cross-reactivity with MT in humans and experimental animals. NH_2 terminal peptide of MT with acetylated methionine was proved to be the epitope of this antibody. The reactivity of this ELISA system with liver, kidney and brain in MT1/2 knock-out mouse was significantly low, but was normal in MT-3 knock-out mouse. The lowest detection limit of this ELISA was a 0.6 ng/mL and the added MT-1 was fully recovered from serum. The mean MT concentration in our preliminary study was 23±4.6 ng/mL in human serum. Cadmium treatment to mice induced significantly higher amount of MT in serum, liver, kidney and spleen as reported previously by different assays.
Conclusion: The proposed competitive ELISA is an easy and specific method for practical use of toxicological studies, determining total MT-1 and-2 simultaneously in serum and other biological specimens of human and experimental animals.

E-53

Investigation and analysis of Zhongxiang City well water heavy metal content

L. Zuo[1], C. Wang[2], L. Zuo[3], D. Peng[4], D. Peng[4]. *[1]Jingmen Center of Disease Control, Jingmen, China, [2]College of Public Health, Wuhan University, Wuhan, China, [3]The First People's Hospital, Jingmen, China, [4]Zhongxiang Center of Disease Control, Jingmen, China,*

Objective: We assessed the heavy metal (Fe, Cu, Mn, Pb, As) content of Zhongxiang City (Hubei, China) drinking water to provide scientific basis for efficient management of local drinking water.
Methods: We randomly selected 267 samples of well water from 13 country monitor sites, and analyzed heavy metal content by the standard method specified in the Chinese National Sanitary Standard of Drinking Water.
Results: In Zhongxiang City, the mean Fe content is 0.37 (0.13-1.19 mg/L), over limit rate is 54.7%, Mn 0.15 (0.03-0.73) mg/L, over limit rate 56.93%; there was a significant difference in Fe and Mn content and over limit rate between different monitor sites ($P<0.01$). In 1.12% of all tested samples, As content is over limit. The content of Cu and Pb is normal in all tested samples.
Conclusion: There is a severe problem of Fe and Mn content over limit in Zhongxiang City drinking water, which should be taken seriously.

E-54

Evaluation of Rapamycin Calibrators and Quality Controls for Potential Commercial Use

C. Heideloff[1], M. Kozak[2], S. Wang[1]. *[1]Cleveland Clinic, Cleveland, OH, [2]Thermo Fisher Scientific, San Jose, CA,*

BACKGROUND Liquid chromatography-tandem mass spectrometry (LC-MSMS) is considered gold standard for measuring many clinical analytes including immunosuppressants. However home-made calibrators by each laboratory cause large lab-to-lab variations.
OBJECTIVE To evaluate rapamycin calibrators and QC materials from Thermo Fisher Scientific (Waltham, MA) using a fully validated LC-MS/MS rapamycin method currently in clinical use.
METHODS Six calibrators with assigned values of 0.9, 2.1, 5.2, 10.0, 26.3, 49.9 ng/mL from Thermo were analyzed along with current calibrators (0.0, 6.7, and 29.0, respectively) purchased from Chromsystems (Munich, Germany) and patient samples. There was one batch per day for a total of 5 days. In each batch we also included four QC with assigned concentrations of 0.0, 3.1, 12.7, and 30.7 ng/mL from Thermo and two QC purchased from Biorad (Hercules, CA) with mean concentrations of 4.1 and 27.8 ng/mL. Sample preparation included vortex mixing 100 µL whole blood, calibrators, or QC with 300 µL acetonitrile-zinc sulfate solution containing internal standard (32-desmethoxyrapamycin). After centrifugation at 15,500 g for 10 minutes, 50 µL of supernatant was injected onto a Cyclone-P TurboFlow column (0.5 x 50mm) for purification followed by a Hypersil Gold C18 analytical column (3.0 x 50 mm) for chromatography on a TLX2 coupled with Quantum Access mass spectrometer (all from Thermo). The mass spectrometer was set at selective reaction monitoring mode (sirolimus: 931.5→864.2 m/z and internal standard: 901.6→834.1 m/z) with positive electrospray ionization.
RESULTS Using Chromsystems calibrators, Thermo QC were 2.83, 12.83, and 30.23 ng/mL, and Biorad QC were 3.72 and 28.68 ng/mL. Using Thermo calibrators, Thermo QC were 3.09, 13.06, and 30.29 ng/mL, and Biorad QC were 3.93 and 28.83 ng/mL. %CV for all values were less than or equal to 9.4%. Recovery ranged from 91.2 to 102.8%. The correlation between the Chromsystems calibrators and Thermo calibrators using 55 de-identified patient samples ranging from 1.03 to 18.82 ng/mL showed a slope of 1.049, an intercept of -0.4453 and an r of 0.9968 with a mean difference of -1.3%.
CONCLUSION The calibrators and QC materials provided by Thermo were in accordance with our validated calibrators and QCs on a fully validated rapamycin LC-MS/MS method.

E-55

The Emergence of Benzylpiperazine (BZP) and Trifluormethylphenylpiperazine (TFMPP) in Sexual Assault and suspected Driving Under the Influence of Drugs (DUID) Cases in Miami-Dade Florida

B. W. Steele, L. J. Reidy, C. Walls. *University of Miami, Miami, FL,*

Objective: To report the incidence and concentrations of benzylpiperazine (BZP) and trifluormethylphenylpiperazine (TFMPP) in urine samples submitted into the toxicology laboratory. This paper will discuss the case results in reference to BZP and TFMPP, their use with other drugs and effects on human performance.
Introduction; Benzylpiperazine is a relatively new psychotropic stimulant. Both BZP & TFMPP were expedited onto schedule I of the Federal controlled substance list in 2002, however only BZP remains. In the state of Florida BZP and TFMPP are not controlled. BZP elevates serotonin and dopamine neurotransmitters concentrations in the brain by blocking the re-uptake of these neurotransmitters. BZP also behaves as a serotonin agonist and releases noradrenaline. TFMPP is a selective releaser of serotonin. The combination of BZP and TFMPP mimics the dopaminergic and serotoninergic effects of amphetamine derivatives, such as euphoria, feeling of wakefulness, and hallucinations. Side effects include vomiting, paranoia and anxiety. Hallucinations occur when BZP and TFMPP are used in combination and is dose dependant. Due to these effects, BZP and TFMPP should be considered in suspected DUID cases or drug facilitated sexual assaults. In 2006 (n=306), 2007 (n=237) and 2008 (n=360) no urine cases contained BZP or TFMPP.
Methods: Urine samples (unpreserved) collected by the police from suspected DUID drivers and sexual assault subjects in Miami Dade Florida from 2009-2010 were screened for BZP by ELISA (Neogen Corporation, KY) and GC-MS. Those screened presumptive positive (50ng/mL cutoff) were reflexed for confirmation. BZP + TFMPP were extracted by a modified basic extraction and derivitized using HFBA and analyzed via GC-MS in select ion monitoring (SIM) mode with MDA D5 used as an internal standard (IS). A calibration range of 50 to 1000ng/mL was prepared with positive and negative controls.
Results: Calibration curve are linear from 50 to 1000 ng/mL with a r^2 value of 0.990 with no interferences from the urine matrix, negative control, other commonly encountered drugs or the IS. Positive controls were within the accepted ± 20% of target values. Seven samples from 2009 screened positive by ELISA or GC-MS screening and were then confirmed by GC-MS SIM. In total 4 out of 270 suspected DUI urines analyzed in 2009 were positive to either BZP and/or TFMPP (=1.4% of cases). Three sexual assaults were positive for BZP and/or TFMPP out of 38 urine cases (8%) submitted in 2009. The results show a wide range of concentrations of BZP and TFMPP in urine samples from suspected DUID drivers and sexual assault victims. The median concentration of BZP was 1919 ng/mL (75 to 108714 ng/mL range). TFMPP was identified in four out of the seven cases. The median concentration of TFMPP was 2643 ng/mL (51 to 5946 ng/mL range). Five out of the seven cases confirmed the presence of other stimulant drugs (e.g. cocaine, amphetamine, MDMA "ecstasy").
Conclusion: This report has identified the emergence of BZP and TFMPP in Miami-Dade County in 2009. Consequently, toxicologists should be aware of the increasing prevalence of these substances, known toxicities and their effects on human performance.

E-56

Evaluation of Carbamazepine, Phenobarbital, Phenytoin and Theophylline TDM Assays on the Abbott ARCHITECT® *c*4000 Clinical Chemistry System

N. Z. Garcia-Perez, A. DeFrance, P. Bathory, C. Tran, D. Petty, L. Hoskins, B. Combs. *Abbott Diagnostics Division, Irving, TX,*

***Objective*:** Verification studies established performance of Carbamazepine, Phenobarbital, Phenytoin and Theophylline enzyme immunoassay therapeutic drug monitoring assays on a clinical chemistry analyzer, the Abbott ARCHITECT® *c*4000 Clinical Chemistry System.
***Methodology*:** The homogeneous enzyme immunoassay technique is used for the analysis of serum and plasma. The ARCHITECT *c*4000 is a fully automated analyzer that can be operated as a stand-alone instrument or integrated with the Abbott ARCHITECT® $i1000_{SR}$ (immunoassay module) to form the ARCHITECT *ci*4100, the newest member of the ARCHITECT family of instruments.
***Results*:** Assay performance (Precision, Sensitivity/LoQ, Linearity and Method Comparison) was characterized using CLSI-derived protocols. Precision (5 days) was determined using a minimum of three levels of controls. LoQ was demonstrated to meet or exceed values previously determined for the ARCHITECT *c*Systems. The analytical range represents the observed linear low and high levels verifying the Linearity claim. Method Comparison was evaluated by assaying serum patient samples (N>100) across the entire analytical range, comparing the ARCHITECT *c*4000 to the ARCHITECT *c*16000. Bias was determined at the medical decision level. Open On-Board and Calibration Stability was demonstrated to meet values previously determined for the ARCHITECT *c*Systems.

Study	Assay Results (ug/mL unless otherwise noted)			
	Carbamazepine	**Phenobarbital**	**Phenytoin**	**Theophylline**
Control Imprecision (Total %CV)	Mean: 3.0; % CV 2.9	Mean: 9.6; % CV 3.4	Mean: 6.4; % CV 5.3	Mean: 4.6; % CV 2.5
	Mean: 8.7; % CV 2.4	Mean: 23.1; % CV 2.5	Mean: 11.9; % CV 4.6	Mean: 13.8 % CV 2.1
	Mean: 13.7; % CV 2.0	Mean: 54.6; % CV 2.8	Mean: 24.1; % CV 4.0	Mean: 29.0; % CV 2.9
Limit of Quantitation (Sensitivity)	0.3 ug/mL with a % CV of 16	0.3 ug/mL with a % CV of 9	0.3 ug/mL with a % CV of 16	0.3 ug/mL with a % CV of 13
Analytical Range, ug/mL	0.2 - 19.1	0.2 - 78.3	0.2 - 37.6	0.2 - 37.5
Method Comparison (vs c16000)	Slope: 1.04	Slope: 1.06	Slope: 0.94	Slope: 1.01
	R: 0.9950	R: 0.9954	R: 0.9903	R: 0.9908
	%Bias: 2	%Bias: 4	%Bias: 3	%Bias: -8
Open On-Board Calibration stability	Open-on-Board: 28 days Calibration stability: 14 days Demonstrated less than or equal to 10 % or +/- 0.5 ug/mL deviation from the control and high linear targets over the testing period	Open-on-Board: 28 days Calibration stability: 14 days Demonstrated less than or equal to 10 % or +/- 1.0 ug/mL deviation from the control and high linear targets over the testing period	Open-on-Board: 28 days Calibration stability: 7 days Demonstrated less than or equal to 10 % or +/- 0.6 ug/mL deviation from the control and high linear targets over the testing period	Open-on-Board: 28 days Calibration stability: 14 days Demonstrated less than or equal to 10 % or +/- 0.6 ug/mL deviation from the control and high linear targets over the testing period

***Conclusion*:** The ARCHITECT *c*4000 Clinical Chemistry System demonstrated acceptable assay performance and achieved or exceeded all pre-established analytical goals. The ARCHITECT *c*4000 assay data support equivalency across ARCHITECT *c*8000, ARCHITECT *c*16000 and AEROSET Systems using the existing product criteria. The common reagent and commodity requirements across the four systems allow the laboratory the flexibility to use any instrument interchangeably depending on the user needs. The ability to integrate the *c*4000 with an *i*1000$_{SR}$ immunoassay module provides the capability to analyze routine clinical requests on a single system.

E-57

Performance and Development of an Immunoassay for Gentamicin on the Abbott ARCHITECT® *i*2000$_{SR}$ and *i*1000$_{SR}$ Analyzers

R. El Kouhen[1], A. Hoffman[1], W. Baugher[1], K. Krishnan[1], C. Rygiel[1], M. Novotny[1], D. Storck-Schattner[1], L. Xu[1], J. Grote[1], B. Merchant[1], Y. Wang[2], N. Naik[2], P. Wong[2], K. Ford[1], D. Wiesner[1]. *[1]Abbott Laboratories, Abbott Park, IL, [2]University Health Network, Toronto, ON, Canada,*

An automated chemiluminescent immunoassay (CMIA) for Gentamicin in human serum or plasma has been developed for use on the ARCHITECT® system worldwide as an aid in the management of patients receiving Gentamicin. The assay is based on paramagnetic microparticle chemiluminescent technology and uses the CHEMIFLEX® technology allowing for excellent precision, sensitivity, and accuracy. The purpose of this study is to evaluate the performance of the Gentamicin assay on both ARCHITECT *i*1000$_{SR}$ and *i*2000$_{SR}$ platforms with multi-center method comparison.

The assay employs a monoclonal antibody specific for Gentamicin and a tracer molecule consisting of Gentamicin covalently linked to acridinium in a competitive format. The assay is linear up to 10 ug/mL Gentamicin, and Limit of Quantitation was determined to be 0.1 ug/mL. Recovery of Gentamicin added to serum ranged from 93.85 - 101.59%; recovery from plasma ranged from 93.34 - 99.35%. Less than 10% interference was observed from bilirubin (20 mg/dL), triglycerides (3000 mg/dL), hemoglobin (500 mg/dL), and protein (12 g/dL). A correlation study was performed comparing patient results obtained using ARCHITECT iGentamicin assay on both *i*1000$_{SR}$ and *i*2000$_{SR}$ platforms to the Abbott AxSYM Gentamicin assay. Regression statistics showed a Slope of 1.02 in correlation between *i*1000$_{SR}$ and *i*2000$_{SR}$ platforms (R = 0.996, N = 106). Correlation to AxSYM showed a Slope of 1.03 with *i*1000$_{SR}$ (R = 0.995, N = 106) and a Slope of 1.00 - 1.09 with *i*2000$_{SR}$ (R = 0.987 - 0.994, N = 54 - 106, 2 sites). Total imprecision across the analytical range (0.1 ug/mL - 10 ug/mL), including within-run, between-run, and between-day variance components, were determined to be 1.99 - 4.80% CV (site 1) and 1.8 - 3.5% CV (Site 2).

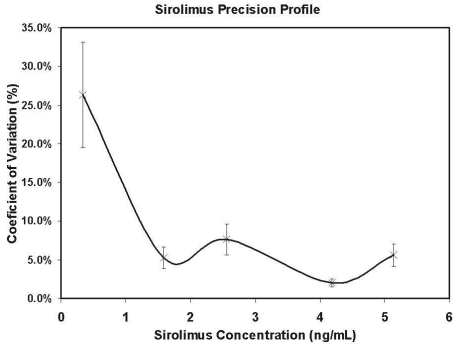
Conclusions: These results demonstrate that the ARCHITECT iGentamicin assay is a robust, precise, and convenient method for measuring the concentration of Gentamicin in serum or plasma.

E-58

Sirolimus Testing Using Siemens Xpand: Accuracy and Turn-around-time for Results

J. Zajechowski, C. Feldkamp, L. Stezar, D. Swiderski, V. I. Luzzi. *Henry Ford Hospital, Detroit, MI,*

Sirolimus is an immunosupressant drug used in organ transplantation. Because of its narrow therapeutic range, Sirolimus concentration must be monitored. In our institution, we evaluated the random access Siemens Xpand® Sirolimus assay. This assay does not require extraction and is an attractive alternative to provide accurate and fast turnaround time (TAT) results. To determine the analytical performance of the Siemens Xpand immunoassay to measure Sirolimus without pretreatment, Sirolimus correlation with LC/MS and IMx, precision, linearity and analytical and functional sensitivity were examined using human whole blood specimens or commercially available control material. For the correlation, Sirolimus was measured in de-identified human samples and analyzed by LC/MS and the Xpand system. Precision, linearity, and analytical and functional sensitivity were performed using More Diagnostics quality control material, or human whole blood. Data was analyzed using Microsoft Excel or EP Evaluator. TAT improvement was measured by comparing TAT before and after implementation of the Xpand assay in the routine testing. Linear regression showed that Sirolimus concentrations measured by the Xpand method, correlated more closely with LC/MS than IMx methods(LC/MS: y = 0.8854x + 1.0478; IMx y = 0.6014x + 1.1743). The CV for the simple precision was 6.2 % at 2.7 ng/mL. Between run precision was 16% at 2.8 ng/mL, 6% at 8.3 ng/mL, and 7% at 18.9 ng/mL. The average percent recovery between 4.9 ng/mL and 30.2 ng/mL was 100 %. The analytical sensitivity was 2 ng/mL. The precision profile validated the manufacturer's claim (figure). The average TAT dropped from 24.7 to 9.7 hours. Correlation and analytical performance data demonstrate that the Sirolimus Xpand immunoassay is an attractive alternative to LC/MS to use in the laboratory. Because there is no sample pre-treatment and batch testing is not required, this assay provides a better TAT for results.



E-59

Relationship between blood alcohol concentration and observable features of intoxication in patients presenting to a county medical center emergency room

K. N. Olson, S. W. Smith, J. S. Kloss, F. S. Apple. *Hennepin County Medical Center, Minneapolis, MN,*

Stages of clinical signs and symptoms of acute alcohol (ethanol) influence and intoxication [effects on the central nervous system] have been related to blood alcohol concentration (BAC). However, few studies have assessed the accuracy of scores of alcohol intoxication among individuals who have been drinking. The purpose of the current study was to assess the relationship between alcohol intoxication scores and a measured BAC. A prospective sample of patients were assessed upon presenting to an urban medical center emergency department where BAC is done routinely on all apparently alcohol intoxicated patients. Prior to BAC measurement, a nurse (80%) or physician (20%) assessed each patient using an alcohol symptom checklist (ASC) which measures eleven physical symptoms of alcohol intoxication. Patients were classified as either chronic alcohol users or non-chronic users. Ethanol was quantitated by either a breath test (Alco-sensor) or whole blood analysis by gas chromatography (GC). 134 subjects were evaluated. 68% were chronic users. The overall mean age was 41.0 years (range 14 to 64 y), with 81% male. Measured BAC ranged from 0 (less than the limit of detection, LoD 0.005 g/dL) to 0.413 g/dL, with

96% > LoD. For all subjects there was a poor correlation (r=0.330) between the measured BAC and the ASC criteria. In subjects that were chronic users the correlation was also poor (r=0.239) and was significantly less compared to the non-chronic users, whose correlation was moderate (r=0.509) (P<0.05). This study demonstrates that the alcohol symptom checklist correlated poorly with the measured blood alcohol concentration in chronic users compared to non-chronic (acute) users of alcohol even when applied by trained medical personnel with experience in assessing patients presenting with clinical features of alcohol intoxication. In chronic users, BAC alone does not correlate with behavior as measured by the alcohol symptom checklist.

E-60

Development of assay kits for the determination of methadone and methadone metabolite EDDP in urine on the RX series analysers.

P. McGivern, E. Donnelly, J. Thompson, P. Armstrong, J. Campbell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background: Methadone is a synthetic diphenylheptanolamine opioid and although it is prescribed to relieve chronic pain, it is primarily used as a substitute for heroin and other opiates in the detoxification and/or maintenance treatments for opioid addiction. The abuse potential of this compound is comparable to that of morphine due to its resembling pharmacological activity. The screening of methadone in urine is relevant for evaluating compliance/misuse. Methadone is metabolized to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine(EDDP) and some subjects metabolise the parent compound so quickly that they appear as negative upon methadone screening. On the other hand, adulteration of urine to disguise additional use of illicit drugs or to fake treatment compliance by spiking small amounts of methadone in urine, can be detected when a positive methadone assay is concurrent with a negative EDDP assay.
Relevance: We report the performance evaluation of two assay kits developed for qualitative or semi-quantitative analysis of methadone and its metabolite EDDP with ready to use reagents for application on the RX series analysers. This is of value as a convenient screening tool in treatment programs or to monitor misuse.
Methodology: The assays are based on competition between drug in the sample and drug labelled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody present in the reagent. Upon binding to the antibody the enzyme activity decreases, active enzyme converts NAD to NADH, resulting in an absorbance change at 340nm that is directly proportional to the amount of drug in the specimen. The assay kits consists of two ready to use liquid reagents. The assays are applicable to the fully automated RX series analysers, RX Daytona and RX Imola. Both systems include dedicated software for data management. Stored calibration curves are used for analyte determination. Within-run and total precision were assessed by testing samples at defined concentration levels, 4 replicates twice a day for 11 days. Two sets of 60 urine samples were screened with the RX series for each assay and confirmed by GC/MS.
Results: Evaluation of the qualitative and semi-quantitative performance of the developed assay kits was based on a cut-off of 300ng/ml and was as follows: the sensitivity of the methadone assay was determined as 4 and 10 ng/ml and for EDDP assay was 7 and 3ng/ml for RX Imola and RX Daytona respectively (assay range up to 1000ng/ml). The precision of the qualitative analysis expressed as %CV(n=88) for both assays was typically less than 6.6 and for the semi-quantitative assessment was typically less than 5.2 for different concentration levels in both systems. For the 60 samples analysed qualitatively and semi-quantitatively for each assay, the percentage agreement with GC/MS was≥80% for methadone and 98.3% for EDDP in both systems.
Conclusion: Data show reproducibility and accuracy of these assay kits for the *in vitro* determination of methadone and EDDP in urine on the fully automated RX series analysers with the added advantage of using ready to use liquid reagents. This is of value as a convenient analytical tool for the monitoring of these compounds.

E-61

Measurement of Urinary Buprenorphine and Norbuprenorphine by Liquid Chromatography-Tandem Mass Spectrometry

D. M. Garby, R. DelRosso, L. A. Cheryk. *Mayo Medical Laboratories, Andover, MA,*

Background Clinically buprenorphine is utilized in the treatment of opioid dependance and as an analgesic in the management of chronic pain. Buprenorphine is a partial agonist of the mu receptor whose clinical effects include sedation, euphoria and analgesia. Being a partial mu receptor agonist, the clinical effects are decreased compared to other treatment options which gives buprenorphine the added advantage of decreased withdrawal symptoms. Buprenorphine is metabolized through N-dealkylation to norbuprenorphine through cytochrome P450 3A4. Norbuprenorphine is an active metabolite possessing one fifth the potency of its parent compound.
Methodology Deuterated stable isotopes (buprenorphine-d4 and norbuprenorphine-d3) were added to 500 µL of human urine as internal standards. Sodium acetate buffer (pH=5.0) and Glusulase® were then added. This mixture was incubated at 50ºC to liberate the conjugates. Buprenorphine, norbuprenorphine and the internal standards were then extracted from the urine sample by liquid/liquid extraction using n-butyl chloride at a basic pH. The organic layer was removed, dried under nitrogen and reconstituted with a solution of deionized water and methanol (95/5 v/v) containing 0.1% formic acid. Buprenorphine, norbuprenorphine and the internal standards were then separated by liquid chromatography (TLX2, Thermo Fisher Scientific, Waltham, Massachusetts) followed by analysis on a tandem mass spectrometer (API 5000, Applied Biosystems, Framingham, Massachusetts) equipped with an electrospray ionization source in positive mode. Ion transitions monitored in the multiple reaction monitoring (MRM) mode were m/z 468.3 → m/z 414.3 and m/z 468.3 → m/z 396.2 for buprenorphine and its ion pair, respectively, m/z 414.2→ m/z 340.2 and m/z 414.2 → m/z 364.2 for norbuprenorphine and its ion pair, respectively, m/z 472.3 → m/z 400.3 for buprenorphine-d4, and m/z 417.2 → m/z 343.2 for norbuprenorphine-d3. Calibrators consisted of 7 standard solutions ranging from 0 to 100 ng/mL.
Results Method performance was assessed using precision, linearity, recovery, matrix interference and specimen stability. Studies were performed using human urine spiked with buprenorphine and norbuprenorphine standards. Intra-run precision (N=20) coefficients of variation (CVs) ranged from 1.1% to 2.0% for buprenorphine and 1.6% to 4.2% for norbuprenorphine. Buprenorphine inter-run precision (N=10) CVs ranged from 3.3% to 4.2%, and from 2.9% to 8.9% for norbuprenorphine. The method demonstrated linearity over the assay range (0.5 to 100 ng/mL) for each analyte, yielding the following equations: observed buprenorphine value = 0.9936*(expected value) - 0.1418, R2 = 0.9996; observed norbuprenorphine value = 0.9945*(expected value) - 0.0026, R2 = 0.9994. Recovery was demonstrated for each analyte by mixing high and low value urine samples. Recovery averaged 96% for buprenorphine and 97% for norbuprenorphine across the assay range. Matrix interference was investigated using a spiked urine specimen and by post column infusion. No ion suppression was detected. A stability study demonstrated that specimens were stable at ambient, refrigerate and frozen (-20ºC or lower) temperatures for up to 14 days.
Conclusion This method provides for the simultaneous and reliable analysis of buprenorphine and norbuprenorphine in urine.

E-62

Multicenter Evaluation of ONLINE DAT Amphetamines II Assay on Roche Hitachi, cobas® Analyzers, and COBAS INTEGRA Systems

A. Verstraete[1], W. Haase[2], D. LeGatt[3], C. Shalapay[3], J. Collins[4], R. Setterstrom[4], I. Domke[5], C. Beck[5], M. Coffing[6]. *[1]Department of Clinical Biology, Ghent University Hospital, Belgium, [2]MEDILYS, Asklepios Klinik Nord, Hamburg, Germany, [3]University of Alberta Hospital/Alberta Health Services, Edmonton, AB, Canada, [4]MEDTOX Laboratories, St Paul, MN, [5]Roche Diagnostics GmbH, Mannheim, Germany, [6]Roche Diagnostics Operations, Inc., Indianapolis, IN,*

Objective: The study goal was to evaluate analytical performance of ONLINE DAT Amphetamines II assay for determination of amphetamines, methamphetamines, and MDMA in urine under routine conditions. Imprecision and agreement with routine immunoassays and reference methods were tested according to standardized protocol in four laboratories.
Methods: The Roche turbidimetric immunoassay, based on kinetic interaction of microparticles in solution (KIMS), utilizes cutoff concentrations of 300, 500, and 1000 ng/mL for semi-quantitative and qualitative methods; 300 and 500 ng/mL cutoffs were tested in this trial. Qualitative mode, 500 ng/mL cutoff (**Hitachi 917, cobas** 6000 analyzer series) assay was compared to Roche Abuscreen OnLine Amphetamines and CEDIA Amphetamine/Ecstasy assays. Semi-quantitative mode, 300 and 500 ng/mL cutoff (**MODULAR** *ANALYTICS* **<P> module, cobas** 6000 analyzer series, COBAS INTEGRA 400, and COBAS INTEGRA 800 systems) assay was compared to Roche Amphetamines/MDMA sensitive and CEDIA Amphetamine/Ecstasy assays. Routine drug-of-abuse screening urine samples were used in all laboratories for method comparison. Discrepant samples were analyzed by LC-MS/MS or GC/MS.

Results: Intra-assay imprecision (21 replicates/run; 5 runs):

Site/Analyzer	Mode	Cutoff (ng/mL)	Samples (ng/mL Amphetamines)	Performance (%CV)
Belgium/ MODULAR *ANALYTICS*<P> **module** and **COBAS INTEGRA 400** and **Germany/cobas c** 501 and **COBAS INTEGRA 800**	Semi-quantitative	500	375 500 625	4.7-8.1 2.8-7.9 2.5-6.9
Germany/cobas c 501	Semi-quantitative	300	225 300 375	4.7-6.3 3.1-5.7 3.8-5.0
Canada/Hitachi 917 and **USA/ MODULAR** *ANALYTICS*<P> **module**	Qualitative* *Calculated based on absorbances.	500	375 500 625	0.7-1.4 0.8-1.3 0.8-1.6

Method Comparison: Of 6140 method comparison analysis points evaluated from screening through confirmation testing, there were 156 discrepant pairs. Among these, 46 were positive with Amphetamines II, but negative by other immunoassays; 10 of the 46 were confirmed positive. Of 110 samples negative with Amphetamines II, but positive by other immunoassays, 107 were confirmed negative. Overall agreement with the Roche method, prior to confirmation was 97.5% and following confirmation, was 99.4%.
Conclusion: Roche ONLINE DAT Amphetamines II assay yielded fewer false positive and false negative results than other immunoassays tested in this study.
This assay is currently under development and has not been cleared for use in the US by FDA.
ABUSCREEN OnLine, CEDIA, COBAS, COBAS C, COBAS INTEGRA, MODULAR and ONLINE DAT are trademarks of Roche.

E-63

Simultaneous UPLC-MS/MS assay for the detection of Levetiracetam and Gabapentin in serum and plasma

K. L. Johnson-Davis[1], J. M. Juenke[2], P. I. Brown[3], G. A. McMillin[1].
[1]University of Utah, Salt Lake City, UT, [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [3]ARUP Laboratories, Salt Lake City, UT,

Background: Levetiracetam (Keppra™) and gabapentin (Neurontin™) are anticonvulsants with novel structures, and suggested therapeutic ranges of 5 to 30 µg/mL and 2 to 10 µg/mL, respectively. Immunoassays are not currently available to support TDM of these anticonvulsant drugs. Here we describe a simple, rapid assay for the simultaneous monitoring of levetiracetam and gabapentin in plasma by UPLC-MS/MS.
Methods: After a protein crash with 50:50 methanol:acetonitrile internal standard solution, 1 µL of supernatant sample is injected onto a Acquity UPLC HSS T3 1.8 um, 2.1 x 50 mm (Waters) column. Elution occurs using a linear gradient of acetonitrile and water each having 0.1% formic acid added. The column is eluted into a Waters Acquity UPLC TQD, operating in a positive mode to detect gabapentin at transition 172.18>154.11, levetiracetam at 171.11>126, and internal standard (3-Amino-2-naphtholic acid) at 188.06>170. Secondary transitions for each analyte are also detected for gabapentin at 172.18>137.06, levetiracetam at 171.11>154, and internal standard at 188.06>115. Runtime is 1.5 minutes per injection. Due to similarity in molecular weights between the compounds chromatographic separation was achieved. Results obtained from patient samples were compared to results generated by established HPLC-UV methods.
Results: Method validation showed an intra-assay imprecision (CV) below 8% and an inter-assay CV below 5% for both analytes at four different values across the range with total imprecision less than 14%. Comparison data with HPLC-UV was excellent, as measured by a linear regression of y = 1.12x - 0.77, r=0.996, Sy,x =0.89 for gabapentin, and y = 0.991x +0.70, r=0.997, Sy,x =2.24 for levetiracetam. The analytical measurement ranges were 1 to 150 µg/mL for gabapentin and 5 to 150 µg/mL for leviteracetam, and no analytical interferences were identified.
Conclusion: A simple, reliable UPLC-MS/MS method was developed and validated for routine clinical monitoring of levetiracetam and gabapentin.

Table 1: Assay Imprecision

Drug	Concentration	Within Run	Between Run	Total Imprecision
	µg/mL	%CV	%CV	%CV
Gabapentin	1.6	6.4	5.6	14.2
	3.6	7.2	2.4	6.2
	7.6	4.4	1.2	2.9
	16.7	4.5	0.5	1.3
Levetiracetam	12.8	4.7	0.7	1.7
	20.0	6.6	0.4	1.1
	68.2	4.3	0.1	0.3
	113.7	6.8	0.1	0.2

E-64

A rapid immunoassay to quantitate paclitaxel in plasma on the Beckman Coulter AU400 using novel monoclonal antiboundy bound nanoparticles

D. Cline, J. B. Courtney, R. L. Harney, Y. Li, H. Zhang, S. J. Salamone.
Saladax Biomedical, Inc., Bethlehem, PA,

Background: Paclitaxel (Taxol®) is the first generation taxoid antineoplastic agent used commonly to treat patients having a variety of solid tumors such as ovarian, breast, and non-small-cell lung carcinomas. As with most cytotoxic agents, paclitaxel has a narrow therapeutic window and wide pharmacokinetic variability. The pharmacokinetic profile is characterized by substantial inter-patient variability of 3 to 13-fold in maximum serum concentration (Cmax), clearance, or area under the concentration vs. time curve (AUC). This variability has critical clinical consequences for severe hematological and neurological toxicities, as well as favorable outcomes. The current chromatographic methods for measuring paclitaxel blood levels require specially trained personnel, use expensive specialized equipment, and have time-consuming sample preparation and runtimes, all of which dissuade routine measurement for dose monitoring.
Objective: To develop an automated nanoparticle immunoassay providing a rapid, simple and cost-effective method to routinely measure paclitaxel levels in patients undergoing chemotherapy.
Methods: Novel and highly selective antibodies to paclitaxel were covalently linked to carboxyl-modified nanoparticles to develop a prototype automated homogenous assay for use on the Beckman Coulter AU®400 clinical analyzer which uses a two reagent system and less than 10 uL plasma to quantify paclitaxel. The degree of aggregation of nanoparticles, inversely proportional to the concentration of paclitaxel in a sample, was monitored at 600 nm. Total imprecision and linearity of the assay were evaluated by testing normal human plasma (NHP) pools spiked with different levels of paclitaxel. Background of assay was also determined by measuring >200 NHP samples. Immunoassay selectivity was characterized by testing metabolites and degradation products of paclitaxel for cross-reactivity at relevant concentrations.
Results: With a quantitative range from 25 to 600 ng/mL, and instrument auto-dilution, plasma samples with paclitaxel concentrations up to 12,000 ng/mL could be determined using this assay. Time-to-first result was 11 minutes and 400 samples per hour could be measured using a stored calibration curve. The total imprecision of NHP pools spiked with paclitaxel at four concentrations across the assay range was <10% CV and the assay was linear from 25 to 600 ng/mL. The background of the assay was <5 ng/mL, as determined using more than 200 NHP samples. The assay cross-reactivities to 6-alpha- and 3'-*p*-hydroxypaclitaxel, the major metabolites of paclitaxel, were 15% and 4%, respectively, and 5% for baccatin III, the major degradation product of paclitaxel. These were shown to have no significant impact on the quantitation of paclitaxel.
Conclusion: This immunoassay is suitable for determining paclitaxel concentrations in plasma with the advantages of speed, small sample size, no sample pretreatment and application on automated instrumentation, providing a tool for optimization of paclitaxel blood levels with pharmacokinetic-guided dosing.

E-65

A rapid immunoassay to quantitate docetaxel in plasma on Beckman Coulter AU400 using novel monoclonal anitbody bound nanoparticles

H. Zhang, D. Cline, J. B. Courtney, R. Harney, Y. Li, S. J. Salamone. *Saladax Biomedical, Inc., Bethlehem, PA,*

Background: Docetaxel (Taxotere®) is a commonly used antineoplastic agent of the taxoid family with broad activity in patients with a variety of solid tumors such as breast cancer, non-small-cell lung cancer, gastric cancer, prostate cancer and head

and neck cancer. As with most cytotoxic agents, docetaxel has a narrow therapeutic window and wide pharmacokinetic variability. The pharmacokinetic profile is characterized by substantial inter-patient variability in total clearance of up to 10-fold and variability in total exposure or area under the concentration vs. time curve (AUC) of up to 7-fold. This variability has critical clinical consequences for severe toxicities and outcomes. The current chromatographic methods for measuring docetaxel blood levels require specially trained personnel, are expensive, time-consuming and therefore not amenable for routine use.

Objective: To develop an automated nanoparticle immunoassay providing a rapid, simple and cost-effective method to routinely measure docetaxel levels in patient specimens.

Methods: Novel and highly selective antibodies to docetaxel were covalently linked to carboxyl-modified nanoparticles to develop a prototype automated homogenous assay to quantify docetaxel. The assay was applied to the Beckman Coulter AU400 clinical analyzer. The degree of agglutination of nanoparticles, inversely proportional to the concentration of docetaxel in a sample, was monitored at 600 nm. Total imprecision of concentrations at medical decision points and linearity of assay were evaluated by testing normal human plasma pools spiked with different levels of docetaxel. Background of assay was also determined by measuring >200 normal human plasma samples.

Results: With a quantitative range from 25 to 500 ng/mL, and instrument auto-dilution, plasma samples with docetaxel concentrations up to 10,000 ng/mL could be determined using this assay. Time-to-first result was 11 mins and 400 samples per hour could be measured using a stored calibration curve. The total imprecision of plasma pools spiked with docetaxel at four concentrations across the assay range was <10% CV and the assay was linear from 25 to 500 ng/mL. More than 200 normal human plasma samples were measured to assess the background of the assay to be <5 ng/mL. Immunoassay selectivity was characterized by testing cross-reactivity of metabolites, degradation products and structurally related compounds of docetaxel at relevant concentrations. The assay cross-reactivity to docetaxel hydroxy-tertbutyl carbamate, the major metabolite of docetaxel, was 12% and <1% for 10-deacetylbaccatin, a degradation product of docetaxel. These were shown to have no significant impact on the quantitation of docetaxel.

Conclusion: This immunoassay is suitable for determining docetaxel concentrations in plasma with the advantages of speed, small sample size, no sample pretreatment and application on automated instrumentation, providing a tool for optimization of docetaxel blood levels with pharmacokinetic-guided dosing.

E-66

Measurement of Tranexamic Acid Levels in Cardiopulmonary Bypass Patients Using a Novel Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry Assay

C. Abou-Diwan, K. Tanaka, R. J. Molinaro. *Emory University, Atlanta, GA,*

Tranexamic acid is a synthetic derivative of the amino acid lysine that possesses potent antifibrinlolytic activity. It has been used to decrease post-operative blood loss in patients with cardiopulmonary bypass (CPB) surgery. Despite the available literature describing the safety and effectiveness of tranexamic acid administration in patients undergoing CPB, some patients suffer from side effects such as seizures of unknown causes. Dosage of patients undergoing surgeries has been empiric with patients receiving a bolus dose of tranexamic acid. There is a paucity of data examining the pharmacokinetics of tranexamic acid levels pre- and post-surgery. Therefore, we have developed a novel method for the identification and quantitation of tranexamic acid in human plasma and CSF using ultra performance liquid chromatography-tandem mass spectrometry for use in pharmacokinetic studies of tranexamic acid. After the addition of aminocaproic acid (ACA**)** as the internal standard, tranexamic acid is extracted from plasma using methanol. Gradient chromatographic separations are performed on a Waters ACQUITY UPLC BEH C18 column using an ammonium acetate/formic acid mobile phase. Tranexamic acid demonstrated a reproducible elution time of 1.68 minutes while ACA, consistently eluted from the column at 1.35 minutes. Both tranexamic acid (precursor ion 158.2 > product ion 95.2) and ACA (precursor ion 132.1 > product ion 114.0) were monitored using positive electrospray ionization in the MRM mode (Waters Quattro Micro) using the hydrogen adduct mass transitions. Both within-run (2.8%-3.7%) and between-run (4.4%-6.0%) imprecision was acceptable using drug-free plasma spiked with known low, medium, and high concentrations of tranexamic acid. Recovery was between 89% and 108% and ion suppression was <13%. The tranexamic acid standard curve for plasma displayed a wide analytical measuring range with linearity up to 10 µg/mL and a limit of quantitation at 100 ng/mL. This novel method was successfully used to measure tranexamic levels in human plasma and CSF from CPB patients and is suitable for pharmacokinetic studies.

E-67

Development of an assay kit for the specific measurement of ethanol in biological samples on the fully automated RX series analysers.

P. McGivern, T. Lipton, P. Armstrong, J. Campbell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background: Ethanol or ethyl alcohol is a psychoactive drug present in alcoholic beverages. It acts as a central nervous system depressant. Ethanol is rapidly absorbed and is mostly metabolized in the liver, the remainder is eliminated by the kidneys and lungs. Ethanol is metabolised to acetaldehyde by the enzyme alcohol dehydrogenase and then to acetic acid by the enzyme acetaldehyde dehydrogenase. Ethanol tests are used in the diagnosis and treatment of alcohol intoxication or poisoning and also in the determination of legal impairment and forensic judgement.

Relevance: We report the development of an assay kit for quantitation of ethanol in serum and urine on the RX series analysers exhibiting specificity for the target compound without interferences with other organic compounds such as acetaldehyde, methanol and other known typical interferents in serum and urine. This is of value for *in vitro* diagnostic use in medical and legal applications.

Methodology: The assay is enzymatic and uses alcohol dehydrogenase, which converts ethanol to acetaldehyde and NAD to NADH, resulting in an absorbance change at 340nm that is directly proportional to the amount of ethanol in the specimen. The assay kit consists of two ready to use liquid reagents. The assay is applicable to the fully automated RX series analysers, RX Daytona and RX Imola. Only 7.7µl and 7.0µl sample volume is required respectively and both analysers generate the first result after 14 minutes. Both systems include dedicated software for data management. Concentrations are calculated from two point calibration. On-board and calibration stabilities were tested by storing the reagents uncapped on the RX series analysers for a period of 28 days. Within-run and total precision were assessed by testing samples at defined concentration levels, 2 replicates twice a day for 20 days. Correlation studies were conducted using commercially available ethanol assays.

Results: Evaluation of the performance parameters shows an assay sensitivity of 10 mg/dl for an assay range of 10-500 mg/dl. The within-run precision and total precision for three different concentration levels (n= 80) and expressed as %C.V. was ≤2.7. The liquid assay reagents present an on-board stability of 28 days at approximately 8^0C and a calibration frequency of 21 days. The assay was compared with commercially available ethanol assays, 40 serum samples and 45 urine samples were tested and the following linear regression equations were generated: Y = 0.91x + 1.99; r = 1.00 and Y = 0.93x - 1.41; r = 1.00 respectively.

Conclusion: This assay kit exhibits specificity and reproducibility for the *in vitro* measurement of ethanol in serum and urine on the fully automated RX series analysers with the added advantage of using ready to use liquid reagents, calibrators and controls. This is of value for alcohol testing in medical and legal settings.

E-68

Multicenter Evaluation of ONLINE DAT Benzodiazepines II Assay on Roche Hitachi, cobas® Analyzers, and COBAS INTEGRA Systems

A. Verstraete[1], W. Haase[2], D. LeGatt[3], C. Shalapay[3], J. Collins[4], S. Ryan[4], D. Wildbredt[5], C. Beck[6], I. Domke[6], M. Coffing[7]. *[1]Department of Clinical Biology, Ghent, Belgium, [2]MEDILYS, Asklepios Klinik Nord, Hamburg, Germany, [3]University of Alberta Hospital/Alberta Health Services, Edmonton, AB, Canada, [4]MEDTOX Laboratories, St Paul, MN, [5]Institute for Laboratory Medicine, Microbiology and Hygiene, Evangelisches Krankenhaus, Bielefeld, Germany, [6]Roche Diagnostics GmbH, Mannheim, Germany, [7]Roche Diagnostics Operations, Inc., Indianapolis, IN,*

Objective: The study goal was to evaluate analytical performance of ONLINE DAT Benzodiazepines II assay for determination of benzodiazepines and their glucuronidated metabolites in urine under routine conditions. Imprecision and agreement with routine immunoassays and reference methods were tested according to standardized protocol in five laboratories.

Methods: Roche turbidimetric immunoassay, based on kinetic interaction of microparticles in solution (KIMS), utilizes cutoff concentrations of 100, 200, and 300 ng/mL for semi-quantitative and qualitative methods. All cutoffs were tested in this trial. Qualitative mode, 300 ng/mL cutoff (**cobas** 6000 analyzer series, **MODULAR** *ANALYTICS* <P> module) was compared to Roche ONLINE DAT Benzodiazepines Plus and Microgenics CEDIA Benzodiazepines w/o β-glucuronidase assays. Semi-quantitative mode, 100 and 200 ng/mL cutoff (COBAS INTEGRA 400, COBAS INTEGRA 800 systems, and **cobas** 6000 analyzer series) was compared to Roche ONLINE DAT Benzodiazepines Plus, Roche Serum Benzodiazepines (UBENZ) and Roche Benzodiazepines (Generation 1) on COBAS INTEGRA system, and Microgenics CEDIA Benzodiazepines (w/ and w/o β-glucuronidase) on Olympus AU400. Routine drug-of-abuse screening urine samples were used for method comparison. Discrepant samples were analyzed by LC-MS/MS or GC/MS.

Results: Intra-assay imprecision (21 replicates/run; 5 runs):

Site/ Analyzer	Mode	Cutoff (ng/mL)	Samples (ng/mL Benzodiazepine)	Performance (%CV)
Canada/**cobas c** 501 and USA/**MODULAR** *ANALYTICS*<P> module	Qualitative* *Calculated based on absorbances.	300	225 300 375	0.2-0.6 0.3-0.7 0.4-0.7
Belgium/COBAS INTEGRA 400 and Hamburg, Germany/ COBAS INTEGRA 800	Semi-quantitative	100	75 100 125	2.5-5.9 1.3-4.2 1.1-6.2
Hamburg, Germany/ COBAS INTEGRA 800 and Bielefeld, Germany/ **cobas c** 501	Semi-quantitative	200	150 200 250	1.5-2.1 1.6-4.2 1.2-4.0

Method Comparison: Of 7044 method comparison analysis points evaluated from screening through confirmation testing, there were 468 discrepant pairs. Among these, 442 were positive with Benzodiazepines II, but negative by other immunoassays; 397 of the 442 were confirmed positive. Of 26 samples negative with Benzodiazepines II, but positive by other immunoassays, 3 were confirmed negative. Overall agreement with the Roche method, following confirmation, was 99%.
Conclusion: Roche ONLINE DAT Benzodiazepines II assay, yielded fewer false negative results than other immunoassays tested in this study.
This assay is currently under development and has not been cleared for use in the US by FDA. CEDIA, COBAS, COBAS C, COBAS INTEGRA, MODULAR and ONLINE DAT are trademarks of Roche.

E-69

Performance Evaluation of DAT Oral Fluid Amphetamines, Methamphetamine, and Opiates Assays on Roche Hitachi

D. Kuntz[1], M. Meixner[1], K. Armstrong[1], B. Masters[2], M. Coffing[2]. *[1]Clinical Reference Laboratory, Inc, Lenexa, KS, [2]Roche Diagnostics Operations, Inc., Indianapolis, IN,*

Objective: The study goal was to evaluate analytical performance of DAT Oral Fluid Assays for determination of amphetamines, methamphetamines, MDMA (Amphetamines and Methamphetamine); and morphine, codeine, 6-acetylmorphine (Opiates), respectively, in oral fluid under routine laboratory conditions. Imprecision and agreement with routine immunoassays and reference methods were evaluated according to standardized protocols.
Methods: Roche oral fluid turbidimetric immunoassays are based on kinetic interaction of microparticles in solution (KIMS). In conjunction with Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI), the assays utilize single cutoff concentrations of 40 ng/mL for Amphetamines and Methamphetamine and 10 ng/mL for Opiates. All assays have semi-quantitative and qualitative applications; only semi-quantitative were used in this trial. **MODULAR** *ANALYTICS* **<P>** module results are compared, with those of OTI Intercept® Micro-plate EIA. A combination of routine drug-of-abuse oral fluid samples and spiked samples were used for method comparison. Discrepant samples were analyzed by LC-MS/MS.
Results: Intra-assay imprecision (21 replicates/run; 3 runs):

Assay	Cutoff	Sample Concentration	Performance	
	(ng/mL)	(ng/mL)	(SD)	(%CV)
Amphetamines	**40**	**20** **40** **60**	**0.64-1.70** **0.52-1.22** **1.11-1.48**	**3.0-7.8** **1.3-3.1** **3.8-5.0**
Methamphetamine	**40**	**20** **40** **60**	**1.24-2.16** **1.48-2.60** **2.24-4.35**	**6.3-11.6** **3.7-7.0** **3.9-7.8**
Opiates	**10**	**5** **10** **15**	**0.26-0.43** **0.32-0.37** **0.32-0.51**	**4.8-9.8** **3.1-4.0** **2.3-3.4**

Method Comparison: 406 specimens were analyzed. All positives, all discordant specimens, and 10% of all negative specimens were confirmed. Overall agreement between Roche and OTI screening methods prior to confirmation was Amphetamines 95%; Methamphetamine 99.3%; Opiates 97.8%. Agreement between Roche method and LC-MS/MS was Amphetamines 99.5%; Methamphetamine 99.5%; Opiates 98.8%.
Conclusion: Roche DAT Oral Fluid assays yielded a high level of agreement with OTI Intercept® Micro-plate EIA (in all cases >95%) and with LC-MS/MS (in all cases >98%) in this study.
These products are not cleared for use in the U.S. A 510(k) submission is pending.
MODULAR is a trademark of Roche. All other product names and trademarks are the property of their respective owners.

E-70

Ethylene Glycol Screening: Adapting the CATACHEM Enzymatic Assay to Minimize False Positives

L. B. Hardy[1], S. L. Fan[1], A. M. Pistorino[1], J. M. Juenke[2], G. A. McMillin[3], G. L. Horowitz[1]. *[1]Beth Israel Deaconess Medical Center, Department of Pathology, Boston, MA, [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [3]University of Utah, Department of Pathology, Salt Lake City, UT,*

Introduction: Ethylene glycol (ETG) is a toxic substance found in automotive anti-freeze. It has been ingested as part of suicide attempts and accidentally by children and animals. ETG, and particularly its metabolic byproducts, can cause renal insufficiency, central nervous system depression, and death. The only currently reliable methods for quantitating ETG in human beings are gas chromatography (GC) and mass spectrometry (MS), techniques not widely available in clinical laboratories. A veterinary medicine enzymatic assay, available through CATACHEM, has been previously evaluated and noted for interfering substances, including propylene glycol (PG) and 1,3-butanediol (BD). Interestingly, the kinetics of the enzymatic reactions differ among the interfering substances and ETG.
Objective: The ethylene glycol enzymatic assay by CATACHEM was evaluated after implementing new method parameters for the Roche Hitachi 917 in an effort to minimize interferences and other false positive reactions as well as to make the assay more practical.
Method: The bacterial enzyme utilized by the method, Glycerol Dehydrogenase, oxidizes ETG in the presence of NAD, causing the production of NADH and an increase in absorbance at 340 nm. The manufacturer recommends the use of "Two-Point End" data analysis, in which the change in absorbance from 306 to 558 seconds is compared to a standard. We chose to take advantage of the true kinetics of the assay by using "Rate A" data analysis, in which the rate of change from 306 to 486 seconds was checked for consistency, which we felt would eliminate interferences.
Results: 1) Recovery studies were done with spiked samples at ten different concentrations of ETG (0 to 370 mg/dL). Both methods of data analysis were linear as high as 250 mg/dL. Between-day precision was tested at two concentrations, with CV and average concentrations (mg/dL) of: 3.4% at 61.7, 1.2% at 227.9. 2) Three different analytes were tested for interference: glycerol (0-32 mg/dL), PG (5-600 mg/dL), and BD (8-64 mg/dL). Glycerol did not interfere with either method. PG and BD gave falsely elevated ETG results at all concentrations using the original method; neither compound gave falsely elevated ETG readings with the new method, though at PG>20 mg/dL and BD>8 mg/dL error messages were generated. 3) Twenty clinical samples with ethanol ordered were tested. Using the original method, four of ten ethanol-positive and one of ten ethanol-negative samples yielded ETG levels >10 mg/dL. Using the modified data reduction, all twenty samples were reported as <10 mg/dL of ETG. 4) For 13 clinical samples, the modified data reduction yielded 3 results with error messages, two of which were ~500 mg/dL, beyond the assay's measuring range. Compared to GC/MS, for the 10 samples without error messages, y = 0.93x - 0.2, r-squared = 0.97; adding the two ~500 mg/dL samples, the correlation was even better (r-squared = 0.99).
Conclusions: The use of a new data analysis method effectively eliminated previously noted interferences. This enzymatic assay for the detection of ethylene glycol is a practical alternative to chromatography-based methods and can be implemented relatively easily in most laboratories on current equipment.

E-71

Development of an Immunoassay for Carbamazepine for the ARCHITECT® *i*2000$_{SR}$ and *i*1000$_{SR}$ Analyzers

B. Sanchez[1], K. Berg[1], C. Repass[1], S. St.John[1], A. Butler[1], W. Baugher[1], Y. Wang[2], N. Naik[2], P. Wong[2], P. Sluss[3], D. Wiesner[1]. *[1]Abbott Laboratories, Abbott Park, IL, [2]University Health Network, Toronto, ON, Canada, [3]Massachusetts General Hospital, Boston, MA,*

Background and Objective: Carbamazepine is used in the treatment of both generalized and partial seizures, due to the rapid control of excessive cerebral electrical discharges and the low incidence of acute and chronic toxicity. Treatment is usually monitored by serum measurements to achieve levels in the range of 2 to 10 µg/mL; toxicity is associated with serum levels above 12 to 15 µg/mL. We report the development and multi-center performance evaluation of an immunoassay for carbamazepine for the ARCHTECT *i*2000$_{SR}$ and *i*1000$_{SR}$ automated immunoassay instruments. In the ARCHITECT

iCarbamazepine assay, sample, anti-carbamazepine coated paramagnetic microparticles, and carbamazepine acridinium-labeled conjugate are combined to create a reaction mixture. The anti-carbamazepine coated microparticles bind to carbamazepine present in the sample and the carbamazepine acridinium-labeled conjugate. After washing, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). An indirect relationship exists between the amount of carbamazepine in the sample and the RLUs detected by the ARCHITECT i System optics. Carbamazepine can be quantified in human serum or plasma and standardization is accomplished with gravimetric USP drug preparations.
Methods: Multi-center studies conducted on the $i2000_{SR}$ and $i1000_{SR}$ included evaluation of precision, limit of quantitation (LoQ), linearity, cross reactivity and comparison of results from patient samples to a reference method. Precision evaluations were completed using a 20-day protocol based on the CLSI protocol EP5-A2. Panels for precision evaluations were carbamazepine drug spiked into normal human serum and commercially available multi-constituent controls. LoQ was determined using four zero-level samples and 5 low-level carbamazepine samples. Linearity evaluation was based on the CLSI protocol EP6-A. Cross reactivity was determined by spiking drug into serum samples containing carbamazepine. Method comparisons were conducted with serum and plasma patient samples using the Abbott AxSYM carbamazepine assay.
Results: Precision total CVs were 1.66-4.37% (range 1.96-18.46 µg/mL). LoQ was determined to range from 0.29 and 0.54 µg/mL. The linear range of the assay was demonstrated from 0.65 µg/mL to 23.77 µg/mL. Cross reactivity to carbamazepine 10,11-epoxide, oxcarbazepine, 10-hydroxycarbamazepine and eslicarbazepine all were less than 5%. Comparison to the AxSYM carbamazepine assay across sites gave the following Passing-Bablok statistics across serum and plasma patient samples: slope ranged from 0.96 to 1.10 and R ranged from 0.969 to 0.981.
Conclusion: These results demonstrate the ARCHITECT iCarbamazepine assay is a robust, precise, accurate, and rapid method for measuring the concentration of carbamazepine in serum or plasma.

E-72

Routine Ultra Fast Liquid Chromatography-Tandem Mass Spectrometry Assay For Simultaneous Measurement Of Immunosuppressants

Y. Gong, G. Ball, G. Hoag. *Dept. Lab Medicine, Vancouver Island Health Authority, Victoria, BC, Canada,*

Objectives: To establish a routine Ultra Fast Liquid Chromatography-Tandem Mass Spectrometry (UFLC-MS/MS) assay for simultaneous measurement of immunosuppressants, including Cyclosporin A (CsA), Tacrolimus (Tacro), Sirolimus (Siro).
Methods: Whole blood samples (100 µl) were mixed with a ZnSO4 precipitation solution containing internal standard (Cyclosporin D and Ascomycin), and centrifuged at 15,000g for 10 min. 25 µl of supernatant was chromatographed on an Agilent Phenyl-Hexyl 1.8µ 2.0x50 mm Column and then monitored by electrospray in positive ionization mode. Two transitions, one as quantifier and one as qualifier, were used for each analyte. Pressure was stable at 6200 psi during the run at 45 °C with a 0.6ml/min flow rate. Linear range, precision, accuracy, recovery, and interference were systematically studied. 60 patient samples were assayed to compare with an established mass spectrometry method.
Results: Total run time was 2.5 min. The limit of quantification for this method for CsA, Tacro, and Siro, was at 11, 0.2, and 0.4 µg/l, respectively. The assay was linear from limit of quantification to 3660, 238, and 515 µg/l for CsA, Tacro, and Siro, respectively (all with r > 0.995). The interassay CVs were 7.1% at 89 µg/l, 3.7% at 4.1 µg/l, and 5.1% at 2.9 µg/l for CsA, Tacro, and Siro, respectively. Similar CVs or lower CVs were observed at higher concentrations. For the accuracy of the method, the mean measured values of EQA survey material at 3 different levels, determined by this method on 10 days, agreed well with the reference values (peer group mean) with bias less than 12%. Method comparison with an established HPLC-MS/MS method showed good correlation for the three analytes. No carryover and no interference by hemolysis, lipemia and icterus were observed.
Conclusions: This simple, fast, and robust UFLC-MS/MS assay is suitable for routine measurement of the immunosuppressants, Cyclosporin A, Tacrolimus, and Sirolimus.

E-73

A Retrospective Cohort Study of Acetaminophen Overdose Patients and an Evaluation of Whether Acetaminophen Elimination Half-life is Associated with Hepatotoxicity

D. C. M. Chan[1], H. K. Lee[2], L. D. Lewis[2]. *[1]Dartmouth-Hitchcock Medical Center, Lebanon, NH, [2]Dartmouth Medical School & Dartmouth-Hitchcock Medical Center, Lebanon, NH,*

Background: Following toxic ingestions, acetaminophen (APAP) associated hepatic damage is a well established phenomenon. Elevation of hepatic enzymes has been traditionally used to evaluate APAP hepatotoxicity. However, elevation of hepatic enzymes is often delayed and in severe intoxications irreversible. Schiødt *et al* (Clin Pharmacol Ther 2002; 71: 221-5) reported that longer APAP elimination half-life was associated with hepatotoxicity in a selected group of patients.
Objectives: This study aimed to investigate whether APAP elimination half-life (amongst other indicators) in patients presenting with actual or potential APAP overdose/poisoning was a better predictor of liver damage than liver enzymes.
Methods: This retrospective cohort study involved all patients who presented to a rural academic medical center with putative APAP overdoses who had at least two plasma APAP concentration measurements performed. The patients were identified *via* Clinical Pharmacology service records. The APAP elimination half-life was calculated using a noncompartmental model (WinNonlin® Pro). Relationships to hepatic transaminases (ALT, AST) and PT/INR were explored to address the hypothesis that the APAP elimination half-life is a predictor of APAP induced hepatic dysfunction.
Results: Between September 1995 and September 2009, 30 patients (7 males; 23 females) met our selection criteria. The APAP elimination half-life was 4.3 h (range 1.1 to 16.9). When the relationship between APAP elimination half-life and hepatic transaminases and PT/INR was explored, APAP elimination half-life exhibited a positive correlation with INR ($r^2 = 0.48$, $p = 0.0002$). No correlation was observed between APAP elimination half-life and hepatic transaminase elevations.
Conclusions: We conclude that in this retrospective study, APAP elimination half-life was not a good predictor of hepatotoxicity based on elevation of transaminases, but was related to hepatic biosynthetic function as measured by PT/INR.

E-74

Monitoring of serum strontium levels after ingestion of 2000 mg of strontium ranelate

T. Franek[1], K. Brabnikova Maresova[2], J. Kukacka[1], J. Stepan[2], R. Prusa[1]. *[1]2nd Faculty of Medicine and Faculty Hospital Motol, Prague 5, Czech Republic, [2]Faculty of Medicine, Charles University, Prague 2, Czech Republic,*

Background. The strontium ranelate represents a therapeutic innovation in the management of osteoporosis and belongs to the group of antiosteoporotic drugs with binary effect. As with calcium, Sr^{2+} may regulate mature osteoclast function directly via calcium-sensing receptor, because Sr^{2+} closely resembles Ca^{2+} in its atomic and ionic properties and because they are both agonists of the calcium-sensing receptor. The aim of study was to evaluate expected therapeutic values of serum strontium after administration of 2000 mg of strontium ranelate.
Subjects. The group of ten healthy subjects, aged 23-26 years, enrolled in this study had to be healthy and not receiving any medication. The subjects were screened by a medical history review and for medications afecting calcium metabolism, and uderwent measurements of bone mineral density and blood and urine chemistry screens. The result of this tests were in the normal physiological range.
Materials and methods. The volunteers, after overnight fasting, received 2000 mg of strontium ranelate. Samples of venous blood were collected before and five times during 5 hours (every hour) following the ingestion. Blood serum was stored at -70°C. Strontium concentration was determined by atomic absorption spectrometry at 460.7 nm on instrument Varian 220 FS (Australia). We used 10-fold diluted serum and measured in acetylene-air flame. Samples with strontium concentrations below 500 µg/L were measured by graphite-furnace AAS with Zeeman background correction (Varian 220Z, Australia). Serum (100 µL) was diluted 1:19. Analytical parameters of the method: CV 5.4 % and bias -11.2 % (on sample with target value 105 µg/L).
Results. Baseline serum strontium levels were 59.44 ± 28.21 ug/L (mean ± SE). Administering of 2000 mg of strontium ranelate caused statistically significant increase in serum strontium levels: at 60 min 4563 ± 370 ug/L (mean ± SE), at 120 min 3926 ± 363 ug/L (mean ± SE), at 180 min 4532 ± 430 ug/L (mean ± SE), at 240 min 4423 ± 530 ug/L (mean ± SE), and at 300 min 4533 ± 522 ug/L (mean ± SE), but there is no statistically significant difference between any of five consecutive measurements in time from one to five hours from ingestion of strontium ranelate.
Conclusion. Monitoring of serum strontium levels might be useful in non-compliant patients, in patients with either side effects or suspected toxic levels. Results of our study contribute to the knowledge of expected therapeutic values in patients with osteoporosis on strontium ranelate therapy.

E-75

Association of G-137C IL-18 Promoter Polymorphism with early Renal Injury in liver transplantation Recipients

y. li, l. wang, b. ying, b. yang. *HUAXI HOSPITAL OF SCU, Chengdu, China,*

Background: Interleukin 18 (IL-18) is a potent proinflammatory cytokine, IL-18 can promote TNF-α, IL-1β, IL-2,IFN-α secretion. ALL those inflammatory cytokines induced by IL-18 can influence the CYP450 and MDR dependent drug disposition and induce

renal toxicity . We investigated the effect of IL-18 -137 (rs187238) and -607(rs1946518) polymorphisms on tacrolimus pharmacokinetics and renal toxicity in liver transplant patients.
Methods: A total of 156 liver transplant patients were enrolled into this study (35 females and 121 males). The mean follow-up was 52 mo (range 6-96 mo). All liver transplant recipients with normal serum creatinine (SCr) .We studied two single-nucleotide polymorphisms in the promoter region of IL-18 gene at the position -137 (rs187238) and -607(rs1946518) by HRM analysis (high-resolution melting curve analysis) in 156 liver transplant recipients , Tacrolimus dosage blood concentration , urine microproteinuria , serum creatinine (SCr) , blood urea nitrogen (BUN), blood glucose, hepatitis C virus Load were also investigated.
Result: The IL-18 G-137C polymorphism in recipients was significantly associated with liver transplant recipients' urine microalbumin , ($P < 0.05$). Recipients with the capacity for high IL-18 production (-137GG) had the highest urine microalbumin . No significant difference was observed in the frequencies of -607 and -137 allelic distribution in patients' tacrolimus concentration/dose (C/D) ratios, blood glucose, blood urea nitrogen (BUN) and hepatitis C virus Load. ($P > 0.05$)
Conclution: This study exclude hepatitis C virus (HCV), hypertension and diabetes mellitus related renal Injury, we identify the IL-18 G-137C (rs187238) is an independent risk factor on early renal glomerulus injury after liver transplantation . this genotype may be a useful marker of early renal Injury after liver transplantation. In such cases, conversion to a less nephrotoxic regimen may be beneficial.

E-76

Methodology Evaluation of Mycophenolic Acid (MPA) Blood Drug Level in Homogeneous Phase Enzyme Immunoassay

R. Zheng, Z. Dong, Y. Tian. *Chinese PLA General hospital, Beijing, China,*

Objective: Mycophenolate mofetil(MMF) is being evaluated for the prevention of rejection in transplanted organs and hydrolyzed to Mycophenolic acid(MPA). In order to know the remaining immunosuppressant dose in blood, we establish methodology platform of MPA blood drug level in plasma using homogeneous phase enzyme immunoassay which is based on competition for MPA antibody binding sites. Furthermore, we determine chemical characters for evaluating reliability of this method in MPA assay reagent.
Methods: The precision, linearity and recovery rate were evaluated according to NCCLS program separately in Hitachi 7600 automatic bio-chemistry analyzer. We choosed 34 renal transplantation samples which were in drug combination with Tacrolimus(FK506) and MPA and compared correlation of the two drugs in patients with renal transplantation.
Results: In the range of 0.1-15μ g/ml, results were in good linearity. The within-run CV of high, middle and low level were 7.08%, 1.49%, 2.16% respectively. The between run CV of high, middle and low level were 8.51%, 5.93%, 3.12% respectively. There was no linear correlation between FK506 and MPA blood drug level when they were in drug combination.
Conclusion: The method is simple, quick, accurate and suitable for monitoring the MPA concentration in patients and is applicable for the pharmacokinetics study of MPA. Because significant individual difference is found among the patients treated with combination immunosuppressive drugs, we should pay more attention to monitoring blood drug level in patients in clinic.

E-77

Pharmacogenetics of Calcineurin Inhibitors (Cyclosporine/Tacrolimus) in renal transplant recipients of North India

R. Singh, A. Srivastava, R. Kapoor, R. D. Mittal. *Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India,*

Background:The calcineurin inhibitors, cyclosporine (CsA) and tacrolimus (Tac), a highly efficacious class of immunosuppressants following solid-organ transplantation, are characterized by narrow therapeutic range and highly variable pharmacokinetic profile. Pharmacogenetics with an aim to identify the inherited basis for inter-individual differences in drug response may provide important information concerning the role of drug metabolizing and drug transporter gene variants in response to immunosuppressive therapy.
Methods:In the present study, association of common polymorphic variants in drug metabolism (*CYP3A4* and *CYP3A5*) and transport (*ABCB1*) were analysed with CsA and Tac pharmacokinetics in renal transplant recipients on CsA (n = 225) and Tac (n = 75) based immunosuppression regimen at 1 week, 1 month, 3 months, 6 months and 12 months post transplantation. Further, the influence of these variants on allograft outcome in terms of rejection episode was analysed and correlated with inter-individual variation in CsA/Tac pharmacokinetics.
Results: Among *CYP3A5* SNPs, recipients with CYP3A5 expresser status (*1/*1+*1/*3) were associated with low dose-adjusted concentrations of CsA/Tac and thereby required higher daily doses to achieve therapeutic concentrations as compared to non-expressers (*3/*3) during the initial period (1-3 months) after renal transplantation. Amongst *ABCB1* SNPs, wild type genotypes of *2677G>T* and *3435C>T* were associated with significantly lower dose-adjusted values and consequently required higher CsA/Tac daily doses. When analysed for association with allograft outcome, the homozygous variant genotypes of *ABCB1 1236C>T*, *2677G>T* and *3435C>T* showed significantly reduced risk of allograft rejection.
Conclusions: Thus, additional pre-transplant genotyping of *CYP3A5*3* and *ABCB1* SNPs in combination with therapeutic drug monitoring may have an important clinical implication in individualizing and optimizing CsA/Tac dose requirement in transplant patients, thereby minimizing allograft rejection .

E-78

Rapid 5-fluorouracil plasma quantification by immunoassay on clinical chemistry analyzers

D. R. Kozo, C. N. Benfield, J. B. Courtney, R. L. Harney, Y. Li, G. D. Lundell, C. J. Spedaliere, S. J. Salamone. *Saladax Biomedical, Inc., Bethlehem, PA,*

Background: 5-Fluorouracil (5-FU) is one of the oldest chemotherapy agents and remains widely used in the treatment of solid tumors. 5-FU is the backbone of colorectal cancer chemotherapy. Studies over the last 30 years have demonstrated wide pharmacokinetic variability of 5-FU which can lead to undue toxicity and suboptimal treatment. Recent clinical studies have demonstrated that managing plasma 5-FU levels and adjusting doses to target steady state concentrations minimizes toxicity and improves outcome.
Methods: An existing nanoparticle immunoassay using a novel antibody to 5-FU was modified to directly quantify 5-FU in patient plasma samples without sample preparation by spin-filters. The assay was adapted to Beckman Coulter AU® analyzers and to the Roche COBAS® c 111 clinical analyzer. The correlation between analyzers, method comparison to a physical method, LoD (limit of detection), LLoQ (lower limit of quantitation), precision, and linearity of the assay were evaluated. Cross-reactivity to related endogenous substances, co-administered and unrelated medications and robustness to interferences were tested.
Results: The spin filter processing of the plasma was eliminated. Sample turnaround time was reduced by >15 minutes. The reportable range was confirmed and the immunoassay was linear, with an LoD of 52 ng/mL and an LLoQ of 85 ng/mL. The immunoassay was precise: <6% total CV around the target medical decision points for FOLFOX and FOLFIRI regimens. Method comparison with LC-MS/MS: Deming slope 1 ± 0.05, R > 0.98. There was no clinically significant cross-reactivity to compounds closely related to 5-FU: <1% for dihydro-5-fluorouracil, eniluracil, thymidine, 5-fluorouridine, uridine, Tegafur, capecitabine and others. Cross-reactivity with uracil was <11%, and would not affect clinical results.
Conclusions: The assay provides the performance required to quantitate 5-FU plasma concentrations. It is easily adapted to other instrument platforms, is precise and correlates well to physical methods. Laboratories could use the assay to introduce an evidence-based approach to optimizing 5-FU dosing with higher through put, simpler methodology and with less labor, space and expense than using physical methods.

E-79

Determination of six drugs of abuse in urine on the RX series analysers with assay kits using multi-analyte calibrators.

P. McGivern, E. Donnelly, J. Thompson, P. Armstrong, J. Campbell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background: For monitoring and regulatory purposes, drug testing by urinalysis is indicated as the use and abuse of drugs has an impact in our society. Drug testing involves initial screening of samples followed by confirmation of positive results with a confirmatory method. The development of rapid and convenient analytical methods is relevant to facilitate the initial screening of samples in test settings.
Relevance: We report the performance evaluation of 6 assay kits developed for qualitative or semi-quantitative analysis of amphetamines, barbiturates, benzodiazepines, cocaine metabolite (benzoylecgonine), methadone and opiates in urine using ready to use multi-analyte calibrators and liquid reagents for application on the RX series analysers. This is of value as a convenient screening tool in test settings.
Methodology: The assays are based on competition between drug in the sample and drug labelled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody present in the reagent. Upon binding to the antibody the enzyme activity decreases, active enzyme converts NAD to NADH, resulting in an absorbance change at 340nm that is directly proportional to the amount of drug in the specimen. The assay kits consists of two ready to use liquid reagents. Urine samples are used neat. The multi-analyte calibrators are ready to use and include the 6 drug classes The assays are

applicable to the fully automated RX series analysers, which include dedicated software for data management. Stored calibration curves are used for analyte determination. Within-run and total precision were assessed by testing samples at defined concentration levels, 2 replicates twice a day for 11 days. At least 60 urine samples were screened with the RX Daytona for each assay and confirmed by GC/MS.
Results: Evaluation of the qualitative and semi-quantitative performance of the assay kits on the RX Daytona showed sensitivity values of 54.34ng/ml (amphetamines, cut-off 1000 ng/ml), 3.82 ng/ml (barbiturates, cut-off 200ng/ml), 45.18ng/ml (benzodiazepines, cut-off 200ng/ml), 11.79ng/ml (benzoylecgonine, cut-off 300ng/ml), 9.73ng/ml (methadone, cut-off 300ng/ml) and 63.83ng/ml (opiates, cut-off 2000ng/ml). The total precision of the qualitative analysis for all the assays, expressed as %CV was typically ≤8.0 and for the semi-quantitative assessment was typically <10.0 for different concentration levels. For the samples analysed qualitatively and semi-quantitatively for each assay, the percentage agreement with GC/MS was ≥ 80% for all the analytes.
Conclusion: Data show reproducibility and accuracy of these assay kits for the *in vitro* determination of six drugs of abuse in urine on the fully automated RX series analysers with the added advantage of using ready to use multi-analyte calibrators and liquid reagents. This is of value as a convenient analytical tool for the screening of urine samples in test settings for monitoring and regulatory purposes.

E-80

Development of an assay kit for the determination of MDMA in urine on the RX series analysers.
P. McGivern, E. Donnelly, J. Thompson, P. Armstrong, J. Campbell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background: 3,4 methylenedioxymethamphetamine (MDMA), commonly known as "ecstasy" is a synthetic drug with stimulant and psychoactive properties. It is an illegal and popularly abused recreational drug. At low doses, MDMA produces euphoria and increased self-awareness. In high doses, the drug can interfere with the body's ability to regulate temperature. Users of MDMA face many of the risks associated to other stimulants including increase in heart rate and blood pressure, muscle tension, nausea, etc. Detection of MDMA or its metabolites in urine indicates use of ecstasy. Drug testing involves initial screening of samples followed by confirmation of positive results with a confirmatory method. The use of quick and convenient analytical methods is indicated for sample screening in test settings.
Relevance: We report the performance evaluation of an assay kit developed for qualitative or semi-quantitative analysis of MDMA in urine on the RX series analysers using ready to use liquid reagents. This is of value as a convenient screening tool in test settings.
Methodology: The assay is based on competition between drug in the sample and drug labelled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody present in the reagent. Upon binding to the antibody the enzyme activity decreases, active enzyme converts NAD to NADH, resulting in an absorbance change at 340nm that is directly proportional to the amount of drug in the specimen. The assay kit consists of two ready to use liquid reagents. Urine samples are used neat. The assay is applicable to the fully automated RX series analysers, RX Daytona and RX Imola which include dedicated software for data management. Within-run and total precision were assessed by testing samples at defined concentration levels, 4 replicates twice a day for 11 days. Stored calibration curves are used for analyte determination. 60 urine samples were screened with the RX analysers and confirmed by GC/MS.
Results: Evaluation of the qualitative and semi-quantitative performance of the developed assay kit was based on a cut-off of 500 ng/ml and was as follows: the sensitivity of the MDMA assay was determined as 13 and 0.05 ng/ml for RX Daytona and RX Imola respectively (assay range up to 1000ng/ml). The precision of the qualitative analysis expressed as %CV (n=88) was typically <3.0 and for the semi-quantitative assessment was typically <8.5 for different concentration levels in both systems. For the 60 samples analysed qualitatively and semi-quantitatively, the percentage agreement with GC/MS was ≥ 90%.
Conclusion: Data shows reproducibility and accuracy of this assay kit for the *in vitro* determination of MDMA in urine on the fully automated RX series analysers. This combined with ready to use liquid stable reagents and calibrators indicates that this assay kit is of value as a convenient analytical tool in test settings.

E-81

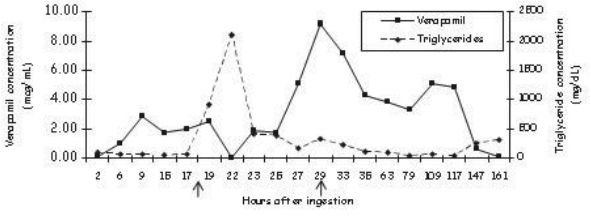
Anti cataract effect of active principle purified from fruit-pulp of *Eugenia jambolana* in galactose fed rats.
R. S. Tanwar, S. B. Sharma, V. P. Gupta. *University College of Medical Sciences, University Of Delhi, Delhi, India,*

Background Inspite of the presence of antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used from ancient period. Aim of the present study was to purify active principle (FIIc) from fruit-pulp of *Eugenia jambolana* and investigate its effect on galactose induced cataract in rats.
Methods Chromatographic purification of crude aqueous extract of fruit-pulp yielded partially purified hypoglycemic principle (FII) which on further purification resulted FIIc. Homogeneity of FIIc was tested by HPLC. Phytochemical investigation of FIIc via UV, NMR and IR spectra suggested that FIIc is a small aliphatic organic compound. Male albino rats fed with 30 % galactose as part of their normal diet were used in the study. Blood glucose and body weight were monitored at regular intervals while cataract were examined through naked eye as well as slit lamp at 40, 60, 100, 115th day after initiation of experiment. Effect of FIIc was investigated by measuring the degree of lens peroxidation and lens antioxidant status. Severity of cataract was determined by measuring the opacity index. FIIc was administered orally at dose of 15 mg/ kg body weight.
Results Administration of FIIc exerted a favorable effect on blood glucose and body weight. FIIc resulted significant ($p<0.05$) reduction in the opacity index and also reduced cataract progression. FIIc significantly reduced the degree of lens peroxidation, increased the levels of reduced glutathione (GSH) and lens catalase and superoxidedismutase activity.
Conclusions Our study suggests that FIIc protects against galactose induced cataract and also administration of FIIc reduced the cataract progression but did not reverse cataractogenesis.

E-82

Retrospective determination of serum verapamil concentrations before and after Intralipid® therapy in an overdose case
D. French[1], P. Armenian[2], W. Ruan[1], A. Wong[1], K. Drasner[1], A. H. B. Wu[1]. [1]*University of California-San Francisco, San Francisco, CA,* [2]*California Poison Control System - San Francisco Division, San Francisco, CA,*

Background: Intralipid® infusion is useful in reversing cardiac and central nervous system toxicity of anesthetic drugs, and recent reports suggest utility for other drug overdoses.
A 47 year old man presented to the emergency department with hypotension and complete heart block 3 hours after a verapamil sustained release overdose. He was given supportive care including calcium and hyperinsulinemia/euglycemia therapy (HIE). Eighteen hours post-ingestion, two 100mL boluses of Intralipid® followed by a 500mL intravenous drip over 30 minutes were administered. Twenty-nine hours post-ingestion, another 1.5mL/kg bolus was given followed by a 0.25mL/kg/min drip for 6 hours.
Objectives: Retrospectively determine serum verapamil concentrations before, during and after Intralipid® administration to determine its effect.
Methods: Serum samples were ultracentrifuged (Airfuge®) to separate serum from lipid. Solid phase extraction was performed (Oasis® MCX) with lamotrigine as an internal standard. High pressure liquid chromatography on a 1090 Hewlett Packard HPLC system was performed using an Agilent Eclipse XDB-C18 column. Isocratic elution was used with a K_2HPO_4:ACN (55:45), pH 3.0 mobile phase at 1.0 mL/min. Detection was accomplished using diode array detection (220nm). Triglycerides were measured using the ADVIA® 1800 Chemistry System.
Results: Extraction efficiency was 99% and the assay was linear from 0-15µg/mL. Within day precision was <1%. Verapamil concentration and triglycerides were measured in 19 serum samples taken between admission and 8 days post-ingestion. After administration of Intralipid® (indicated by arrows in figure), there is an increase in triglyceride concentration, and a corresponding decrease in verapamil concentration.Conclusions: Intralipid® reduced the serum verapamil concentration significantly, indicating utility of this treatment. Since the verapamil concentration increased after the Intralipid® infusion was stopped, continuing the infusion might have been beneficial, perhaps because the patient took the extended release form of verapamil.



E-83

Comparison of nicotine and nicotine metabolite concentrations in paired specimens of meconium and umbilical cord tissue, and correlation with maternal smoking histories

S. J. Marin[1], R. D. Christensen[2], V. L. Baer[2], M. Merrell[3], G. A. McMillin[4]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]McKay-Dee Hospital, Intermountain Healthcare, Ogden, UT, [3]ARUP Laboratories, Salt Lake City, UT, [4]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT,*

Smoking during pregnancy is associated with several adverse fetal/neonatal outcomes, including preterm birth, low birth weight, respiratory distress, and an increased mortality rate. Nicotine readily crosses the placenta and concentrates in fetal blood, amniotic fluid, and breast milk. Withdrawal symptoms can occur in neonates during the first days of life, but the etiology is usually unclear without an accurate history or definitive evidence of nicotine exposure. Therefore, detecting in utero exposure to nicotine might assist with neonatal and social management strategies. Meconium is currently the specimen of choice for detecting in utero drug exposure because it is thought to reflect maternal drug use during the last trimester of pregnancy. However, meconium is not always available for testing, or may not be available for several days after birth. In contrast, umbilical cord tissue is always available at birth. Here we report patterns of nicotine and metabolite (cotinine, 3-hydroxycotinine, nornicotine) concentrations, as well as presence of the tobacco alkaloid anabasine, in paired specimens of meconium and umbilical cord tissue collected from neonates of mothers who admitted to smoking and/or exposure to second-hand smoke during pregnancy. Concentrations of the analytes were compared with the smoking histories provided by the mothers. Mothers were consented and specimens deidentified according to an IRB-approved protocol.

METHODS: Eighteen paired specimens of meconium and umbilical cord tissue (0.25 g meconium and 1.0 g cord tissue) were homogenized and subjected to solid phase extraction in duplicate. LC-MS/MS was performed with a Waters Alliance Quattro-Micro system, using positive electrospray ionization and multiple reaction monitoring. Deuterated analogs for all analytes were included as internal standards. Two ion transitions were monitored for each analyte and internal standard. Quantitation was achieved using four point linear calibration curves. Matrix-matched calibrators and controls were prepared from pooled previously qualified drug-free specimens. The analytical measurement range was 4.0-2,000 ng/g in meconium and 0.5-1,000 ng/g in cord tissue for nicotine and trans-3-hydroxycotinine, and 2.0-2,000 ng/g in meconium and 0.25-1,000 ng/g in cord tissue for cotinine, nornicotine and anabasine.

RESULTS: Nicotine, cotinine and 3-hydroxycotinine were detected in meconium, along with small amounts of nornicotine. Cotinine and 3-hydroxcotinine were also detected in umbilical cord tissue, but at concentrations lower than those in meconium. Nicotine and nornicotine were not detected in umbilical cord tissue at significant concentrations, and anabasine was not detected in either specimen type. In general, concentrations of cotinine and 3-hydroxycotinine correlated with the mother's smoking pattern in both specimen types. Analyte concentrations were lowest for specimens collected from mothers who admited to second hand smoke exposure only, or who quit smoking early in pregnancy.

CONCLUSIONS: This work demonstrates that umbilical cord tissue is a sensitive matrix for detecting prenatal nicotine exposure. Moreover, umbilical cord tissue has the distinct advantages that it is available on all deliveries and can be sent immediately for testing.

E-84

Analysis of 21 benzodiazepines, Zolpidem and Zopiclone in serum or plasma using UPLC/MS/MS with liquid-liquid extraction

S. J. Marin[1], M. Roberts[2], R. Davis[1], G. A. McMillin[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Waters Corporation, Atlas Park, Manchester, United Kingdom, [3]Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT,*

Benzodiazepines are the most frequently prescribed drugs in the western world. They are indicated for a variety of disorders including: anxiety, insomnia, agitation, muscle spasms and alcohol withdrawal. They work primarily due to their interaction with the $GABA_A$ receptor. Tolerance, physical and psychological dependence or addiction and misuse or abuse can occur. Accurate methods of detection and quantitation are necessary for clinical management of vulnerable populations. METHODS: Twenty-seven serum/plasma specimens de-indentified using an IRB approved protocol were analyzed for 13 benzodiazepines by a previously published LC/MS/MS method using solid phase extraction (SPE) (1). These specimens were used in the validation of a UPLC/MS/MS method designed to detect and quantify 21 benzodiazepines, and two common sleep aids, zolpidem and zopiclone. Aliquots of matrix-matched calibrators (1, 10 and 100 ng/mL), controls, and samples (from patients, or prepared spikes) were subjected to liquid-liquid extraction (LLE) and analyzed using UPLC/MS/MS. Deuterated analogs for 16 of the analytes were included as internal standards. A gradient UPLC method using a Waters Acquity with a C18 column and a mobile phase of water:methanol with formic acid was used for separation. A Waters TQD mass spectrometer was operated in positive electrospray mode. Two ion transitions were monitored for each analyte and one transition was monitored for each internal standard using multiple reaction monitoring (MRM). Linearity and accuracy were evaluated using spiked samples within the analytical measurement range. Recovery was determined using pre- and post spiked serum. Ion suppression was evaluated by comparing responses from analytes spiked into six different batches of extracted blank sera to those spiked into mobile phase. RESULTS: The limit of detection (LOD) was defined as the concentration which gave a signal to noise (S:N) ratio > 7:1 (for both qualifier and quantifier transitions). The LOD was 5 ng/mL for four of the benzodiazepines, and 1 ng/mL for all other analytes. The ULOQ was 1,000 ng/mL for all analytes. All spiked sample concentrations were within ±15% of the target concentration for all analytes. Total recoveries ranged from 62 to 89% at 50 ng/mL. The linear correlation for 27 serum/plasma specimens analyzed for 13 benzodiazepines determined from the previously published method (39 positive results total for all analytes) was $y = 1.033x + 5.427$, $r^2 = 0.988$. In addition, zolpidem was detected in four of the specimens and zopiclone was seen in one, but these analytes were not quantitated in the previously published method. Conclusions: A new UPLC/MS/MS method for the quantitation of 21 benzodiazepines and two sleep aids was validated and compared to an existing LC-MS/MS method. The new method added 9 analytes and improved sensitivity without increasing the run time.

1. Marin SJ *et al. Journal of Analytical Toxicology* (2008) 32: 491-498

E-85

Development of an improved Lithium assay* using a crown formazan dye on the Siemens Dimension® clinical chemistry system

F. M. Celano, T. Q. Wei. *Siemens Healthcare Diagnostics Inc., Newark, DE,*

Background: Lithium is used primarily to treat the manic phase of affective disorders, mania, and manic-depressive illness. Because of its narrow therapeutic range and toxicity at high concentrations, therapeutic monitoring is crucial in the management of patients on Lithium therapy.

Relevance: Recent Guidelines of the German Federal Medical Council (or RiliBÄK) imposed rigorous regulations on the precision and accuracy of the Li assays, especially at the low end of the assay range. The Siemens Dimension® Lithium assay is under the development of a revision to improve precision and accuracy specifically targeted at the low Lithium concentrations.

Methodology: This assay employs a crown formazan dye, 7-nitro-2, 12-dicarboxyl-16, 17-dihyro-5H, 15H-dibenzo[b,i] [1,11,4,5,7,8] dioxatetraaza cyclo-tetradecine. The dye rapidly reacts with lithium ion (Li^+) in an alkaline water and dimethylsulfoxide mixture and has an absorbance change that is measured bichromatically at 540 and 700 nm. The method processing parameters, such as photometer read times, mixing duration and dye blank correction were further optimized on Xpand®, EXL™ and RxL systems, therefore, making the precision and accuracy performance of this assay more robust.

Results: The method is linear to 5 mmol/L and uses 10 µL of serum or sodium heparin plasma. Time to first results and assay throughput are comparable to the current commercial Dimension Lithium method. Repeatability and within-lab reproducibility were measured to be 2.3 and 2.4%CV at 0.47 mmol/L, 2.6 and 2.9 %CV at 0.56 mmol/mL, 1.8 and 1.8%CV at 0.78 mmol/mL respectively per the CLSI EP15-A2 protocol on the Dimension RxL system. Similar performance was obtained on the Dimension Xpand and EXL platforms. Split sample correlation (n=66) between the commercial lithium (Li) assay and the improved lithium assay (ILi) on Dimension RxL system gave a linear regression fit of: $ILi = 1.005 \times Li - 0.003$, $r^2=0.997$, range = 0.00 - 5.00 mmol/L. Ten survey samples tested recovered within the acceptable limits as published at the German Society for Clinical Chemistry and Laboratory Medicine (DGKL) website.

Conclusion: The revised Dimension Lithium method provides accurate, reproducible, and rapid measurements of lithium on the Dimension clinical chemistry systems.

*Product under development, not available for sale

E-86

Performance of a New Automated Fentanyl Immunoassay in Chronic Pain Patients

M. L. Snyder, P. Jarolim, M. Fernandez, S. E. Melanson. *Brigham and Womens Hospital, Boston, MA,*

Background: Fentanyl, a synthetic opioid agonist with high potency and short duration of action, is increasingly used in outpatient settings to manage chronic pain. Urine drug screens are frequently performed on patients undergoing chronic pain treatment to monitor compliance and/or detect the use of other prescribed, undisclosed, or illicit substances. However, commonly used opiate immunoassay screens do not detect fentanyl and until

recently fentanyl specific automated immunoassays were not available in the US. For this reason, more labor intensive and complex chromatographic-mass spectrometry or ELISA-based testing was required.
Objective: To evaluate the performance of a newly developed automated fentanyl enzyme immunoassay in patients treated for chronic pain.
Methods: Leftover urine samples (n=120) from patients treated for chronic pain were analyzed using the new automated qualitative fentanyl enzyme immunoassay (EIA) (Immunalysis Corporation, Pomona, CA) on the Olympus AU480 analyzer (Beckman Coulter Inc., Brea, CA). A 2-ng/mL fentanyl calibrator was used to distinguish positive from negative. All specimens were sent to an outside lab for confirmation using an ELISA with a lower 1-ng/mL cutoff followed by reflex testing of all positives by LC-MS. Spike and recovery experiments and endogenous (glucose, albumin, urea and creatinine) and exogenous (ethanol, stimulants, depressants, barbiturates, tricyclics, opiates, anticonvulsants and antidepressants) interference studies were performed. Medical records were reviewed to determine concomitant drug use.
Results: The EIA method compared well with our outside laboratory: 96.0% (24/25) positive agreement and 100% (95/95) negative agreement. Fentanyl and norfentanyl concentrations ranged from non-detectable to 400 and 1100 ng/mL, respectively. The one specimen that tested falsely negative by EIA gave a raw signal (98) very close to the positive cutoff signal (100) (Fentanyl = <1 ng/mL; Norfentanyl = 39 ng/mL). No false positive results were obtained. We confirmed the manufacturer's claim of <1% cross-reactivity for norfentanyl and found no cross-reactivity at 1000 times the cutoff. However, the EIA yielded a positive result in a specimen with 20 ng/mL norfentanyl and non-detectable fentanyl, suggesting that another metabolite such as despropionlyfentanyl with a manufacturer reported 8% crossreactivity may contribute to positive results with the EIA method. Negative urine spiked with 1, 2, 3, and 4 ng/mL of fentanyl produced signals that were 56%, 88%, 139%, and 203% of the 2 ng/mL calibrator cutoff signal, respectively. Coefficients of variation (CVs) using manufacturer supplied 1-ng/mL and 4-ng/mL control materials, were 36% (n = 20) and 15.1% (n = 20), respectively. The low and high control signals were always below and above the 2-ng/mL calibrator threshold signal, respectively. No significant exogenous or endogenous interferences were found. Notably many of the 95 negative specimens contained other medications and/or drugs of abuse such as opiates, methadone, cocaine, propoxyphene, benzodiazepines and cannabinoids.
Conclusions: The Immunalysis urine fentanyl EIA is a simple and quick screening method with high sensitivity and specificity in patients with chronic pain who are often prescribed other potentially interfering substances, such as opiates or other analgesics. Implementation of this fentanyl screen should facilitate testing in clinical laboratories that do not perform confirmatory testing.

E-87

Therapeutic drug monitoring experience of oxcarbazepine with epileptic patients in a natural clinical setting.

K. Park, D. Seo, S. Hong, S. Lee. *Samsung medical center, Seoul, Korea, Republic of,*

Background : Oxcarbazepine (OXC) is one of the newer antiepileptic drug (AEDs) as monotherapy and adjunctive therapy for epilepsy. There is little data about serum level monitoring and pharmacokinetics of OXC in Korean patients. The purpose of this study is to determine the relationship between OXC dose with serum OHC concentration and analyze the pharmacokinetic interaction of OHC in terms of age, sex, weight, and other AED used concomitantly in Korean epileptic patients. In clinical setting, population pharmacokinetic modeling and clinical drug response are analyzed.
Methods : We retrospectively analyzed 257 cases of OXC TDM in 204 patients with 143 males and 114 females. The Serum levels of the OXC were evaluated by means of HPLC. The age range was 3 ~ 80 years (31.14 ± 16.90 years, mean ± SD) and the weigh range was 10 to 95 kg (63 ± 16 kg , median ± SD). There were OXC monotherapy (79 cases, 31%), polytherapy of OXC with EIAED (178 cases, 28%), and polytherapy of OXC with non-EIAED (105 cases, 41%). In regard to serum level, we calculated OHC-OXC-CDR (concentration to dose ratio) by dividing the OHC concentration (mg/L) by the OXC daily dose (mg/day) adjusted body weight (kg). The statistical study was carried out using PASW for window version 17.0. software.
Results : The mean serum concentration of OHC was 14.5 ± 8.3 ug/mL (range of 0.22 ~ 49.88 ug/mL) at the mean dose of 913.31 ± 488.67 mg/day (range of 75~3000 mg/day) corresponding to a mean dose of 15.77 ± 8.40 mg/Kg/day. The serum concentration of OHC is correlated with OXC dose per body weight (r = 0.5298, p value < 0.0001). The OHC-OXC CDR was 1.02 ± 0.57 (range of 0.01~4.31) with an inter-individual coefficient of variation of 56%. Regarding the association with patient age, weight, and sex, no correlation with serum OHC concentration or OHC-OXC CDR were found. Regarding association of co-medication, significant difference in OHC-OXC CDR were observed among OXC monotherapy group, OXC with EIAED group, and OXC with non-EIAED group (1.06 ± 0.67 in OXC monotherapy group, 0.79 ± 0.39 in OXC with EIAED group, 1.14 ± 0.56 in OXC with non-EIAED group, p < 0.0001). And ADRs such as Hyponatremia and skin rash were not involved in serum OHC concentration.
Conclusions : Linear relationship was found between the serum concentration of OHC and OXC dose per body weight. The OHC-OXC CDR has no correlation with age, sex, weight and seizure type. And the OHC-OXC CDR was decreased when was administrated in combination with EIAED. After all, OXC dose per body weight and co-medicated AED can be used as a reliable index for accessment of serum OHC concentration, OHC-OXC CDR, and OHC clearance in Korean epileptic patients.

E-88

The research of renal toxicity lesion of CsA and FK506 in allo-liver recipients

J. T. Tang[1], L. L. Wang[1], L. X. Li[1], B. Cai[1], Y. G. Zhou[1], H. Wu[2], L. N. Yan[2]. *[1]Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China, [2]The Center of Liver Transplantation, West China Hospital of Sichuan University, Chengdu, China,*

Objective:To analyze renal toxicity of the CsA and FK506 in allo-liver recipients during different periods after transplantation and explore the difference of renal toxicity lesions in allo-liver recipients with CsA or FK506 treatments.
Methods: Apply EMIT to make treatment drugs monitoring and immunoturbidimetric assay to detect B2-M,A1M,MA,TRF,IgG in urine in 138 allo-liver recipients. Analyze the results of urine micro-proteins in FK506 group and CsA group to make comparison of the characteristics of renal toxicity lesions between two groups, and explore the relationship of renal toxicity lesions and administration time.
Results:Urine micro-proteins in allo-liver recipients treated by calcineurin inhibitor (CNI) all abnormally increased, and the abnormal rate of renal tubular proteinuria was the highest (up to 39.13%) in group of 0-6 months after CNI treatment; the abnormal rate of renal tubular and glomerular /compounded proteinuria was the highest (up to 60%) in the group of 2-3 years after CNI treatment; the abnormal rate of renal glomerular proteinuria was the highest (up to 17.39%) in the group of 7-12 months after CNI treatment; the highest total abnormal rate of proteinuria was up to 95% in the group of 2-3years after CNI treatment. Urine micro-proteins were significantly higher in FK506 treatment group than CsA treatment group during 0-1 year and 1-3 years after transplantation. Statistic analysis showed that the abnormal rate of renal compounded proteinuia was the highest in recipients whatever in FK506 or CsA treatment group(P<0.025), and the abnormal rate of renal tubular proteinuria was higher than that of renal glomerular proteinuria (P<0.025). There was no significant difference in types of urine micro-proteins between FK506 and CsA treatment groups(P>0.05).
Conclusions: The abnormal increasing A1M reached to the peak in 0-6 months group and 2-3 years group after transplantation, and the abnormal high MA and TRU came to the peak at 1-2 years group and 7-12 months group after transplantation. MA and TRU varied with post-transplant time, increasing and then decreasing, which indicated that during CNI treatment, immunosuppressants firstly caused the renal tubule lesions, while renal glomerulus lesions were slightly; the lesions of renal glomerular aggravated with the long-term CNI treatment. Non- difference of IGU between different post-transplant period groups indicated that the renal toxicity of CNI mainly caused lightly or moderate renal tubule lesions, and not or occasionally lead to severe renal glomerular lesions. All urine micro-proteins were significantly higher in FK506 group than that in CsA group (P<0.05), which indicated that the renal toxicity of FK 506 was more severe than CsA.

E-89

Development of an assay kit for the determination of cannabinoids in urine on the RX series analysers.

P. McGivern, E. Donnelly, J. Thompson, P. Armstrong, J. Campbell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background: Cannabinoids are a group of compounds found in the plant *Cannabis sativa*. The psychoactive component of marijuana is Δ^9-tetrahydrocannabinol (THC), which is rapidly metabolised to the principle inactive metabolite 11-nor-Δ^9-THC-9-carboxylic acid (11-COOH-THC). THC produces effects of euphoria and sedation and an altered time sense. The illicit use of marijuana as a recreational drug has led to the development of analytical methods to detect consumption. The use of rapid and convenient analytical methods is of value for sample screening in test settings as only samples giving a positive result need to be further assessed by a confirmatory method.
Relevance: We report the performance evaluation of an assay kit developed for qualitative or semi-quantitative analysis of cannabinoids in urine on the RX series analysers using ready to use reagents. This is of value as a convenient screening tool in test settings.
Methodology: The assays are based on competition between drug in the sample and drug labelled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody present in the reagent. Upon binding to the antibody the enzyme activity decreases, active enzyme converts NAD to NADH, resulting in an absorbance

change at 340nm that is directly proportional to the amount of drug in the specimen. The assay kits consists of two ready to use liquid reagents. The urine samples are used neat. The assay is applicable to the fully automated RX series analysers, RX Daytona and RX Imola which include dedicated software for data management. Within-run and total precision were assessed by testing samples at defined concentration levels, 4 replicates twice a day for 11 days. Stored calibration curves are used for analyte determination. 60 urine samples were screened with the RX analysers and confirmed by GC/MS.

Results: The evaluation of the qualitative and semi-quantitative performance of the developed assay kit was based on a cut-off of 50 ng/ml. The specificity of the assay expressed as %cross-reactivity was 100% for 11-nor-Δ^9-THC-9-carboxylic acid, 63% for 11-hydroxy- Δ^9-THC and 25% for THC. The sensitivity of the assay was determined as 11.2 and 2.06 ng/ml for RX Daytona and RX Imola respectively (assay range up to 200 ng/ml). The precision of the qualitative and semi-quantitative analysis expressed as %CV (n=88) was typically <20% for different concentration levels in both systems. For the 60 samples analysed qualitatively and semi-quantitatively, the percentage agreement with GC/MS was >80%.

Conclusion: Data show reproducibility and accuracy of this assay kit for the qualitative and semi-quantitative determination of cannabinoids in urine on the fully automated RX series analysers. This is of value as a convenient analytical tool for sample screening in test settings.

E-90

Development of a highly sensitive and specific polyclonal antibody to salicyluric acid: potential applications in monitoring of aspirin use or misuse?

P. Lowry, P. Innocenzi, M. E. O. Benchikh, R. I. McConnell, S. P. Fitzgerald. *Randox Laboratories, Crumlin, United Kingdom,*

Background: Aspirin is one of the most used drugs, indicated for the treatment of fever and pain and also used clinically to treat stroke, myocardial infarction, angina, colorectal cancer and rheumatoid arthritis. Its effectiveness as a therapeutic drug is constrained by a narrow concentration range. Once ingested, aspirin is rapidly hydrolysed to salicylic acid whose main metabolite is salicyluric acid, which makes up approximately 75% of the excreted product. As the level of salicylic acid increases, the urinary excretion concentration of salicylic acid increases relative to salicyluric acid.The therapeutic level of salicylic acid in blood of a healthy adult is generally considered to be 150-300mg/l. This level corresponds to urinary excretion ratio of salicylic acid to salicyluric acid of about 1:7.5. Due to the availability of aspirin in numerous over-the-counter products and its widespread therapeutic use, the monitoring of its use or misuse is relevant. For this purpose, the use of antibodies recognising specifically the metabolite salicyluric acid, enables the development of non-invasive immunoassays based on the ratio of salicylic: salicyluric acid as indicator for monitoring aspirin concentration and to detect aspirin overdose in urine samples.

Relevance: We report the development of a highly specific polyclonal antibody to salicyluric acid. This is of value for the development of non-invasive immunoassays using urine as sample, via calculation of the ratio salicylic acid: salicyluric acid for applications in therapeutic drug monitoring and toxicology settings.

Methodology: Salicyluric acid was modified with a cross-linker and conjugated to bovine thyroglobulin (BTG). The resulting immunogen was administered to an adult sheep and polyclonal antiserum was generated. IgG was extracted from the antiserum and evaluated via microarray-based, competitive chemiluminescent immunoassay using a biochip platform. Signal detection, data processing and storage were carried out using the Evidence Investigator analyser.

Results: Initial evaluation of the developed polyclonal antibody show specific recognition of salicyluric acid (%cross-reactivity 100%) and cross-reactivity<1.0% with other compounds including salicylic acid, 4-aminosalicylic acid, hippuric acid, aspirin, gentisuric acid, methylsalicylate, ibuprofen, gentisic acid, diflusinal, benzoic acid, acetylsalicylic acid,salsalate, paracetamol. The IC50 value was determined as 0.07µg/ml. Intra-assay precision (n=3) expressed as %CV was <8% for different concentration levels.

Conclusion: These results indicate that the polyclonal antibody generated is highly sensitive, specific for salicyluric acid and suitable for the development of non-invasive immunoassays using urine as sample and based on the ratio salicylic acid: salicyluric acid as indicator of aspirin consumption to monitor its use or misuse.

E-91

Evaluation of the DAU assays on VITROS® 5,1 FS

T. Bertsch[1], F. Degel[1], J. Aufenanger[2], O. Sonntag[3]. *[1]Institut für Klinische Chemie, Nürnberg, Germany, [2]Institut für Laboratoriumsmedizin, Ingolstadt, Germany, [3]Ortho Clinical Diagnostics, Neckargemünd, Germany,*

Background: The analytical performance of the novel VITROS® 5,1 FS assays for drugs of abuse testing were evaluated. In this study we did comparison to CEDIA assays and confirmed the results by highly sophisticated GC/MS methods. The aim of the study was to show the reliability of the assays under routine conditions.

Methods: The within-run imprecision was determined by measuring two different urine samples with low and high concentration of the drug of interest. The day-to-day imprecision was assessed by using quality control material at different levels provided by the manufacturer. We evaluated the assays for amphetamine, barbiturates, benzodiazepines, cocaine, methadone, opiates, phencyclidine and cannabinoids. About 100 urine samples from daily routine were used to compare with CEDIA® (Microgenics) and with GC/MS methods.

Results: The coefficients of variations (CV) for imprecision within-run ranged from 1.1 % (barbiturates) to 7.3 % (amphetamine) at low concentration, while the CV for the imprecision from day-to-day ranged from 1.5 % (phencyclidine) to 8.6 % (cannabinoids) at low concentration. The overall comparison with the other methods was good. Discrepant results between the two immunochemical methods were rare and could be explained by GC/MS to result from differences in crossreactivity (e.g. lorazepam in benzodiazepine assay, MDMA in the amphetamine assay) or from variations near the respective cut-off -values. Surprisingly, using the same cut-off-value of 100 ng/mL, there was no bias in the qualitative results (positiv/negative) between the VITROS methadone assay and the CEDIA EDDP assay, targeting the primary metabolite of methadone. GC/MS analyses of the positive samples showed in all cases the presence of both substances, so, no case of poor metabolizer or of abusive addition of the mother substance in order to simulate compliance was included.

Conclusion: The assays for DAU on the VITROS 5,1 FS showed good imprecision and high reliability. A good agreement with even high sophisticated methods like GC/MS was found.

E-92

A rapid method for simultaneous determination of five antiepileptic drugs by liquid chromatography-tandem mass spectrometry

M. R. Al-Turkmani, T. Law, M. D. Kellogg. *Children's Hospital Boston and Harvard Medical School, Boston, MA,*

OBJECTIVE: To develop and evaluate performance characteristics of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous quantitative determination of five antiepileptic drugs in serum.

BACKGROUND: Therapeutic drug monitoring of antiepileptic drugs (AED's) is widely practiced to assess therapeutic compliance, determine efficacy, and prevent toxicity. Methods for concurrent measurement of newer AED's in a single sample are limited. Here we describe a rapid LC-MS/MS-based assay for simultaneous determination of lamotrigine (LTG), levetiracetam (LVT), topiramate (TPM), zonisamide (ZNS), and the active monohydroxy derivative (MHD) of oxcarbazepine in serum.

METHODS: Calibration curves were constructed using six standards of LTG, LVT, TMP, ZNS and MHD at 0, 2, 5, 10, 30 and 60 µg/mL. The antiretroviral drug ritonavir was used as an internal standard (5 µg/mL in methanol). Three levels of control material at 3, 15, and 40 µg/mL were used to monitor the assay. Sample preparation involved adding 160 µL of internal standard solution to 20 µL of serum, followed by vortex mixing for 5 seconds. The mixture was incubated at room temperature for 5 minutes and centrifuged for 2.5 minutes at 1300 rpm (1.57 rcf). Two µL of the extract were injected onto a Supelco LC18-DB column on a Shimadzu liquid chromatography system connected to an Applied Biosystems API 3000 triple quadrupole mass spectrometer. Solvent A (0.1% formic acid and 15 mmol/L ammonium acetate in water) and solvent B (100% methanol) were maintained at a flow rate of 0.4 mL/min during the run. Mobile phase composition was 98% solvent A and 2 % solvent B from 0 to 2 minutes. The compounds were eluted with 90% solvent B (10% A) from 2.01 to 3.30 minutes and 100% solvent B from 3.31 to 3.8 minutes. The column was reconditioned with 98% solvent A (2% B). Total run time was 5 minutes per sample. Detection and quantitation of the analytes were carried out using electrospray ionization (ESI)-MS/MS operated in the positive-ion mode. Two transitions were monitored for each compound in the multiple reaction monitoring (MRM).

RESULTS: Within-run and between-day imprecision values were less than 10 % for all tested AED's. The assay was linear throughout the entire calibration range (0 -60 µg/mL) with a correlation coefficient (r^2) value higher than 0.99 for all AED's. Functional sensitivity was 1 µg/mL for all drugs and the analytical recoveries ranged between 92 and 108 %. Split sample comparison with immunoassays (TPM, ZNS) and high performance liquid chromatography (HPLC) assays (LTG, MHD, LVT) performed by an independent reference laboratory demonstrated good agreement with r^2 values of 0.91, 0.94, 0.93, 0.91, and 0.83 for LTG, LVT, TPM, ZNS, and MHD, respectively. Interference studies demonstrated no cross-reactivity with 40 other therapeutic drugs, including commonly prescribed AED's.

CONCLUSION: We have developed a rapid and accurate LC-MS/MS method for simultaneous determination of multiple AED's using small sample volume and a simple extraction procedure.

E-93

A Modified Method for Free Phenytoin Analysis on the cobas® c501 - Comparison to Abbott TDxFLx®

C. Shalapay, R. Yousofi, D. LeGatt. *University of Alberta Hospital, Edmonton, AB, Canada,*

Objective and Relevance: Phenytoin, a widely used anticonvulsant drug, is highly bound to plasma proteins. Alteration in the protein binding of phenytoin can result in a clinical response which is not mirrored by total phenytoin concentration. In these instances, monitoring the free drug level may correlate more accurately with clinical effectiveness. The imminent discontinuation of the free phenytoin assay on the TDxFLx® (Abbott Diagnostics), led us to search for a rapid immunoassay that could be adapted to another existing analyzer. The cobas® c501 analyzer (Roche Diagnostics) was recently purchased to perform drugs of abuse testing, therapeutic drug monitoring (TDM) and some esoteric chemistry tests. Currently, there is no application protocol for a free phenytoin assay on the cobas c501. The aim of this study was to develop a user-defined application protocol and to evaluate performance of a free phenytoin assay by modification of the Roche total phenytoin assay.

Methodology: The Roche total phenytoin method was modified by using the COBAS FP free phenytoin calibrators developed for use on the COBAS INTEGRA 400/700/800 system in conjunction with the Roche total phenytoin reagents for the cobas c501 system. The reagents were transferred to a cobas c pack MULTI cassette and user-defined application parameters were developed to maximize the analytical performance. The assay is based on the kinetic interaction of microparticles in solution (KIMS). Imprecision was evaluated by a modified CLSI protocol, using 3 levels of controls, assayed twice daily. Linearity and limit of quantitation (LOQ) were also investigated. A patient comparison was done using specimens (n = 41) from patients undergoing routine TDM. Ultrafiltrates were prepared according to the established Abbott protocol using Centrifree® micropartition filters (Amicon) and analyzed on the cobas c501 and TDxFLx.

Results: Total imprecision CVs for free phenytoin (means of 4.0, 8.0 and 11.8 µmol/L) were 3.3, 2.9 and 2.6%, respectively. Linearity was established to 16.0 µmol/L with an LOQ of 0.4 µmol/L. This is comparable to with the performance of the TDxFLx free phenytoin assay which has an analytical measuring range of 0.4 to 16.0 µmol/L. Autodilution was validated to establish a clinical reportable range of 0.4 to 32.0 µmol/L. The Cobas/TDxFLx patient specimen comparison showed good correlation (R^2= 0.961). Deming regression analysis demonstrated a small proportional bias at the upper end of the analytical measuring range (y = 0.9025x + 0.3745). Results were categorized in relation to the established therapeutic range and plotted in a 3-way contingency table. Concordance along the diagonal confirmed that this bias is not clinically significant.

Conclusion: The user-defined free phenytoin application described here for the cobas c501 is innovative, using the Roche c501 total phenytoin assay with the COBAS FP free phenytoin calibrators developed for the COBAS INTEGRA system. This further enhances the versatility of the cobas c501 for routine TDM of this drug in the clinical setting.

E-94

Emit® Methotrexate Assay on the Roche cobas® c501 analyzer - A Viable Alternative to the Abbott TDxFLx® Method

C. Shalapay, R. Yousofi, D. LeGatt. *University of Alberta Hospital, Edmonton, AB, Canada,*

Objective and Relevance: Methotrexate is an antineoplastic/antimetabolite drug used for treatment of leukemia, non-Hodgkin's lymphoma, lung cancer, breast cancer, severe psoriasis and sarcoidosis. Methotrexate dosing is varied, ranging from low-dose oral therapy to high-dose infusion therapy with leucovorin rescue. Routine therapeutic drug monitoring (TDM) is essential for clinical management in the latter dosing situation. With Abbott slated to phase out the TDxFLx® analyzer (Abbott Diagnostics) sometime in the next year, it is important the laboratory validate an alternate rapid immunoassay for methotrexate on another existing analyzer. The cobas® c501 analyzer (Roche Diagnostics), recently purchased to perform drugs of abuse testing, therapeutic drug monitoring (TDM) and some esoteric chemistry tests was the best candidate in our situation. The Syva® Emit® Methotrexate enzyme immunoassay (Siemens Diagnostic Healthcare Inc.) is available for the quantitative analysis of methotrexate. Siemens provides instructions for using this assay on several analyzers but there is no application protocol for the cobas c501. The aim of this study was to develop a user-defined application protocol for methotrexate and to evaluate the performance of the assay on this analyzer.

Methodology: The Syva Emit methotrexate assay is a homogeneous enzyme immunoassay technique. The methotrexate ready to use reagents were transferred to a cobas c pack MULTI cassette and user-defined parameters were developed for the cobas c501. Imprecision was evaluated by a modified CLSI protocol, using 3 levels of controls, assayed twice daily. Linearity and limit of quantitation (LOQ) were investigated. Specimens (n = 40) were selected from patients undergoing routine therapeutic drug monitoring and the results were compared to those from the TDxFLx analyzer.

Results: Total imprecision CVs for methotrexate controls (means of 0.34, 1.16 and 1.20 µmol/L) were 3.7, 5.5 and 5.0 %, respectively. Linearity was established to 2.00 µmol/L with an LOQ of 0.05 µmol/L (CV = 8.7%). Dilution protocols were validated to establish a clinical reportable range of 0.05 to1800 µmol/L. The Cobas/TDxFLx patient specimen comparison showed good correlation (R^2 = 0.9877). Deming regression analysis demonstrated a proportional bias at the upper end of the analytical measuring range. (y = 0.8655 + 0.3963). The patient correlation between the two methods using serum concentrations ≤ 5.0 µmol/L did not demonstrate a proportional bias. The clinical utility of the assay should not be impacted by this bias because the purpose of most methotrexate TDM protocols is to monitor serum concentrations during high dose-infusion therapy until they are < 0.05 µmol/L.

Conclusion: This user-defined application for the *Emit* Methotrexate immunoassay enhances the versatility of the cobas c501 for routine TDM of this drug in the clinical setting. Furthermore, this application provides laboratories with a viable alternative for methotrexate TDM since the most widely used method, the Abbott TDxFLx method, will soon be discontinued.

E-95

Evaluation of MULTIGENT PETINIA TDM Assays on the Abbott ARCHITECT® *c*4000 Clinical Chemistry System

K. B. Sikder[1], A. DeFrance[1], S. Shaw[1], N. Wilson[2], T. Engle[2], S. Orr[2], B. Shull[2], J. Dwenger[2], D. Petty[1], F. Knox[1], T. Sparks[1], L. Hoskins[1], P. Bathory[1], C. Tran[1], P. L. Smith[1], J. Dave[1], T. Sloan[1]. *[1]Abbott Laboratories, Irving, TX, [2]Thermo Fisher Scientific, Indianapolis, IN,*

***Objective*:** Establish performance of particle-enhanced turbidimetric inhibition immunoassay (PETINIA) TDM assays on the Abbott ARCHITECT® *c*4000 Clinical Chemistry System.

***Methodology*:** The ARCHITECT *c*4000 Clinical Chemistry System uses the same reagents calibration processes, reaction modes, photometric technology and Integrated Chip Technology® (ICT) as the Abbott ARCHITECT® *c*8000 and *c*16000 Systems and AEROSET® Systems. *c*4000 can be operated as a stand-alone instrument or be integrated with the ARCHITECT® $i1000_{SR}$ as the *ci*4100. Assay performance (Precision, Sensitivity, Linearity and Method Comparison) was characterized using CLSI-derived protocols. Precision (20 days) was determined using three levels of controls.

Results: Precision (20 days) was determined using three levels of controls. LoQ was demonstrated to meet predetermined goals for the ARCHITECT Systems. The analytical range represents the observed linear low and high levels verifying the Linearity claim. Method Comparison was evaluated by assaying serum patient samples across the dynamic range, comparing ARCHITECT *c*4000 to *c*8000 and to a predicate device per original 510(k) FDA submission.

Study	MULTIGENT TDM Assay Results						
	Amikacin (µg/mL)	Digoxin (ng/mL)	Gentamicin (µg/mL)	Quinidine (µg/mL)	Tobramycin (µg/mL)	Valproic Acid (µg/mL)	Vancomycin (µg/mL)
Control Imprecision (Total SD/ Total %CV)	L1 = 6% L2 = 3% L3 = 2%	L1 = 3% L2 = 1% L3 = 1%	L1 = 3% L2 = 2% L3 = 2%	L1 = 1% L2 = 1% L3 = 1%	L1 = 0.5 (Total SD) L2 = 2% L3 = 3%	L1 = 2% L2 = 1% L3 = 1%	L1 = 0.1 (Total SD) L2 = 1% L3 = 1%
Sensitivity: (LoQ) Limit of Quantitation	≤ 2.0	< 0.15	≤ 0.5	≤ 0.2	≤ 0.2	≤ 6.0	≤ 1.1
Analytical Range	2.0 to 50.0	0.15 to 5.00	0.5 to 10.0	0.2 to 8.00	0.2 to 10.0	12.5 to 150.0	1.1 to 100.0
Method Comparison vs c8000 vs Roche Hitachi 917	Slope: 1.09 r: 0.99 Slope: 1.06 r: 1.00	Slope: 0.90 r: 0.99 same	Slope: 1.00 r: 1.00 same	Slope: 1.03 r: 0.99 Slope: 0.91 r: 1.00	Slope: 1.06 r: 1.00 Slope: 0.96 r: 0.99	Slope: 0.96 r: 1.00 same	Slope: 0.92 r: 1.00 Slope: 0.93 r: 1.00

***Conclusion*:** The ARCHITECT *c*4000 Clinical Chemistry System demonstrated acceptable performance characteristics that met or exceeded all pre-established analytical goals. Method correlation data supports the equivalence of the ARCHITECT *c*4000, *c*8000, and *c*16000 and AEROSET Systems. The use of common reagents and commodities across the four systems allows the flexibility to use any system for TDMs depending on user needs. The ability to integrate the *c*4000 with an $i1000_{SR}$ immunoassay module provides the capacity to analyze most routine clinical chemistry and immunoassay tests on one system.

E-96

Clinical Toxicology Multi-Targeted Screening and General Unknown Screening on an LC/MS/MS System with Automatic Library Searching for Compound Identification

A. Schreiber[1], D. Caraiman[1], M. Jarvis[1], A. Taylor[1], L. Sapp[2], T. Sasaki[3]. *[1]AB Sciex, Concord, ON, Canada, [2]AB Sciex, Framingham, MA, [3]AB Sciex, Foster City, CA,*

Background: LC/MS/MS has become a very valuable screening tool in clinical toxicology. By using LC/MS/MS, sample preparation can be simplified and run times shortened, resulting in increased throughput and faster sample turnaround times. Sample preparation is very generic, allowing for screening across a wide variety of compound classes using a single sample preparation procedure and run.

Because of widespread use and abuse of drugs, comprehensive screening for detection of pharmaceutical and illicit drugs is an important part of clinical toxicology analyses and is often divided into two categories: multi-targeted screening and general unknown screening.

Typically, there is a relatively small subset of substances that are heavily used and abused, making a targeted approach the most useful because of its ability to detect drugs at low concentrations and relative ease of data processing and interpretation. However, use of newer designer drugs can be a problem. If these newer drugs have not been identified as frequently used or abused substances, they will not be on the list of target compounds and their use can remain undetected. General unknown screening is therefore a complementary screening technique. GUS has the ability to detect and identify or classify unexpected drugs, drug analogs, and drug metabolites.

Methods: Multi-Targeted Screening (MTS) applications of QTRAP® technology allow the screening for hundreds of drugs using Multiple Reaction Monitoring (MRM) with high selectivity and sensitivity. The use of the *Scheduled* MRM™ algorithm further enhances the screening capabilities. In addition, automatically acquired Enhanced Product Ion (EPI) spectra are recorded for compound identification by searching against a mass spectral library containing more than 1250 compounds.

General Unknown Screening (GUS) methods enable the unambiguous identification of a large range of compounds without the input of preliminary information. On a QTRAP® LC/MS/MS system the GUS workflow has been simplified to the following: Untargeted full scan Enhanced MS (EMS) followed by EPI based on specific criteria. Subsequently, library searching against a large database is performed to determine whether any of the components are identifiable.

Results: Several drug-positive urine samples obtained from a clinical toxicology lab at a local hospital were investigated with the aim of evaluating the potential of both GUS and MTS on a 3200 QTRAP® system. Results were compared regarding selectivity, sensitivity and the risk for false positive and negative results.

Targeted screening detected more drugs in the samples versus the general unknown approach, as expected, however GUS had the advantage of detecting unexpected compounds. Additionally, for unexpected drugs the GUS workflow was capable of identifying these as potential metabolites or structural analogues based on comparisons of fragmentation patterns to an MS/MS spectral library.

Conclusions: Using simple, generic sample preparation protocols, both multi-targeted and general unknown screening were successfully implemented using the 3200 QTRAP® LC/MS/MS system with Cliquid® Software. The two screening methods provide different advantages, and have proven to be truly complementary in the arena of clinical toxicology. The flexibility of the QTRAP® platform allows laboratories to perform both categories of screening on a single instrument.

E-97

Evaluation of two Chemistry Analyzers for Urine Drug Screen testing in the Emergency Department

C. D. Thompson[1], C. J. Clark[1], G. A. McMillin[2], C. M. Lehman[2], K. L. Johnson-Davis[2]. *[1]ARUP Laboratories, Salt Lake City, UT, [2]University of Utah, Salt Lake City, UT,*

Introduction: Exposure to drugs and toxins is a major cause for the rising number of emergency room visits each year. To support the needs of the emergency department (ED), the National Academy of Clinical Biochemistry recommends Tier I STAT qualitative testing, with a turnaround time of 1h or less. The analytical method that is commonly used for STAT testing is immunoassay, performed with automated chemistry analyzers. Although this methodology can meet the turnaround time requirement, it is limited in its sensitivity and specificity when compared to chromatographic assays.

Purpose: The purpose of this study was to compare two automated immunoassay systems to determine which platform would provide better quality cocaine, opiate, and amphetamine testing to support the University of Utah Hospital ED. The goal was to select the platform associated with the fewest number of false positive/negative results.

Method: Forty patient urine samples were confirmed either positive or negative by mass spectrometry for cocaine, opiates, and amphetamines. Split sample analyses of these specimens were performed on both the Roche COBAS INTEGRA®400 plus and Ortho Vitros® 5,1 FS instruments. The Roche COBAS Integra analyzer utilizes kinetic interactions of microparticles and measures the change in light transmission. The Ortho Vitros instrument is a competitive immunoassay that utilizes antibodies and drug-labeled enzyme (glucose-6-phosphate dehydrogenase) to measure the change in absorbance at 340 nm. The results from the two chemistry analyzers were compared to mass spectrometry results. **Results:** Method comparison results are summarized in the table below. (TP) = true positive; (TN) = true negative; (FP) = false positive; (FN) = false negative; (MS) = mass spectrometry.

Conclusions: The Vitros Fusion analyzer generated fewer false positive and false negative results for opiate and amphetamine testing.

Table: Summary of Results

Test	Analytes Detected	Cutoff ng/mL	MS	Ortho Vitros® 5,1 FS		Roche Integra® 400plus	
Cocaine	Benzoylecognine	300	TP 12	TP 12	FN 0	TP 12	FN 0
			TN 28	TN 15	FP 13	TN 15	FP 13
Opiates	Codeine Morphine Dihydrocodeine Hydrocodone Hydromorphone	2000	TP 28	TP 27	FN 1	TP 15	FN 13
			TN 11	TN 11	FP 0	TN 11	FP 0
Amphetamines	(d,l) Amphetamine Methamphetamine	1000	TP 23	TP 19	FN 4	TP 19	FN 6
			TN 17	TN 17	FP 0	TN 16	FP 1

E-98

Application Of Levetiracetam (LTA) Monitoring To Standard Laboratory Analyzers

G. J. Abraham, H. Gang, N. Qureshi. *Analytical Diagnostic Lab, Brooklyn, NY,*

BACKGROUND AND METHODS: Levetiracetam (Keppra) (LTA)is an anticonvulsant approved for use as adjunctive therapy in the treatment of epilepsy. It is currently monitored by labor intensive LC-MS methods. To simplify and standardize the monitoring of LTA for diagnostic laboratories a homogeneous EIA method has been developed. We tested this EIA assay on our current analyzer, the ROCHE COBAS 501C and compared results with our reference laboratory method (Quest Laboratories).

The EIA LTA (ARK Diagnostics, Inc) assay is based on the competition between the drug in the specimen and LTA labeled with G6PDH for binding to the antibody reagent. As the latter binds antibody, the enzyme activity decreases. In the presence of drug from the specimen, free enzyme activity increases and this increase is directly proportional to drug concentration. This free enzyme activity then converts the coenzyme nicotinamide adenine dinucleotide - NAD to NADH, which is measured by its UV absorbance.

In our laboratory the LTA assay was carried out on the Cobas 501C analyzer, under standard operating conditions and using the reaction parameters provided by ARK Diagnostics, Inc.

PROCEDURE AND RESULTS: twenty routine patient laboratory specimens were assayed by the reference lab and on our own Cobas 501C instrument. The comparative data of 501C vs. Quest are as follows:

Range of assayed specimens : 0.2-102 ug/mL.

*Regression (Deming) and correlation : S*lope 1.246 (C.I. 1.14-1.34), intercept 2.86, Standard error of the estimate 5.86, R=0.9874.

Precision: Level 1 (mean 11.67 ug/mL) CV 3.7%, Level 2 (mean 108.43 ug/mL) CV 2.2%.

DISCUSSION AND CONCLUSIONS: The homogeneous EIA LTA assay had been previously tested on the Hitachi 917 analyzer by ARK Diagnostics, Inc. Our present studies have further demonstrated that this LTA method can be successfully applied on other standard laboratory analyzers, and therefore may eventually find wide acceptance in the clinical laboratory field where a number and a variety of other analytical platforms are being used.

We thank N. Qureshi, MS, for his skilled analytical benchwork and ARK Diagnostics, Inc., for their cooperation in the project.

E-99

Monoamine oxidase B A644G intron 13 SNP and clinical outcome under antidepressant therapy

J. Staeker, B. Laika, S. Leucht, S. Heres, W. Steimer. *Klinikum rechts der Isar, TU München, Munich, Germany,*

Background: Genetic variations in the Monoamine oxidase B (MAOB) gene may lead to a changed antidepressant treatment response.
Objective: Investigation of the influence of MAOB A644G intron 13 SNP on clinical outcome under antidepressant therapy.
Patients and Methods: A total of 86 depressive inpatients (n= 56 female and n= 30 male) treated with various antidepressants were included. They were evaluated psychopathologically by the PD self rating scale and the clinical global impressions scale (CGI) at admission to hospital and 4 weeks thereafter. Side effects were assessed by using a modified version of the dosage record and treatment emergent symptoms scale (DOTES). Genotyping was performed using an as yet unpublished RT-PCR method. Since the gene encoding MAO-B is located on the X-chromosome data analyses were done separately for male and female patients by PASW Statistics 17 .
Results: When analyzing the influence of this SNP on all patients receiving at least one antidepressant but no antipsychotic medication the female G-allele carriers had a significantly higher CGI-S improvement after 4 weeks of treament (Kruskal-Wallis-Test: AA:0.54±0.78,AG:1.30±1.13,GG:1.71±1.1, p=0.047, n=47). There was no association with treatment response in male patients.They, however, suffer from significantly more side effects if carrying a G-allele (KW-Test: DOTES sum score 1.33±1.44 vs. 0.40±0.91, p=0.042, n=27). Baseline characteristics (age, BMI, smoking habits, diagnosis, baseline CGI and PD rating) did not differ among the genotypes.
Discussion: These results suggest that a GG-genotype may be advantageous for female patients. In men on the other hand carriers of the A-allele seem to suffer less from adverse effects under antidepressant therapy than G-allele carriers. Gender differences of the MAOB A644G intron 13 SNP effects have been discussed earlier by Kelada et al. [1]. The mutation has also been associated with varying gene expression: higher transcriptional activity of the G-allele in vitro [2] but higher mRNA levels of the A-allele in vivo (only males examined, [3]). Furthermore, mechanisms of MAOB gene regulation seem to be variable in brain and in platelets in the same individual [4]. The A→G substitution may also lead to gender specific enzyme activity. In contrast to our results a study of Tadic et al. [5] showed that women with AA-genotype responded faster and more pronounced than AG/GG-carries whereas no association between intron 13 SNP and clinical outcome was found in men.
Conclusion: Our study suggests a correlation between MAOB A644G intron 13 SNP and clinical outcome in antidepressant medication. The effects are, however, limited and not in concordance with a previous study [5]. Further studies are needed to draw firm conclusions regarding the effects of the MAOB A644G intron 13 SNP on outcome in antidepressant treatment.

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E-100

Low Hydrocodone Excretion Detected in Patients on Oxycodone Medications

R. West, A. J. Pesce, C. West, C. Mikel, S. Latyshev, B. Crews, P. Almazan. *Millenium Laboratories, San Diego, CA,*

Physicians treating pain patients are aware that some of these patients may use opioids not prescribed by them because the patients are "doctor shopping". Therefore, urine drug tests ordered as part of the monitoring of patients on chronic opioid therapy are closely examined for the presence of non-prescribed opiates. The advent of liquid chromatography tandem mass spectroscopy (LC-MS/MS) has made it possible to quantitatively measure excreted opiate concentrations over a 10,000 fold range. With this dynamic range it is possible to quantify impurities in opiate formulations as well as the target drug and its metabolites. In a previous study we showed codeine, as a small impurity of morphine preparations, could be observed in these patients (West et al Ther Drug Monit 2009;31:776-778). In this work, using the same analytical methods, we examined the urinary excretion patterns of 2854 patients prescribed oxycodone for the presence of hydrocodone. For samples with oxycodone excretion greater than 50,000 ng/mL, hydrocodone was found 35% of the time. (n = 74). For samples with oxycodone less than 50,000 ng/mL, hydrocodone was found 9% of the time. (n = 2780**)**. This is consistent with a report from AIT Laboratory in Practical Pain Management, Sept 2009 which described detection of hydrocodone in oxycodone products. Purdue Pharma, makers of Oxycontin ®, stated that hydrocodone may be present in an amount up to 0.15% in their product (Personal communication). These findings complicate the interpretation of urine drug testing results in these patients. Concentrations of codeine less than 100 ng/mL in patients on morphine, and hydrocodone concentrations less than 150 ng/mL in patients on oxycodone may reflect impurities in the respective morphine and oxycodone medications, and not "doctor shopping".

E-101

ARK™ IMMUNOASSAY FOR GABAPENTIN

J. Nguyen, B. Moon, A. Orozco, K. Kasper, J. Valdez. *ARK Diagnostics, inc, Sunnyvale, CA,*

BACKGROUND: Gabapentin (1-(aminomethyl)cyclohexaneacetic acid) concentrations can be used as an aid in management of patients treated with gabapentin. Gabapentin (Neurontin®, Pfizer) is an anti-epileptic drug indicated as adjunctive therapy in the treatment of partial seizures with and without secondary generalization in patients over 12 years of age with epilepsy. Neurontin is also indicated as adjunctive therapy in the treatment of partial seizures in pediatric patients age 3 - 12 years. It is also indicated for the management of postherpetic neuralgia in adults. Overall, in patients treated with therapeutic doses, serum gabapentin concentrations occur mostly in the order of 2-20 µg/mL (P.N. Patsalos et al. 2008. Epilepsia 49:1239-1276).
OBJECTIVE: To evaluate the performance of the new ARK™ Gabapentin Assay on the ROCHE HITACHI® 917 automated clinical chemistry analyzer.
METHODS: The ARK Gabapentin Assay is a homogenous enzyme immunoassay for quantifying gabapentin in human serum or plasma. The assay was evaluated on the ROCHE HITACHI® 917. The assay is calibrated using a six point calibration curve (0 to 40 µg/mL) where increasing reaction rate correlates to increasing gabapentin concentration. Performance of the assay was determined by assessing precision, limit of quantitation, analytical recovery and method comparison. Five-day verification within-run precision on tri-level controls was 4.2% CV (2.50 µg/mL), 2.9% CV (8.00 µg/mL) and 3.9% CV (20.00 µg/mL). Total precision performed on the same controls were 5.2% CV (2.50 µg/mL), 4.0% CV (8.00 µg/mL), and 6.3% CV (20.00 µg/mL). Limit of Quantitation (LOQ) was 0.50 µg/mL (11.0 %CV). Other antiepileptic drugs tested did not significantly affect the quantification of gabapentin. Since gabapentin is an amino acid cross-reactivity with this class of compound was also tested and no significant interference was observed. Analytical recovery in serum was within 10% of the nominal values from 2.00 to 40.00 µg/mL. Method comparison of the ARK assay versus HPLC was conducted for 100 specimens spanning 1.0 to 34.0 µg/mL: using Passing Bablok regression analysis ARK = 0.98 HPLC - 0.15 (r^2 = 0.96). Proficiency samples from the Heath Control scheme demonstrated recovery within 10% of spiked values for samples within the range of measurement.
Conclusions: The ARK Gabapentin Assay is suitable for the quantitative measurement of gabapentin in serum and plasma. All reagents, calibrators, and controls are supplied as stable liquids; ready-to-use. This assay correlated with HPLC and is well-suited for routine TDM use on automated clinical chemistry analyzers.

E-102

Evaluation of the Abbott ARCHITECT Tacrolimus Assay.

A. M. Fukuchi, K. A. Kelly, A. W. Butch. *UCLA Medical Center, Los Angeles, CA,*

Objective. The analytical performance of the Abbott ARCHITECT Tacrolimus assay was evaluated and compared to the Abbott IMx Tacrolimus II assay.
Relevance. The Abbott ARCHITECT Tacrolimus random access assay replaces the Abbott IMx Tacrolimus batch assay that has been discontinued by the manufacturer.
Methods. The ARCHITECT assay requires a whole blood specimen pretreatment step with methanol/zinc to precipitate protein and extract the drug, followed by a 30-minute immunoassay using mouse anti-tacrolimus antibody-coated paramagnetic microparticles and an acridinium tracer. Total imprecision was determined by analyzing whole blood (EDTA-anticoagulated) patient pools and commercial controls in duplicate a total of 11 days. Functional sensitivity was determined by analyzing whole blood patient samples (duplicate analysis, 10 days) with tacrolimus concentrations ranging from 0.2 and 4.0 ng/mL. Linearity and recovery was determined by adding known concentrations of tacrolimus to whole blood from an individual not receiving tacrolimus. Three hundred whole blood samples from patients on tacrolimus therapy that had received an allograph transplant (100 heart, 100 liver, 100 kidney) were used for the method comparison. Passing-Bablock regression, Spearman correlation correlations, and Bland-Altman plot analysis were performed using Analyse-it statistical software.
Results. Total assay imprecision (%CV) for ARCHITECT-1 was 4.5% (mean = 10.9 ng/mL) and 5.7% (mean = 20.5) for patient pools, and was 3.9% (mean of 4.8) and 4.4% (mean of 22.4) for commercial controls. Total imprecision for the ARCHITECT-2 was 4.4% and 3.4% for patient pools and 3.9% and 4.4% for commercial controls, respectively.

The functional sensitivity (20% CV) as measured at the upper 95% confidence interval was 0.5 and 0.3 ng/mL for ARCHITECT-1 and 2, respectively. Both instruments were linear to 30 ng/mL and exhibited acceptable recovery of tacrolimus (within ±7%) at concentrations between 5 and 25 ng/mL. Patient comparison studies (n = 300) revealed the following relationships: ARCHITECT-1 = 1.00(IMx) + 0.40 (r = 0.98, average bias = 0.41); ARCHITECT-2 = 0.98(IMx) + 0.49 (r = 0.98, average bias = 0.35); and ARCHITECT-1 = 0.98(ARCHITECT-2) + 0.10 (r = 0.99, average bias = -0.06). The two methods were also compared in different transplant groups (n = 100 for each transplant type) and the ARCHITECT was found to exhibit a positive bias of 0.17/0.13, 0.43/0.47, and 0.62/0.44 ng/mL (ARCHITECT-1/ARCHITECT-2) for heart, liver and kidney transplant samples, respectively.

Conclusions. The ARCHITECT Tacrolimus assay has comparable analytical performance compared with the IMx Tacrolimus II assay and has the advantage of random access for improved laboratory workflow. Although a slight positive bias was observed for the ARCHITECT compared with the IMx, the transition to the ARCHITECT from the IMx should be smooth and not have an impact on patient management.

E-103

ARK™ IMMUNOASSAY FOR LAMOTRIGINE

K. Chung, A. Orozco, J. Valencia, B. Moon, K. Kasper, J. Valdez. *ARK Diagnostics, inc, Sunnyvale, CA,*

BACKGROUND: Lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-*as*-triazine) concentrations can be used as an aid in management of patients treated with lamotrigine. Lamotrigine (LAMICTAL®, GlaxoSmithKline) is an anti-epileptic drug indicated as adjunctive therapy in patients ≥2 years with partial seizures, primary generalized tonic-clonic seizures or generalized seizures of Lennox-Gastaut syndrome, and monotherapy in patients ≥16 years with partial seizures. It is also indicated in patients ≥18 years with bipolar disorder. Overall, in patients treated with therapeutic doses, serum lamotrigine concentrations mostly in the order of 2.5-15 µg/mL have been reported (P.N. Patsalos et al. 2008. Epilepsia 49:1239-1276). The incidence of toxicity increases with concentrations >15 µg/mL.

OBJECTIVE: To evaluate the performance of the new ARK™ Lamotrigine Assay on the ROCHE HITACHI® 917 automated clinical chemistry analyzer.

METHODS: The ARK Lamotrigine Assay is a homogenous enzyme immunoassay for quantifying lamotrigine in human serum or plasma. The assay was evaluated on the ROCHE HITACHI® 917. The assay is calibrated using a six point calibration curve (0 to 40 µg/mL) where increasing reaction rate correlates to increasing lamotrigine concentration. Performance of the assay was determined by assessing precision, limit of quantitation, analytical recovery and method comparison. Five-day verification within-run precision on tri-level controls was 1.9% CV (2.00 µg/mL), 2.6% CV (12.00 µg/mL) and 5.0% CV (25.00 µg/mL). Total precision performed on the same controls were 4.4% CV (2.00 µg/mL), 5.7% CV (12.00 µg/mL), and 6.0% CV (25.00 µg/mL). Limit of Quantitation (LOQ) was 0.75 µg/mL (4.15% CV). Analytical recovery in serum was within 10% of the nominal values from 1.00 to 30.00 µg/mL. Other antiepileptic drugs tested did not significantly affect the quantification of lamotrigine. Method comparison of the ARK assay versus HPLC was conducted for 184 specimens spanning 1.0 to 38.8 µg/mL: using Passing Bablok regression analysis ARK = 0.97 HPLC + 0.58 (r^2 = 0.97). Proficiency samples from the Heath Control scheme demonstrated recovery with 10% of spiked values for samples within the range of measurement.

CONCLUSIONS: The ARK Lamotrigine Assay is suitable for the quantitative measurement lamotrigine in serum and plasma. All reagents, calibrators, and controls are supplied as stable liquids; ready-to-use. This assay correlated with HPLC and is well-suited for routine TDM use on automated clinical chemistry analyzers.

E-104

Evaluation Of The Tacrolimus And Sirolimus In Liver And Renal Transplant Recipients

P. Romano, M. E. Mendes, J. A. Costa, V. R. M. Batista, N. M. Sumita. *Central Laboratory Division & Laboratories of Medical Investigation (LIM-03) of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil,*

Introduction: The therapeutic monitoring of immunosuppressants Sirolimus (Rapamycin) and tacrolimus (FK506) is indispensable to maintain stable levels of these drugs, avoiding graft rejection in the transplanted patient in the case of low dosage, or toxicity in high dosage, and allowing monitoring of individual treatment.

In the 80's, the introduction of the potent immunosuppressive drugs CsA and FK506 reduced the incidence of rejection episodes after solid organ transplantation.

Sirolimus inhibits the response to interleukin-2 (IL-2) and thereby blocks activation of T- and B-cells. In contrast, tacrolimus inhibits the production of IL-2. CsA and FK506 have distinct chemical structures but similar mechanisms of action, inhibiting the transcription of the first signal for T-lymphocyte activation.

The major side effects associated with CsA and FK506 therapies are nephrotoxicity and neurological disturbances. However, clinical studies demonstrate that FK506 is a potent alternative to CsA due to its lower nephrotoxicity and reversible neurotoxicity when the dosage is decreased. The chief advantage sirolimus has over calcineurin inhibitors is that it has low toxicity towards kidneys**.**

Objective: The aim of this study was to evaluate the performance chemiluminescence's immunoassay with magnetic microparticles (CMIA-Abbott Laboratories) for determination sirolimus and tacrolimus in whole blood, comparing with methods in use in the laboratorial routine.

Materials and Methods: Specimes collection in EDTA whole blood tubes collection. Sample preparation involved blood samples pretreated with Zinc sulfate solution .These samples were vortex-mixed and centrifuged. The supernatant was dispensed into the respective reaction cells and automated analysis was performed on Architect System (Abbott) . The CMIA was performed according manufacturer´s instructions.

Comparison of liver and renal patients samples measured by: CMIA Abbott against CMIA (Dimension RXL Max- Siemens) to Tacrolimus and CMIA against MEIA II(IMx analyzer Abbott)to Sirolimus.

Results: The assay was found to be linear up to 28.6 ηg/mL (Sirolimus) and 24.2 ηg/mL (Tacrolimus). The functional sensitivity was 0.00 ± 0.014 ηg/mL (Sirolimus) and 0.01± 0.010 ηg/mL (Tacrolimus).

The within-run precision ranged:

Tacrolimus: 1.81±0.12% to sample with a concentration of 1.81 ηg/mL and 2.7±0.41% for concentration of 15,08ng/mL

Sirolimus:2.5±0.16% to sample with a concentration of 6.5 ηg/mL and 2.2±0.39% for concentration of 18,0 ng/mL

The between-run precision ranged:

Sirolimus: 4.25±0.09% to sample with 2.00 ηg/mL and 4.06±0.39% for concentration of 8.20ng/mL.

The recovery test ranged from 92.8% to 6.5 ηg/mL (sirolimus) and 99.3% to 8.20 ηg/mL (tacrolimus).

The carryover was 0.074 above the error limit 0.414 to sirolimus and 0.053 above the error limit 0.306 to tacrolimus.

The comparison method to MEIA II X CMIA (Architect/Abbott) was r = 0.9586 (sirolimus) and r =0.9575 to comparison CMIA (Abbott) X CMIA (Siemens) to Tacrolimus.

The ANOVA were used to compare results of Tacrolimus to IMX/Abbott versus Architect/Abbott versus Dimension RLX Max/Siemens (p = 0.513).

Conclusion: The CMIA assay is a simple, fast and convenient alternative to evaluate imunossupressors in the human whole blood. There were good levels of correlation between the methods studied. These parameters are useful in therapeutic monitoring for the management of transplant patients receiving sirolimus or Tacrolimus therapy.

E-105

GST Polymorphisms of Anti Tubercular Treatment Induced Hepatotoxicity in Indians

S. rana, S. K. Sharma, R. P. ola, S. K. sinha, S. K. arora, J. K. kamboj, V. P. singh, K. singh. *PGIMER, Chandigarh, India,*

Introduction: Drug induced hepatotoxicity in general, is outcome of dynamic processes involving toxic metabolite generation and its detoxification in the liver. Glutathione (GSH) plays an important protective role as an intracellular free radical scavenger by conjugating with toxic reactive metabolites that are generated from biotransformation of drugs and xenobiotics. Thus, it plays an important role in preventing ATT- induced hepatotoxicity. Glutathione -S- transferase (GST) catalyzes these conjugation reactions. Deficiency in GST activity, because of homozygous null mutations at GSTM1 and GSTT1 loci, may modulate susceptibly to ATT-induced hepatotoxicity.

Aim: Therefore, the multiplex PCR protocol was used to determine the prevalence of deletion / addition genotypes of both GSTM1 and GSTT1 genes in ATT-induced hepatotoxicity patients.

Materials and Methods: In the present study, a total of 100 north Indian adult patients of tuberculosis on ATT were enrolled. DNA used for polymorphic analysis was isolated from lymphocytes of these patients by using phenol-chloroform extraction method. The genetic polymorphic analysis for GSTM1 and GSTT1 genes were determined simultaneously by using multiplex PCR method with ubiquitous ß-globulin gene as an internal control. The presence or absence of the GSTT1 (459 base pairs) and GSTM1 (215 base pairs) amplification was determined in the presence of the control ß-globulin gene (268 base pairs).

Results: Mean age of 100 patients was 45.6 years (range 18-70 years). Out of 100 patients, 16 (16%) were ATT-induced hepatotoxicity and 84 (84%) were of non-hepatotoxicity. Out of 16 hepatotoxicity patients, 5 (31.3%) showed GSTM1, 4 out of 16 (25.0%) GSTT1 and 7 out of 16 (43.7%) GSTT1/GSTM1/ß-globulin. While13 out of 84 (15.5%) showed

GSTM1, 28 out of 84 (33.3%) GSTT1, and 43 out 84 (51.2%) GSTT1/M1/ß-globulin in non-hepatotoxicity patients.
Conclusion: This study showed that GSTM1 was significantly higher in hepatotoxicity patients as compared to non-hepatotoxicity patients.

E-106

Simultaneous quantitative determination of Ethyl Glucuronide and Ethyl Sulphate in human urine using UPLC/MS/MS

R. Watts[1], J. Button[2], M. Wood[1], J. B. Clarke[1]. *[1]WATERS CORPORATION, MANCHESTER, United Kingdom, [2]St George's University of London, London, United Kingdom,*

Simultaneous quantitative determination of Ethyl Glucuronide and Ethyl Sulphate in human urine using UPLC®/MS/MS
Objective: To develop and validate a simple and rapid UPLC/MS/MS method for the simultaneous quantitative determination of ethyl glucuronide (EtG) and ethyl sulphate (EtS) in human urine that can be used to detect or monitor recent ethanol intake within a clinical, forensic or workplace setting.
Methods: Human urine samples (50µL) were prepared following centrifugation (12000rpm for 10 minutes) by a simple dilution (1:20) using 0.1% formic acid and the addition of deuterated internal standards (EtG-D5 and EtS-D5). Chromatography was performed using a Waters® ACQUITY UPLC system; analytes were separated on a Waters ACQUITY UPLC HSS C18 (2.1 x 150mm, 1.8µm) column using a gradient elution over 4 minutes with a mixture of 0.05% formic acid in water (A) and acetonitrile (B). A Waters TQ detector (tandem mass spectrometer) was used for analysis with electrospray ionisation in negative mode (ESI-). Two separate MRM transitions were used for each compound (m/z 221 > 85, 221 > 75 for EtG and m/z 125 > 97, 125 > 125 for EtS) and each transition was optimised to achieve maximum sensitivity.
Results: For both compounds, responses were linear over the investigated range (0.25-100mg/L for EtG, 0.05-20mg/L for EtS). Intra and inter-assay precision and accuracy were assessed at four spiked QC levels (0.75, 2.5, 7.5 and 50mg/L for EtG, 0.15, 0.5, 1.5 and 10mg/L for EtS) and were found to be good, with precision CV's < 10% and accuracy between 97-112%. The cut-offs applied for EtG and EtS analysis were 0.5 and 0.1mg/L with limits of detection being 0.15 and 0.04mg/L, respectively. Possible matrix effects were assessed by spiking blank prepared patient samples (n = 6) with both compounds and comparing the responses against the equivalent concentration of solvent standard solution. The average matrix effects were found to be acceptable (-16% for EtG and -7% for EtS). Both compounds were shown to be stable in prepared samples over a period of 24 hours when stored on the ACQUITY UPLC at 5oC. The method was applied to the analysis of samples (n = 39) which were previously analysed for EtG only by Microgenics DRI® EtG Enzyme Immunoassay and all samples showed good correlation (r2 = 0.977). The quantifier/qualifier ion ratios for both compounds were monitored for all calibrators, QC's and samples and were found to be within ±20% of the desired ion ratios.
Conclusion: This method provides a simple and rapid solution for the simultaneous quantification of EtG and EtS within 5 minutes. The developed method was shown to be accurate, precise and linear over the desired analytical range. The method was successfully applied to the analysis of real samples and provides the ability to detect or monitor recent ethanol consumption in a clinical setting.

E-107

ATP production in CD4+ T cells as a predictor for infection and allograft rejection in pediatric liver transplant recipients

G. Brandhorst[1], S. Schulz-Jürgensen[2], M. Burdelski[2], M. Oellerich[1]. *[1]Dept. of Clinical Chemistry, University Medicine Goettingen, Goettingen, Germany, [2]Dept. of Pediatrics, University Hospital Schleswig-Holstein, Kiel, Germany,*

Background: Biomarkers of immune cell response are needed for monitoring the minimization of immunosuppressive therapy. Within the scope of this study we investigated ATP production in CD4+ T cells as a pharmacodynamic marker for cell-mediated immune response in stable pediatric liver transplant recipients.
Methods: Fifty transplant patients and 31 outpatients without liver disease were included. Twenty-six transplant patients received cyclosporine (CSA), 22 tacrolimus (TCL), 10 mycophenolate mofetil (MMF), and 1 everolimus (some patients received drug combinations). The reactivity of the immune system was measured as rise of intracellular ATP (iATP) in CD4+ T-lymphocytes following PHA-stimulation (Cylex® ImmuKnow®).
Results: Median iATP concentrations were 251 ng/mL in samples from liver transplant recipients and 385 ng/mL for the pediatric controls. A highly significant difference between the two groups could be demonstrated (p=0.001). No significant correlation between drug trough concentrations and iATP concentrations was found, neither for CSA (r=0.172, p=0.527) nor for TCL (r=0.024, p=0.918). ATP concentrations in patients with clinically significant EBV reactivation were in the low response range (2 patients, ATP concentrations 181 and 221 ng/mL). Patients with allograft rejection had significantly higher ATP concentrations as compared to the event-free subgroup (4 patients, median ATP 444 ng/mL, p=0.035).
Conclusion: These preliminary results indicate that the ImmuKnow® assay might provide independent information to identify either over- or underimmunosuppression in pediatric liver transplant patients. Further investigations are necessary to validate and extend these findings.

E-108

Optimization and Validation of a High Throughput LC-MS/MS Method for Tacrolimus, Sirolimus and Cyclosporine A

N. Babic, J. A. Burrus, E. H. Mokhtari, C. Gloria, M. Hainzinger, S. J. Zibrat, K. T. J. Yeo. *University of Chicago, Chicago, IL,*

Objective: We optimized and validated a high throughput LC-MS/MS method for simultaneous determinations of Tacrolimus (Tac), Sirolimus (Sir) and Cyclosporine A (CsA). We then compared our method to immunoassay methods from two manufacturers, CEDIA (Thermo Fisher Scientific, Freemont, CA) run on Roche P Modular and Abbott IMx/TDx (Abbott Park, IL). Two sets of commercially available calibrators, IRIS (Recipe, Munich, Germany) and Chromsystems (Munich, Germany), were also evaluated on the LC-MS/MS.
Methods: The method was developed on a Thermo TSQ Ultra triple-quadrupole mass spectrometer attached to a TLX-2 Cohesive LC-system. Whole blood specimens, controls and standards were extracted using 0.1 mM $ZnSO_4$ solution containing internal standards, ascomycin and cyclosporine D. The sample was homogenized, centrifuged and further purified using a Cyclone-P TurboFlow column with separation performed on a C-18 Hypersil Gold analytical column (Thermo Fisher Scientific, Waltham, MA).
Results: Inter-assay CVs were: Tac, 11.0% (3.3 ng/mL), 6.0% (6.9 ng/mL) 6.0% (13.7 ng/mL); Sir, 12.0% (3.8 ng/mL), 8.0%(11.9 ng/mL), 7.0%(19.3 ng/mL); CsA, 5.0-7.0% (62.5-1276 ng/mL). Analyte measuring range was verified to 47 ng/mL (Tac), 85 ng/mL (Sir) and 2000 ng/mL (CsA). Functional sensitivities were 2.0 ng/mL for Tac and Sir and 15 ng/mL for CsA. Comparison studies are shown in Table 1.
Conclusion: We optimized and validated a high-throughput assay for simultaneous measurement of the three most common immunosuppressive drugs. This assay offers additional accuracy and precision over currently available immunoassays since it measures parent drug. The use of calibrators from different manufacturers can affect the extent of bias between the LC-MS/MS and immunoassay methods.

Table 1. Summary of three-way method comparison and calibrator comparison.

Calibrators	Correlation Equation	r^2	n	Range
IRIS	[TAC IMx] = 0.98 [TAC LC-MS/MS] + 0.05	0.93	70	0.83-25.2
	[TAC CEDIA] = 1.08 [TAC LC-MS/MS] + 0.05	0.90	70	0.83-25.2
	[TAC CEDIA] = 1.13 [TAC IMx] - 0.38	0.90	70	1.6-26.9
	[SIR IMx] = 0.77 [SIR LC-MS/MS] + 0.60	0.95	75	0.22-22.4
	[CsA IMx] = 1.28 [CsA LC-MS/MS] + 4.85	0.96	68	20.3-711
	[CsA CEDIA] = 1.22 [CsA LC-MS/MS] - 19.98	0.95	61	20.3-711
	[CsA CEDIA] = 0.97 [CsA IMx] - 25.63	0.97	61	4.72-734
Chromsystems	[TAC IMx] = 1.11 [TAC LC-MS/MS] - 0.18	0.89	52	0.95-17.8
	[TAC CEDIA] = 1.16 [TAC LC-MS/MS] + 0.02	0.85	52	0.95-17.8
	[SIR IMx] = 0.76 [TAC LC-MS/MS] + 0.18	0.93	75	0.84-22.0
	[CsA IMx] = 1.32 [CsA LC-MS/MS] + 20.10	0.96	68	6.91-682
	[CsA CEDIA] = 1.27 [CsA LC-MS/MS] - 6.63	0.95	61	6.91-682

E-109

Non-Dioxin Like Polychlorinated Biphenyl (NDL-PCB) Body Burdens in the US Population by Race/Ethnicity and Age Groups for 1999-2004 (National Health and Examination Survey: NHANES)

J. Barletta, A. Bartolome. *University of Maryland, Baltimore, MD,*

Although manufacture and use of PCBs were banned in the US in 1977, PCB exposure is a continuing concern due to widespread distribution in the environment and their persistence as ubiquitous micro-contaminants. NDL-PCBs are endocrine disrupters; able to alter thyroid and sexual hormones, as well as elicit neurological, immunological, and carcinogenic effects.
Specific indicator NDL-PCBs which are representative of many PCB congeners (i.e., 28, 52, 101, 138, 153, and 180) comprise the majority of total NDL-PCBs in food, and are the most frequently detected congeners in the NHANES data.
We wanted to determine the NDL-PCB body burden levels of the US population relative to age and race/ethnicity for the three NHANES reported 2-year periods (Period A:1999-2000, Period B:2001-2002, and Period C:2003-2004). We report the median levels of NDL-PCB indicator congeners (138, 153, and 180) in the non-institutionalized US

population for these years.
For Mexican Americans, Non-Hispanic Whites, and Non-Hispanic Blacks, median levels of NDL-PCB 153 for ages 12-20 years was 21.8, 23.6, and 22.8 ng/gm; 9.7, 8.4, and 8.1 ng/gm; and 4.1, 5.6, and 6.4 ng/gm (for yearly periods a, b, and c) respectively.
No significant differences were detected between Mexican American, Non-Hispanic Whites, and Non-Hispanic Blacks for NDL-PCBs 138, 153, and 180. All race/ethnicities showed a continuously significant downward trend from 1999-2004.
In contrast, for all participants older than age 20, there were significant differences between all 3 race/ethnicity groups for all yearly periods. For Mexican Americans, Non-Hispanic Whites, and Non-Hispanic Blacks, median levels of NDL-PCB 153 for ages greater than 20 years was 26.1, 39.7, and 46.6 ng/gm; 19.3, 44.8, and 52.9 ng/gm; and 15.0, 33.7, and 35.7 ng/gm (for yearly periods a, b, and c) respectively.
For those participants older than 20, Non-Hispanic Blacks had significantly higher NDL-PCB body burdens than Non-Hispanic Whites, who in turn had significantly higher body NDL-PCB body burdens than Mexican Americans. As with participants younger than 21, all race/ethnicities showed a continuously significant downward trend from 1999-2004.
In conclusion, the median levels of NDL-PCBs for all participants from the latest data analyzed (2003-2004) displayed significantly lower levels (21.2-24.3% decreases) than those participants from the earliest (1999-2000) data analyzed . Multivariate regression analyses demonstrated a positive and significant relation between NLD-PCB body burden and age (>20 years). However, no significant differences were seen between NDL-PCB body burden and ethnic groups for age (<21 years).
Biomonitoring is the most powerful tool to determine human exposure to persistent pollutants after long-term exposure. These analyses are of paramount importance for the US population to track changes and monitor correlations of the ubiquitous presence of these environmental toxins to potential negative health effects. The analysis of data 6-10 years ago raises questions of its usefulness to the present time.
It would be of interest to be able to analyze the NLD-PCB body burden levels of the US population for the years following 2004; however, no data has yet been released by NHANES for these subsequent years, nor is there information on when future releases will become available.

E-110

ARK® Topiramate Assay on the Ortho-Clinical Diagnostics VITROS® 5,1 FS Chemistry System

E. Hardy[1], J. Valdez[2]. *[1]St. Mary's Hospital Laboratory, Madison, WI, [2]ARK Diagnostics, inc., Sunnyvale, CA,*

BACKGROUND: Topiramate (2,3:4,5-Di-*O*-isopropylidene-□-D-fructopyranose sulfamate, Topamax®) is used as adjunctive therapy or monotherapy for patients with partial onset or primary generalized tonic-clonic seizures as well as Lennox-Gastaut syndrome. Topiramate is also indicated for the prophylaxis of migraines. Recently, the International League Against Epilepsy issued updated guidelines for the TDM of AEDs (P.N. Patsalos et. al, 2008. *Epilepsia,* 49(7):1239-1276). Situations in which AED measurements are most likely to be of benefit include (1) to establish a clinical benchmark for individual therapeutic concentration which can be used at subsequent times to assess potential causes for a change in drug response; (2) to aid diagnosis of clinical toxicity; (3) to assess compliance; (4) to guide dosage adjustment in patients when pharmacokinetic variability exists (e.g., children, the elderly, patients with associated diseases, drug formulation changes, drug-drug interaction, pregnancy, or dose-dependent pharmacokinetics).
OBJECTIVE: To evaluate the performance of the new ARK™ Topiramate Assay on the Ortho-Clinical Diagnostics VITROS® 5,1 FS Chemistry System.
METHODS: The ARK Topiramate Assay is a homogenous enzyme immunoassay for quantifying topiramate in human serum or plasma. The assay was evaluated on the VITROS® 5,1 FS using an open channel for application of user-defined method. The assay was calibrated using a six point calibration curve (0 to 60µg/mL) where increasing reaction rate correlates to increasing topiramate concentration. Performance of the assay assessed precision, limit of quantitation, linearity, recovery, endogenous interference, specificity and method comparison to FPIA.
RESULTS: Precision of tri-level controls was 2.4% CV (2.5 µg/mL), 3.4% CV (9.8 µg/mL), and 1.9% CV (37.0 µg/mL). Limit of Quantitation was 1.5 µg/mL. The assay was linear from 1.2 to 60.0 µg/mL. Analytical recovery was within 10% for nominal values 1.5 to 45.0 µg/mL. Endogenous substances (lipids, bilirubin, hemoglobin and albumin) and other AEDs did not interfere with measurement of topiramate at the levels tested. Passing Bablok regression analysis for method comparison between ARK and FPIA resulted in a correlation of 0.98 and slope of 1.09 (n=67).**Conclusions:** Analytical utility of the ARK Topiramate Assay for quantifying topiramate in serum and plasma was demonstrated on the Ortho-Clinical Diagnostics VITROS® 5,1 FS Chemistry System.

E-111

A parallel study of Immunalysis and Microgenics Oxycodone kits on the Olympus AU680 Analyzer

J. Flanagan, C. LeMay, J. Sweningson. *Marshfield Clinic, Marshfield, WI,*

Objective: The objective of this study was to evaluate the acceptability of the oxycodone reagent kit by Microgenics on the Olympus AU680 analyzer. An ~ 14% false positive rate for oxycodone using the Immunalysis reagent kit was observed during the initial evaluation of a newly purchased Olympus AU680 analyzer. Because all screen positive specimens are confirmed by a Gas Chromatography/Mass Spectrometry (GC/MS) method according to our laboratory protocol, this high degree of false positives posed an unacceptable impact on workload and misuse of resources. The Microgenics kit was evaluated in comparison with the Immunalysis kit by running them in parallel on the Olympus AU680 Analyzer.
Methods: The Olympus AU680 analyzer was calibrated using the kit-specific oxycodone calibrator (100 ng/mL). A specimen is designated as positive when the concentration is greater or equal to 100 ng/mL (cutoff concentration). Two levels of positive controls, 75 ng/mL and 125 ng/mL, were used for the intra- and inter-assay precision studies. A positive control at 125 ng/mL and certified negative urine were used for the accuracy/differentiation study. Serial dilutions of standards ranging from 0% to 2000% of the calibrator were used for the linearity study. 109 GC/MS-confirmed patient samples were used for the comparison study, which included 29 oxycodone false positives using the Immunalysis reagent, 21 negatives containing neither opiates nor oxycodone, and 59 positives containing oxycodone/oxymorphone from 50 ng/mL to ~70,000 ng/mL. The highest positive patient sample was also used for the carryover and hook effect studies.
Results: Both the Microgenics and Immunalysis kits performed satisfactorily on intra-precison (N = 10, mean = -18 and - 20; SD = 2.6 and 2.0; %CV = -14.4 and -9.8 for Microgenics and Immunalysis at -25% of the control,respectively. N = 10, mean = 26 and 0.1, SD = 3.8 and 2.3, %CV = 14.0 and 2337 for Microgenics and Immunalysis at +25% of the control, respectively.) and inter-precision (Microgenics reagent only, N = 15) studies. Both reagent kits accurately differentiated positive and negative specimens.
No carryover or hook effect was observed. For the linearity study, both assays were moderately linear up to 125 ng/mL. The signals started to flatten beyond this concentration for both assays. The observed signal differences between 125 ng/mL and the cutoff for the Immunalysis and Microgenics kits were 8.3 and 26, respectively. All 29 Immunalysis false positive samples were negative when assayed with the Microgenics reagent. The results for the rest of specimens agreed with those obtained using GC/MS for both the Immunalysis or Microgenics reagent kits. The rates of concordance with the results using GC/MS for the Immunalysis and Microgenics kits were 73% and 99%, respectively.
Conclusions: We confirmed that the Immunalysis oxycodone reagent cross-reacts with hydrocodone and hydromorphone, and the Microgenics oxycodone reagent kit is more specific than the Immunalysis kit in identifying oxycondone/oxymorphone containing specimens. The accuracy, precision, carryover, and hook effect studies were all within the acceptance criteria of our policy. Thus, the Microgenics oxycodone reagent kit was validated for use on the Olympus AU680 Analyzer.

E-112

Monitoring Tacrolimus Levels In Whole Blood By CEDIA Method

O. -. Portakal, G. Haseliık. *Hacettepe University, Ankara,Turkey,*

Tacrolimus (FK 506) is a potent immunosuppresive agent used in renal and liver transplantations. Tacrolimus affects through inhibition of phosphatase activity of calcineurin which is required for T cell proliferation. It causes reduction of interleukin-2 production by T-cells, so generally used to prevent acute rejections. Therapeutic drug monitoring is required to make optimization of drug efficiency. The purpose of this study was to evaluate the analytical performance of CEDIA method for Tacrolimus measurement. For this aim, Tacrolimus levels in whole blood were measured simultaneously by CEDIA (Microgenics, Ca, US) and MEIA (Abbott, Ca, US) methods with a manual pretreatment step, and linearity, limit of detection and quantitiation (LoD/LoQ), recovery and imprecision were determined. In linearity assay, high concentration calibrator was diluted by ½ - 1/32 and the slope of linear regression curve was found to be 1.05. For determination of LoD/LoQ, the lowest calibrator were measured 10 times and LoD/LoQ were calculated as 0,87/1,20. To detect proportional systematic error (PE), low and high calibrators were added into serum pool and recovery was observed between 94.87% and 108.96%; PE was found to be lower than E_A. Within run and between days imprecision were 4.94% and 4.92%, 6.42% and 4.63%, and 3.25% and 7.22 ng/ml in low, medium and high concentrations, respectively. To detect constant systematic error (CE), 4.5 mg/ml EDTA was added into high and low serum pool and in both CE was found to be lower than E_A. In method comparison, coefficient of correlation was 0.97 with 0.96 of slope and -0.05 of intercept by linear regression analysis. Systematic error and total error was lower than E_A. We conclude that CEDIA method is a suitable method for monitoring Tacrolimus level.

E-113

Automated Homogeneous Enzyme Immunoassay for the Detection of Fentanyl and Norfentanyl in Human Urine

S. Mitra, V. Bodepudi, F. Johansson, L. Anne. *Thermo Fisher Scientific, Fremont, CA,*

The objective of the study was to develop a homogeneous enzyme immunoassay for the semi-quantitative determination of Fentanyl in human urine. Fentanyl is a synthetic opiate analgesic similar to morphine. Fentanyl is 50-100 times more potent than morphine. It is prescribed mainly for patients with chronic pain and is generally used to manage pain after surgery. Fentanyl is prescribed as intravenous anesthetic (Sublimaze®), transdermal patch (Duragesic®), and transmucosal Lozenge form (Actiq®). The fentanyl dose in the Duragesic ranges from 2.5-10 mg and in Actiq, it ranges from 0.2 mg-1.6 mg. The fentanyl patches have become the potential targets for abuse and diversion. These patches are sold on the street as Apache, China white, jackpot and Tango. Half-life of Fentanyl is 3-12 hours. More than 90% of the dose is eliminated as N-dealkylated and hydroxylated metabolites.

Microgenics DRI® Fentanyl Enzyme Immunoassay uses a monoclonal antibody that is highly specific to both the parent drug fentanyl and its major metabolite norfentanyl. The assay is based on competition between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug from the urine sample for a fixed amount of antibody binding sites. In the absence of free drug from the sample, the antibody binds the enzyme labeled drug causing a decrease in enzyme activity. Active enzyme converts NAD to NADH resulting in an absorbance change that can be measured spectrophotometrically at 340 nm. The phenomenon creates a direct relationship between drug concentration in urine and enzyme activity.

The assay consists of two reagents-antibody/substrate reagent and enzyme conjugate reagent. The calibrator levels are 0, 10, 25, 50 and 100 ng/mL. The three control levels are 5, 15, and 35 ng/mL. Reagents, calibrators and controls are liquid ready-to-use. Norfentanyl is used as the calibrator drug. The performance of the assay was evaluated on the Olympus AU680 analyzer using the following parameters to generate the calibration curve: sample volume 25 µL, reagents 1 and 2 at 70 µL each, assay method Rate1, measuring cycles 13-17 and calibration type POLYGONAL. The assay range is 5-100 ng/mL. Within-run precision for the three controls ranged from 1.1-4.3% and total precision ranged from 0.9-8.0%. The limit of detection (LOD) was 0.5 ng/mL. No significant interference was observed from endogenous substances. The assay demonstrated 100% cross-reactivity to norfentanyl and significant cross-reactivity to the hydroxylated metabolites of fentanyl and norfentanyl with minimal cross-reactivity to other opiate compounds. Comparison of the immunoassay results (n=200) with LC-MS/MS showed >95% agreement between the two methods.

DRI Fentanyl Assay is a simple, precise and convenient method for the detection of Fentanyl and its metabolites in human urine.

E-114

Efficiency of Intralipid® extraction is largely dependent upon the partition constant of a drug, and may help predict clinical efficacy of lipid rescue for toxicological emergencies.

W. Ruan, D. French, A. Wong, K. Drasner, A. Wu. *University of California, at San Francisco, San Francisco, CA,*

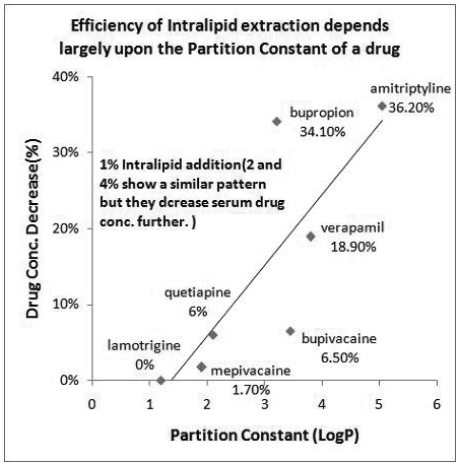
Background: Recently, an increasing number of cases reported using intravenous lipid emulsion (Intralipid®) in treating severe local anesthetic cardiac and CNS toxicity. Although the mechanism of action is not completely understood, its predominant effect likely results from extraction of lipophilic toxins from aqueous plasma and affected tissues. Lipophilicity of a drug is determined by its partition constant (LogP), a measure that estimates the distribution of a drug to hydrophobic compartments such as lipids relative to hydrophilic compartments such as plasma. Hence, the efficiency of Intralipid® extraction may be dependent upon the partition constant of a drug.

Objectives: Determine whether using the partition constant of a drug allows prediction of the efficiency of Intralipid® extraction.

Methods: Each drug was spiked into drug-free-serum at 2µg/ml, followed by the addition of Intralipid® at 1%, 2% or 4%. After incubation at 37ºC, serum samples were ultracentrifuged (Airfuge®) to separate serum from Intralipid®. Solid-phase extraction was performed (Oasis®MCX) in the presence of an internal standard. High pressure liquid chromatography (HPLC) on a Hewlett-Packard-1090 system was performed using an Agilent Eclipse-XDB-C18 column in conjunction with appropriate mobile phases. Detection was accomplished using diode array detection at proper wavelengths.

Results: Spiked drug concentrations before and after Intralipid® (1%, 2% or 4%) were compared. For most drugs, including lamotrigine, mepivacaine, quetiapine, verapamil and amitriptyline, there was a linear correlation between the partition constant (LogP) and the percentage reduction in concentration. However, Bupivacaine and Bupropion deviated significantly from this linear curve (see figure). Since the LogP value is traditionally based on the ratio of H_2O/Octanol, it is possible the solubility of Bupivacaine and Bupropion in Intralipid® differs significantly from their solubility in Octanol.

Conclusion: The partition constant of an overdosed drug can largely be used to predict the efficiency of Intralipid® extraction, and will likely predict its clinical efficacy.



E-115

ABCB1 and CYP3A4/5 variants are associated with blood levels of tacrolimus in renal transplant recipients

R. D. C. Hirata[1], A. C. Rodrigues[1], C. Felipe[2], H. T. S. Junior[2], P. C. Salgado[1], B. C. Florencio[1], C. Alves[1], E. Y. F. Luo[1], C. Fajardo[1], J. O. M. Pestana[2], D. Zetchaku[2], G. Spinelli[2], N. Olive[2], M. H. Hirata[1]. *[1]Faculdade de Ciências Farmacêuticas, Sao Paulo, Brazil, [2]Hospital do Rim e Hipertensão Fundação Oswaldo Ramos, Sao Paulo, Brazil,*

Background: Tacrolimus is an immunossupressive drug largely used in renal transplant recipients, however it thas a narrow therapeutic range. Variants in drug metabolizing enzymes and transporters genes have been associated with variability in blood concentration of tacrolimus and higher risk of acute rejection or adverse effects. The effect of ABCB1, CYP3A4 and CYP3A5 gene polymorphisms on dose and blood concentrations of tacrolimus and rejection rate after renal transplantation were investigated.

Methods: Data from ninety-nine renal transplant recipients (age: 44 ± 12 years; 72% male and 28% female) with renal transplantation from Sao Paulo city, Brazil, that received the immunosuppressive regimen containing tacrolimus, prednisone and mycophenolate sodium for 3 months were obtained. Tracolimus dose was adjusted to reach blood concentrantion between 5 and 15 ng/mL. Blood samples were collected for tacrolimus concentration (C0) measurements after 7, 28, 60, and 90 days and for DNA extraction at pre-transplant. Tacrolimus concentration was measured by ARCHITECT assay (Abbott Laboratories). Genomic DNA was extracted by a salting out method and CYP3A4 (c.1026+12G>A), CYP3A5 (*1D), and ABCB1 (c.1236C>T, c.3435C>T and c.2677G>T/A) gene polymorphisms were identified by TaqMan® Real-time PCR, using TaqMan MGB probes (VIC™ and FAM® dye-labeled).

Results: Tacrolimus mean dose (D) was significantly reduced (D= 7 vs 28 days: 0.182± 0.0583 *vs* 0.117±0.067 mg/kg/day, $p<0.05$ and 28 *vs* 60 and 90 days: 0.117±0.067 vs 0.089±0.047, 0.071±0.038 mg/kg/day, $p<0.05$). The mean concentration to dose ratio (C0/D) significantly increased along the 3 months (C0/D= 7 days: 0.182±0.058 $ng.mL^{-1}$/mg *vs* 60 days: 1.631±0.989 $ng.mL^{-1}$/mg; 90 days: 1.589±0.979 $ng.mL^{-1}$/mg; $p<0.05$). The genotype frequencies of the ABCB1, CYP3A4 and CYP3A5 gene polymorphisms were in Hardy-Weinberg equilibrium. Carriers of CYP3A5*1D/*1D have received lower doses and presented higher blood levels of C0/D of tacrolimus than homozygous for wild type and heterozygous after 7, 28, 60 and 90 days of the immunosuppressive therapy ($p<0.05$). This was also found for CYP3A4 CC carriers ($p<0.05$). For ABCB1 variants c.2677T/A allele was the only variant associated with higher C0/D after tacrolimus treatment after all periods of treatment and lower dose after 7 and 28 days ($p<0.05$). Twenty-five subjects experienced acute rejection during the first three months of transplantion during treatment of tacrolimus. Despite these variants lead to higher blood levels of tacrolimus, they were not associated with the incidence of acute rejection ($p>0.05$).

Conclusions: These results reveal that homozygous for CC genotype for CYP3A4 and AA for CYP3A5*1D variant and carriers of c.2677T/A ABCB1 allele need lower doses of tacrolimus to reach blood levels on the therapeutic range. Despite we have not found association between these variants and acute rejection a larger study will be necessary to confirm this hypothesis.

E-116

Comparison of Three Cyclosporine Methods

M. G. Rush[1], R. Mathieu[2], C. L. Wiley[1]. *[1]Providence Sacred Heart Medical Center and PAML, Spokane, WA, [2]PAML, Spokane, WA,*

Background: Although Cyclosporine A (CsA) has been in use as an immunosuppressant for organ transplants for over 30 years, variable absorption, a narrow therapeutic index and unpredictable pharmacokinetics have necessitated therapeutic drug monitoring. Assay methods to monitor cyclosporine levels include RIA (radioimmunoassay), HPLC (high performance liquid chromatography), monoclonal and polyclonal immunoassays employing fluorescence polarization, chemiluminescence or homogeneous enzyme techniques, and LC-MS/MS (liquid chromatograph-tandem mass spectrometry). Assays have also incorporated both monoclonal and polyclonal antibodies to cyclosporine making comparison of methods challenging. Until recently, HPLC was the only method which could accurately measure the parent compound.

Objective and Methods: Our objective was to evaluate the analytical performance of three methods for detecting cyclosporine levels in whole blood. Seventy-nine EDTA-anticoagulated whole blood samples were tested by 3 methods; Waters HPLC, Abbott i1000SR chemiluminescent microparticle immunoassay (CMIA) and LC-MS/MS using a Waters MassTrak Immunosuppressant kit with an API 4000 equipped with the Shimadzu SIL 20AHT/20ACHT Prominence HPLC. Samples were refrigerated until HPLC analysis and all analyses were conducted within 24 hrs.

Results: The results were analyzed using EP Evaluator® Release 8 (see Table).

Assay Data	n	Mean	SD	Median	Minimum	Maximum
HPLC	79	136	78.0	126	38	579
Abbott i1000SR	79	152	107.5	126	25	837
LC-MS/MS	79	104	75.9	86	32	640
METHOD COMPARISON - Deming Regression Analysis						
Assay	**n**	**Slope**	**95% CI of Slope**	**Intercept**	**95% CI of Intercept**	**R2**
HPLC vs. Abbott	79	1.40	1.31 to 1.48	-37.2	-50.6 to -23.8	0.96
HPLC vs. LC-MS/MS	79	0.97	0.91 to 1.04	-28.5	-38.8 to -18.1	0.96
Abbott vs. LC-MS/MS	79	0.70	0.67 to 0.74	-3.1	-9.5 to 3.3	0.98

Conclusions: This study demonstrates how closely HPLC and LC-MS/MS match each other; both assays measure the parent compound. Abbott's chemiluminescent method reports higher results, as expected. This non-specific method cross-reacts with cyclosporine metabolites. All methods yielded high correlation coefficients. These method comparisons should aid clinicians when deciding which methodology to use in following their patients' levels of cyclosporine.

E-117

Mining of NHANES 2005-2008 data to determine age, gender and race specific reference intervals for blood lead, cadmium and mercury

J. Chan[1], G. S. Cembrowski[1], H. E. Hoffman[2], M. S. Cembrowski[3]. *[1]University of Alberta Hospital, Edmonton, AB, Canada, [2]Occupational Health, University of Alberta, Edmonton, AB, Canada, [3]Northwestern University, Evanston, IL,*

Background NHANES 2005-2008 is a subset of the National Health and Nutrition Examination Survey which is being conducted on US civilian noninstitutionalized individuals. We have been abstracting chemistry and hematology data from NHANES and publishing graphical reference interval summaries on www.mylaboratoryquality.com. As there is a lack of consensus about the reference limits of trace elements in ambulatory populations, especially in children and minorities, and as trace element testing has become an important constituent of NHANES evaluations, we decided to generate reference interval data from newly acquired NHANES trace elements data.

Methods We selected two sub-populations of individuals who had whole blood trace element testing as part of NHANES: 1) non-smokers with a poverty income ratio [PIR] ≥ 1 (the US poverty threshold is 1 and individuals with PIR <1 are considered impoverished) and 2) those with a PIR <1. The trace elements were performed at the Division of Laboratory Sciences, National Center for Environmental Health, and Centers for Disease Control and Prevention. Whole blood lead, mercury and cadmium concentrations were determined using inductively coupled plasma mass spectrometry. Inorganic mercury in blood was measured using stannous chloride as a reductant. The test results were stratified by gender, age and race (Mexican American [MA]), nonHispanic Black [NHB] and nonHispanic White [NHW]). The 90, 95 and 97.5 percentiles were determined for each of the population subgroups.

Results The Table summarizes the 95 percentile limits for lead (ug/L).

Conclusions As expected, there are obvious racial, socio-economic and even gender influences on blood lead concentration. There are similar correlations in cadmium and mercury. Not only should this information be used to assess and more rigorously define reference intervals of ambulatory populations, we should also use this information to drive social and economic change in order to systematically reduce exposure to toxic trace elements.

	MNHW		FNHW		MNHB		FNHB		MMA		FMA	
Age, y	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
12 to 14	2.6	3.4	1.3	1.6	2.8	7.8	1.6	4.3	2.0	3.2	1.6	1.8
14 to 18	2.2	2.4	1.3	1.7	2.5	2.7	1.6	2.0	2.3	3.3	1.7	3.8
18 to 25	1.9	2.7	1.1	1.3	2.2	2.2	1.2	1.4	3.9	7.7	2.1	2.5
25 to 45	2.9	3.4	1.5	1.9	2.7	5.2	1.7	3.1	5.6	5.3	2.3	3.6
45 to 65	4.0	5.2	3.0	3.2	4.0	7.7	4.0	4.0	4.3	7.0	4.2	4.8
65+	5.2	5.8	4.1	3.6	6.1	8.3	5.1	4.7	5.9	7.7	2.8	4.3
N	1688	317	1741	308	802	273	814	339	663	366	662	390

E-118

The measurement of imatinib by liquid chromatography-tandem mass spectrometry

M. E. Franklin, S. Lee, P. I. Pillans, P. J. Taylor. *Princess Alexandra Hospital, Brisbane, QLD, Australia,*

Introduction: Imatinib mesylate is a tyrosine kinase inhibitor used in the treatment of chronic myeloid leukaemia (CML). It inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines, as well as fresh leukaemic cells from CML patients. Although the drug does not cure the disease, it is able to achieve long-term control of CML in the majority of patients. Several studies have now shown that a trough imatinib plasma concentration of 1.0 μg/ml is needed to inhibit the molecular pathway that drives the disease. The purpose of monitoring plasma imatinib concentrations is to ensure that this trough target is maintained. The aim of this study was to develop a liquid chromatography-tandem mass spectrometry method (LC-MS/MS) that is suited for use in the therapeutic drug monitoring of imatinib.

Methods: Plasma samples (25 μL) were mixed with precipitation reagent (containing labelled internal standard, d_8-imatinib, 25 ng/mL; 1 mL) in 96 round-well plates. The plates were vortex mixed and centrifuged. An aliquot (5 μL) was injected and chromatography was performed on an Allure PFP Propyl analytical column (50 x 2.1 mm, 5 μm, Restek) under gradient conditions at a flow rate of 0.45 mL/min. The mobile phases consisted of 2 mmol/L ammonium acetate and 1 mL/L formic acid in water (solvent A), and 2 mmol/L ammonium acetate and 1 mL/L formic acid in methanol (solvent B). The gradient program started at 50% B, increasing to 100% B between 0.8 and 3.7 minutes. After 6 minutes the gradient was stepped back to 50% B and the column re-equilibrated for 2.5 minutes. Mass spectrometric detection was by selected reaction monitoring (imatinib *m/z* 494.2→394.2; internal standard *m/z* 502.4→394.2) using positive electrospray ionization conditions (0.7 kV). Linearity was tested over the range of 0.05 to 5.0 μg/mL. Accuracy and precision were determined using quality controls at 0.3, 1.5 and 3.0 μg/mL. Matrix effects were also examined quantitatively, in a single batch, by measuring 24 different sources of imatinib free plasma spiked at concentrations of 0.3, 1.5 and 3.0 μg/ml (n = 8 at each concentration).

Results: Under the mass spectrometric conditions the predominant precursor ion was the protonated molecular ion, $[M+H]^+$. Total chromatographic run time was 8.5 min. The assay had an analytical range of 0.05 to 5.0 μg/mL ($r^2 > 0.999$, n = 8). Inter- and intra-day accuracy and imprecision for quality control samples (0.3, 1.5, 3.0 μg/mL) were 96.3 to 108.3% and ≤6.5% (n = 8), respectively. The lower limit of quantification was 0.05 μg/mL. At this concentration, the inter-day accuracy and precision was 96.4% and 6.2%, respectively. The intra-batch accuracy and precision for the spiked matrix effect samples were 104.5 to 108.4% and ≤2.8% (n = 8), respectively.

Conclusion: We report an analytically suitable mass-spectrometry based assay for imatinib measurement. The approach which utilizes simple sample pre-treatment in conjunction with high chromatographic retention enables the method to be suitable for routine use in the therapeutic drug monitoring of imatinib.

E-119

A Rapid and Efficient Method to Analyze Carisoprodol and Meprobamate in Blood and Urine by LCMSMS

F. N. Nunes-Daniel, S. Piwarski, C. Jacobs, D. Boehme. *Ventura County Forensic Sciences Laboratory, Ventura, CA,*

Carisoprodol and its metabolite meprobamate are frequently encountered in clinical and forensic toxicology due to widespread prescription and abuse. Previous published analysis of these compounds in blood and urine involved extractions as part of sample preparation. The objective of this study was to develop and validate a rapid, simple, and efficient method to detect and quantify carisoprodol and meprobamate in a small volume of blood or urine, using high-performance liquid chromatography/tandem-mass spectrometry with positive electrospray ionization. Blood samples were subjected to protein precipitation with cold acetonitrile, followed by centrifugation and dilution of the supernatant with mobile phase. Urine samples were diluted with mobile phase, followed by centrifugation to remove any particulates. Carisoprodol-d_7 and meprobamate-d_7 were used as internal standards. A 10uL aliquot of sample was injected on a liquid chromatograph system. Separation was carried out on a Hypersil Gold™ column (50mm x 2.1 mm i.d., 3 µm) using a gradient of methanol/water with 0.1% formic acid. The run time was 5 minutes. The linear range for carisoprodol and meprobamate in both matrices was demonstrated to be 0.25-25mg/L. The correlation coefficients were 0.999 for both analytes in both matrices. The limit of detection for both drugs was 0.25mg/L. There was no carryover at 25mg/L, the highest concentration tested. The carisoprodol and meprobamate accuracies in blood were 97-110% and 86-104%, respectively. The carisoprodol and meprobamate accuracies in urine were 98-105% and 98-108%, respectively. The intra and inter-assay precision in blood at three concentration levels (n=25) across the linear range for carisoprodol and meprobamate ranged from 0.1-11.9% and 0.9-10.5%, respectively. The intra-run precision in urine at three concentration levels (n=8) across the linear range for carisoprodol and meprobamate ranged from 2.0-3.0% and 2.5-4.3%, respectively. A matrix interference study was performed using eight different sources of blood and urine and no ion suppression was observed at the expected retention times of carisoprodol and meprobamate. Additionally, no interferences from related and unrelated compounds were observed. A parallel study was conducted using post mortem blood samples previously tested by a reference laboratory. The reference laboratory used gas chromatography/flame ionization detector to quantify carisoprodol and meprobamate. There were no qualitative differences and the concentrations in agreement ranged from 86-105% and 86-110% for carisoprodol and meprobamate, respectively. A parallel study was conducted using post mortem urine samples tested qualitatively in-house by gas chromatography/mass spectrometry. All the qualitative results previously obtained were confirmed by this method.

E-120

Clinical Utility of an LC-MS/TOF Seizure Panel in Emergency Department's Seizure Patients

R. R. L. Gerona, A. H. B. Wu. *San Francisco General Hospital/ UC- San Francisco, San Francisco, CA,*

Background: Approximately 1% of the 120 million patients coming to the emergency department (ED) annually do so because of seizures. Of these, around 6% are drug-related. Hence, the development of a seizure panel with a fast turn-around time can potentially impact the management of thousands of ED patients. The challenge in coming up with a seizure panel for use in the emergency department is to find a single method that can simultaneously analyze a chemically diverse set of analytes in a wide dynamic range with a fast turn-around time. Current advances in the mass accuracy, sensitivity and dynamic range of LC-MS/TOF can aptly respond to this challenge.

Objective: To develop an LC-MS/TOF seizure panel to aid in diagnosing ED seizure patients.

Methods: We set-up a rapid, semi-quantitative LC-MS/TOF assay to detect 11 chemically diverse drugs reported by the California Poison Control Center to be the most common causes of drug-induced seizures. The assay was developed using Agilent's HPLC (1200)-Time-of-Flight Mass Spectrometer (6230) with electrospray ionization in positive mode. Serum samples (100µL) from ED patients with documented seizures were analyzed after protein precipitation. Separation of the analytes was achieved by gradient elution using Agilent's Eclipse Extend Plus C18 1.8µm 10 x 2.1mm column. The raw data obtained for each sample was analyzed using Agilent's MassHunter Qualitative and Quantitative Analysis software to determine what and how much analyte(s) is present in the patient's serum.

Results: The over-all turn-around time for the analysis of one sample is approximately 40 min, of which 10 min is accounted for by the LC-MS/TOF run. The limit of detection for the 11 analytes ranges from 0.1ng/mL to10 ng/mL. The measurable linear concentration ranges for the analytes span the relevant clinical ranges and are as low as 1ng/mL. The concentration dynamic range for each analyte is approximately 200. Based on five injections per run and five runs, % CV values range from 2.0- 5.5% for within run precision and 2.5- 10.0% for between run precision. Recoveries for the 11 drugs spiked in drug free serum varied from 85 to 105%. The method is currently being tested on ED patients referred by the California Poison Control Center and the San Francisco General Hospital. Of six cases referred so far, results from the assay have made significant impact on the management of three patients in real time. One particular example is a case involving a 50 year old female who had an overdose of an herbal sleeping aid. We found high levels of diphenhydramine in her serum instead that guided attending physicians in her management to recovery.

Conclusion: We have developed an LC-MS/TOF method for a seizure panel intended to detect and semi-quantitatively measure 11 drugs in ED patients documented to have seizures. The method has a fast turn-around time, and high analytical sensitivity and precision. In its initial stages of being tested on ED patients, the assay has already made an impact on the management of three patients with drug-induced seizures in real time.

E-121

Measurement of Ethanol Use in a Chronic Pain Population

B. Crews, C. Mikel, R. Guttierrez, R. West, C. West, A. Pesce. *Millennium Laboratories, San Diego, CA,*

Determining ethanol use in the pain patient population being treated with chronic opioid therapy is critically important to the treating physician. Historically, urine drug screening for ethanol was the only method available. Laboratory findings of urinary ethanol can be complicated by the fermentation of glucose. Recently ethyl glucuronide and ethyl sulfate, which are metabolites of ethanol, have been used to identify alcohol use. These metabolites are present in urine up to 72 hours after ingestion of alcohol. *Purpose of the Study*: In an attempt to establish whether ethanol-positive urine samples can be specific for ethanol use, we examined over 10,000 consecutive urines from pain patients for the presence of ethanol, ethyl glucuronide, ethyl sulfate, and glucose. *Method*: The method used to detect ethanol was an enzymatic assay (Microgenics DRI ethanol). The method used to detect ethyl glucuronide and ethyl sulfate was liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Thermo Fisher Aria Transcend and TSQ Quantum Ultra QqQ instrument. The analytical range was from 500 ng/mL to 10,000 ng/mL with a coefficient of variation of less than 15% at the upper and lower limits of quantitation. *Results*: We observed 1 to 2% of patients with ethanol values above 20 mg/dL. Of these patients, 35% of the patients were negative for ethyl glucuronide and ethyl sulfate (cutoffs = 500 ng/mL). 50% of these patients had glucose levels greater than 100 mg/dL, and several had ethanol levels which were non-physiologic. *Limitations:* Limitations of the study include the lack of demographic data beyond treatment with opioids for chronic pain. *Conclusions*: Fermentation did occur in urine samples shipped via overnight for analysis. Glucose may not be present in fermented samples due to complete conversion; however these findings suggest that the presence of ethyl glucuronide and ethyl sulfate may be used to distinguish between fermentation of glucose into ethanol and positive ethanol due to alcohol consumption.

E-122

Optimization of an HPLC assay for S-warfarin quantification

M. P. Borgman[1], K. Reynolds[2], R. Valdes[1], M. W. Linder[1]. *[1]University of Louisville, Louisville, KY, [2]PGXL Laboratories, Louisville, KY,*

Background: Recent interest in understanding the pharmacogenetic influences on oral anticoagulation with warfarin have lead to an increased demand for assays to measure warfarin enantiomers in patient samples. Several methods for plasma warfarin analysis are available in the published literature, however commercial availability of S-warfarin quantitation is rather limited. Therefore, our goal to support pharmacogenomics studies was to develop an HPLC method capable of quantifying this active warfarin enantiomer. Considerations are method of plasma extraction, throughput, resolution of chromatography and availability of reagents, such as internal standard (IS).

Methods: We reviewed the literature and found two predominant methods, one which employed solid phase extraction, but non-commercially available IS (Henne et al., Journal of Chromatography B, 1998) and a more recent method employing liquid-liquid extraction techniques, and commercially available IS (Osman et al., Journal of Chromatography B, 2005). Both methods were initially followed as described in the literature using our HPLC system comprised of a Waters 1525 pump set at 1 ml/min with a Waters 2487 dual wavelength UV detector set at 312 nm. The column employed was the (R,R)-Whelk-O1 Kromasil analytical chiral column protected by a Hypurity C-4 guard column. Optimizing our chromatography system for chiral separation was achieved through altering the composition of a 0.5% acetic acid (HOAc) and acetonitrile (ACN) mobile phase such that we varied ACN from 40% to 90%. Standards of racemic warfarin and oxybenzone (IS) dissolved in ACN were used. Additionally, extraction of warfarin enantiomers and IS

from human plasma was achieved using Waters Sep-pak C-18 cartridges, washing with methanol and 1M HCL, and eluted in 75% methanol in water.
Results: Chromatography was initially evaluated for both methods using a combination of unextracted racemic warfarin and IS standards. Under published mobile phase conditions (40% ACN : 60% HOAc), warfarin and IS were retained on the column. Increasing ACN between 80-90% caused co-elution of R-warfarin and IS. Optimum chromatographic separation of warfarin enantiomers was achieved with conditions set at 30% HOAc/70% ACN. Under these conditions S-warfarin, R-warfarin and IS eluted at 6.3, 7.1 and 8.0 minutes, respectively. Peak width at the highest concentration tested (5 mg/L) was less than one minute. Chromatography results based on the published solid phase extraction technique yielded artifacts which co-eluted or interfered with IS detection. The optimized extraction method resulted in an extraction efficiency of 65% for S-warfarin with no baseline derangements in blank plasma over the time frame where peaks of interest elute. A standard curve was generated for extracted plasma standards from 0.1 to 5 mg/L giving a correlation coefficient of 0.9998 (conc = 2.2934(peak area) - 0.1065).
Conclusions: We have improved upon the literature methods in terms of reproducibility of chiral chromatography results, ease and pureness of extraction from human plasma, and employed a commercially available internal standard used previously by Osman et al. now optimized with a solid phase extraction method. Together, we have developed an assay for chiral separation and quantitation of S-warfarin that suits the needs of our pharmacogenomics research.

E-123

Drug Monitoring and Toxicology: A Rapid Method for the Monitoring of Rufinamide by HPLC-UV

P. H. Tang. *Cincinnati Children's Hospital Medical Center, Cincinnati, OH,*

Background: Rufinamide is a new antiepileptic drug with a triazole derivative structure. It has shown promise as adjunctive treatment for Lennox-Gastaut Syndrome and may have some role in localization related epilepsies as well. US FDA has recently approved the use of drug for the adjunctive treatment of seizures associated with Lennox-Gastaut Syndrome and as adjunctive treatment for partial-onset seizures. Therapeutic drug monitoring of antiepileptic drug concentration in plasma is helpful to physicians in evaluating patient compliance with treatment, in providing guidance to achieve well-tolerated and effective dosing, and in identifying drug-drug interactions when drugs are given as polytherapy. Plasma rufinamide is generally measured by high-performance liquid chromatographic (HPLC) method. In some HPLC methods, multi-step extraction techniques and extensive sample pretreatment are used. Simple and reliable HPLC procedures based on direct HPLC injection after sample deproteinization or even without sample pretreatment have not been reported. Previously, measurement of plasma rufinamide ordered by the physicians here at the Cincinnati Children's Hospital Medical Center was performed at the reference laboratory. The results turnaround time were not always satisfied and service of therapeutic drug monitoring was, therefore, lagging.
Objective: The need for a quick measurement of rufinamide in plasma samples in a simplified manner and the need for a cost-effective procedure prompted the development of a rapid HPLC method. Here, a simple and reliable HPLC method is described for determination of rufinamide concentrations in a small volume (100µL) of plasma that is suitable in pediatric practice.
Methods: Sample was vortex-mixed with the internal standard (trimethadione) and methanol for 1 minute and centrifuged at 10,350 g for 10 minutes at room temperature. The supernatant was transferred to an autosampler vial, a portion of supernatant (20µL) was injected directly onto the HPLC system. Using a mixture of phosphate buffer, methanol, and acetonitrile as mobile phase, the derivatization products were analyzed on a 5-µm Microsorb-MV reversed-phase C18 column (250 x 4.6 mm) column using UV detection at 199 nm. Quantification was based on a 5-point calibration using peak height measurements of rufinamide and internal standard.
Results and Discussion: The method achieved a linear concentration range of 1-50 mg/L, which covered the proposed therapeutic range of 5-48 mg/L. The limit of detection was 0.2 mg/L. Both within-run and between-run precision for three fortified controls (10, 20, and 40 mg/L) in plasma were lower than 5%. Analytical recoveries were greater than 95%. No interference was observed from the most commonly administered antiepileptic drugs (primidone, carbamazepine, ethosuximide, felbamate, lamotrigine, levetiracetam, oxcarbazepine, phenobarbital, phenytoin, topiramate, valproic acid, and zonisamide). The method was compared to a reference laboratory HPLC assay using 30 samples ranging from 4 to 45 mg/L. The correlation showed a slope of 1.04, an intercept of 0.2 mg/L and an *r* of 0.93.
Conclusion: This method is simple and easy to perform with excellent reproducibility, requires no solid-phase extraction and one step deproteinization prior to chromatography. It is suitable for routine analysis of rufinamide in human plasma samples.

E-124

Serum Uric Acid Levels as an adjunct in the assessment of certain Psychiatric Disorders

J. R. Peela[1], A. M. Jarari[1], S. S. Dastagir[1], E. A. Salh[2], A. Hai[1], A. K. Rawal[1].
[1]Department of Biochemistry, Faculty of Medicine,Al-Arab Medical University,, Benghazi, Libyan Arab Jamahiriya, [2]Department of Biochemistry, Faculty of Pharmacy,Al-Arab Medical University,, Benghazi, Libyan Arab Jamahiriya,

The increasing number of latent and manifest hyperuricemia is important concerning differential diagnosis in neurological and psychiatric diseases. The pathological importance of hyperuricemia in these diseases is particularly unknown. Previous studies have shown that uric acid estimation in cerebrospinal fluid was made with neurological and psychiatric diseases. On an average the uric acid level in cerebrospinal fluid is 1:10 of the blood uric acid level. High levels for example were found after L-Dopa treatment and epileptic seizures and parkinsonism. Monitoring serum uric acid levels, a relatively inexpensive and easily available test, may prove to be a useful adjunct in the assessment of certain indices of certain psychiatric illness. There is evidence of dysregulation of the antioxidant defense system in schizophrenia. Some studies have shown uric acid, a potent antioxidant, is reduced in the plasma of patients with schizophrenia. Tranquilizers like 1,4 -benzodiazepine (purinergic) are shown to decrease xanthine oxidase activity initially and may cause fluctuations in serum uric acid levels. In the present study an attempt was made to understand the effect of tranquilizers on serum uric acid levels in different psychiatric conditions.40 cases (22 males and 18 females) of MDP (Maniac depressive psychosis) and Schizophrenia who were undergoing treatment with different tranquilizers from the psychiatric hospital, Benghazi, Libya and 20 healthy controls (12 males and 8 females) not using any tranquilizers were taken for study. The serum uric acid levels were significantly elevated ($p < 0.05$) in these patients receiving treatment with tranquilizers. The levels of uric acid in male patients were significantly higher when compared with females and also over male controls ($p < 0.01$).In female patients uric acids levels are raised but not significant ($p > 0.05$) over control females. This preliminary study does indicate that serum uric acid levels do change with the administration of these drugs. The nature, gender, duration and the type of drugs used and their individual effects on different psychiatric disorders will be discussed.

Thursday AM, July 29

Poster Session: 9:00 am – 12:00 pm
Pediatric/Fetal Clinical Chemistry

E-125

Can We Use the Glucose Challenge Test [GCT] More Efficiently?

D. Lantz[1], C. Schromm[2], E. S. Pearlman[3]. *[1]East Boston Neighborhood Health Center (EBNHC), Boston, MA, [2]Codman Square Community Health Center, Boston, MA, [3]Boston Medical Center, Boston, MA,*

Currently patients are screened for the risk of gestational diabetes mellitus (GDM) through the use of a GCT that uses the the plasma glucose concentration (PGC) one hour after ingestion of a 50 gram glucose load (with no fasting requirement) as the decision parameter. If the PGC is greater than 140 mg/dL the patient is referred for a three hour OGTT with a 100 gram glucose load. This two stage process is inconvenient for patients and the question arose from clinicians as to whether a value for the GCT result could be defined when GDM could be diagnosed without additional testing. We reviewed the results of 197 consecutive patients referred for a 3 hour OGTT of whom 59 met the ADA criteria for GDM. All PGCs were determined using a Dade Xpand chemistry analyzer (Siemens; Newark, DE) according to the manufacturer's instructions. The median age of the non-GDM patients was 29 YO [range: 16-46] while for the GDM patients was 33 YO [range: 21-45; p<.01 median test]. The median gestational age (GA) at the time of OGTT was not significantly different for GDM vs. non-GDM patients and the median value for the entire group was 27.43 weeks [range: 6.71-36.56 weeks]. There were 18 patients (16 non-GDM) for whom a 3 hour OGTT was ordered with GCT result </= 140 mg/dL. Four of the latter had PGCs of exactly 140 mg/dL. The median GCT result for the non-GDM patients was 150 mg/dL [range 98-216 mg/dL] and 163 mg/dL for the GDM patients [range: 116-320 mg/dL; p<.005 median test]. The results with different cut-points illustrate the trade-off between number of OGTTs saved and number of false positive diagnoses of GDM. Deciding to diagnose GDM with GCT results =/> 200 mg/dL would result in only 2 FPs but would save only 5 OGTTs. Using GCT thresholds of 175, 180, 190 and 200 mg/dL, the number of OGTTs saved was 17 (28.9%), 13 (22%), 7 (11.9%) and 5 (8.5%) respectively while the number of FP diagnoses of GDM was 11, 6, 4 and 2 at the respective thresholds. Thus the choice of cut-point depends on the false positive rate one is willing to accept and this in turn depends on whether, for example, treatment with oral hypoglycemic agents is contemplated. Although the data set is small is suggests that a cut-point of 180 mg/dL would affect a >20% decrease in the in OGTTs at a FPR [FP/(FP+TP)] of 9.2% [6/(59+6)]. Alternative strategies could include using a multivariate statistical technique such as discriminant analysis with easily accessible parameters such as patient age, GA and patient weight/BMI to develop a classification score or developing a screening procedure based on fasting gluose data.

E-127

Prevalence of anemia and iron deficiency among children attending public daycare centers in Brasília, Federal Capital of Brazil.

S. F. FONSECA[1], G. C. RODRIGUES[2], V. F. LOZANO[2], L. F. A. NERY[2], S. S. S. Costa[2], C. M. Araujo[2], S. T. A. Gomes[2]. *[1]Unb, Brasilia, Brazil, [2]Sabin Laboratory, Brasilia, Brazil,*

Iron-deficiency anemia is the nutritional deficit most commonly found in less-developed regions. In Brazil, the frequency of such cases is increasing, and today it is the most prevalent nutritional deficiency, surpassing even energy-protein malnourishment. The main consequence of the lack of iron in pre-school children is psychomotor deficit, traces of which may be noticed even after the deficiency has been adequately treated. Due to its high prevalence and its serious consequences, combating iron-deficiency anemia is one of the priorities established by the professionals responsible for the planning of Nutrition Programs in Public Health, which are supported by political aid and the social commitment Brazil has assumed to reduce iron-deficiency anemia. The Ministry of Health has mandated the fortification of corn flower and wheat flour with iron, since these are food items that are easily accessible to the population and are economically viable to the country, and has implanted the National Program of Iron Supplements for groups that are at risk. The objective of this study was to verify the prevalence of iron-deficiency anemia in children that attend public daycare centers in the Federal District, Brazil.
MATERIAL AND METHODS:Transverse study in which all children from one to 15 years old, that attend four public daycare centers in Brasilia and its suburbs were invited to participate. After receiving a completed informed consent form from the family members or those responsible for each child, 10mL of blood in sterile vacuum tubes were collected, divided into two tubes; one tube containing EDTA and another containing a separator gel. The hematological values were determined using an automated blood cell counter (*Sysmex* XE 2100, Roche), and the level of serum ferritin was evaluated by chemiluminescence (*ADVIA Centaur*® CP System, Siemens). The operational definition of anemia was established by the WHO, adopting the cut-off hemoglobin level of 11.0 g/dl for children under six years and 12 g/dl for children older than six years and adolescents. Those with ferritin values < 20 ng/ml were considered to be iron deficient.
RESULTS AND CONCLUSION: The sample group consisted of 437 children with ages ranging from 1 to 15 years, averaging 7.8 years; 232 boys and 205 girls. The average hemoglobin level in the general population was 12.8 g/dl and the average ferritin level was 45 ng/ml. Anemia was present in 49 children (11.2%) and iron deficiency in 27 children (6.3%). Despite the prevalence of iron-deficiency anemia being inferior to the levels reported in the national literature (which may reflect on the preventive measures put into practice in the country since 2004), the majority of children with iron deficiency (74.1%) were under five years old or were adolescents, and therefore, in the groups of higher risk. Twenty-two children were referred to a pediatrician for evaluation and determination of the cause of their anemia.

E-128

Compound heterozygous mutations in two classic CAH female patients

J. Tian[1], G. Yang[2], F. Zheng[1], Y. Zhang[1], G. Song[1]. *[1]Zhongnan Hospital of Wuhan University, Wuhan, China, [2]Wuhan University, Wuhan, China,*

Background: Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive disorder mainly caused by defects in the steroid 21-hydroxylase gene (CYP21A2). CYP21A2 is a microsomal cytochrome P450 required for the synthesis of cortisol and aldosterone. The reduced synthesis of cortisol and aldosterone leads to excessive androgen production. The CYP21A2 gene consists of 10 exons and 9 introns spanning about 3kb. Females affected with severe, classic 21-hydroxylase deficiency are exposed to excess androgens prenatally and are born with virilized external genitalia (classic Simple Viriliaing (SV) CAH) .
Objective: The objective of this study is to find the inheritance defections of the family.
Subjects and Methods: We recruited two sisters with classic SV CAH, the eld sister is seventeen years old and the younger sister is ten years old. Their parents were clinically and biochemically unaffected with high level of Testo. The father's Testo level was 11.35ng/ml, and the mother's Testo level was 1.23 ng/ml in ovulation period. The two sisters' ACTH-stimulated plasma 17-OHP concentrations were 100 ug/dl and 50 ug/dl separately. The Testo level of the elder daughter was 6.07 ng/ml, and that of the little daughter was 3.97 ng/ml. The PRL of the elder daughter was 56.62 ng/ml, and that of the little daughter was 32.43 ng/ml.Their blood pressure were all normal. Genomic DNA was extracted from peripheral blood leukocytes using protease K-phenol. We use nested PCR and 7 pairs of specific primers to amplify CYP21A2 gene. The PCR products were directly sequenced. **Results:** We found that the father had a heterozygous mutation p.I173N in exon 4, the mother had a heterozygous mutation IVS2-13A/C>G in intron 2, and the two sisters both had a compound heterozygous mutations of p.I173N in exon 4 and IVS2-13A/C>G in intron 2.
Conclusions: Obviously they inherited the p.I173N-bearing paternal allele and the IVS2-13A/C>G bearing maternal allele. And a compound heterozygous mutations of p.I173N at exon 4 and IVS2-13A/C>G at intron 2 may induce sever CAH in those patients.

E-129

The Relationship of 7a-hydroxylase gene polymorphism with serum lipid levels in China children

C. Wang[1], C. ZHANG[2], J. LIANG[1], c. WANG[3], l. Jin[1]. *[1]School of Public Health, Wuhan University, Wuhan, China, [2]School of Public Health,Wuhan University, Wuhan, China, [3]Wuhan University, Wuhan, China,*

Objective: Cholesterol 7α-hydroxylase (CYP7A1) is a rate-limiting enzyme for bile acid biosynthesis from cholesterol. To evaluate the relationship between CYP7A1 gene and lipid, we analysis the polymorphism in nucleotide A-204C of CYP7A1 gene and the serum lipid levels children in China children.
Methods 216 healthy children (boys127, girls 89, aged 6-9 year) were randomly selected in Hubei province. Venous blood samples were collected to fast for at least 12h. The polymorphism in nucleotide A-204C of CYP7A1 gene was detected by using PCR-RFLP. Plasma TG and TC were measured by enzymatic methods, HDL-C andLDL-C were determined by homogeneous methods, apoA1, apoB and LP(a) were tested by immunoturbidimetric method , all using anautomatic chemistry analyzer (Aeroset, USA). Coefcients of variation for measurements were each less than 5%.
Results:The frequencies of AA, AC and CC genotypes were 6.0%, 26.4% and 67.6%, respectively. These data fit Hardy-Weinberg equilibrium (χ^2 = 4.953, P>0.05). No signicant differences both genotypes and alleles were found between boys and girls. The table 1 showed: the levels of LDL-C, TC, LP(a) with AA genotype were lower than those

in other genotypes (P<0.05),but the levels of HDL-C with CC genotype were higher than those in other genotypes (P<0.05).There were no significant differences of the genotypes in TG while significant differences among the three genotype in apoA level.

Conclusion: CC genotype is most common in subjects. There was significant correlation between the polymorphism of the CYP7A1 gene and lipid levels in China children.

Table1 The serum lipid levels across the CYP7A1- A-204C genotypes in 216 healthy China children **()**

Lipids	genotype			P		
	AA(n=13)	AC(n=57)	CC(n=146)	AAvsAC	AAvsCC	CCvsAC
HDL-C (mmol/L)	1.08±0.25	1.12±0.21	1.25±0.19	0.51	0.01*	<0.01*
LDL-C (mmol/L)	1.40±0.58	1.73±0.52	1.80±0.43	0.02*	<0.01*	0.30
TC (mmol/L)	2.84±0.67	3.74±0.75	3.46±0.56	0.03*	<0.01*	0.07
TG(mmol/L)	0.92±0.55	0.98±0.70	0.84±0.47	0.76	0.58	0.11
ApoA (mmol/L)	1.14±0.42	1.32±0.21	1.44±0.20	0.01*	<0.01*	<0.01*
ApoB (mmol/L)	0.50±0.13	0.52±0.13	0.53±0.10	0.07	0.03*	0.65
Lp(a) (mg/dL)$^{\Delta}$	34.40 (11.90-64.50)	80.60 (23.75-195.10)	93.20 (20.35-188.78)	0.05*	0.03*	0.89

Note: *P<0.05,

Δ the data is not normal distribution, the average of Lp(a) was showed by median and quartile range

E-130

Ethnic Differences in Thyroid Tests during the First Trimester of Pregnancy

S. L. La'ulu[1], W. L. Roberts[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah, Salt Lake City, UT,*

Changes occur in thyroid function during pregnancy and complicate the diagnosis of thyroid dysfunction. The objective of our study was to establish thyroid reference intervals (RI) by ethnic group during the first trimester of pregnancy. Surplus first trimester maternal serum screen specimens were collected from 540 Asians, 549 blacks, 601 Hispanics, and 878 whites ranging from 15-46 years of age (median=31 years) with gestational age ranging from 10-13 weeks (median=12.4 weeks). Samples from 129 healthy subjects were also obtained for comparison (63 female, 66 male, 18-62 years of age, median age=30). Thyrotropin (TSH), free (FT4), and total (TT4) thyroxine, free (FT3), and total (TT3) triiodothyronine, thyroglobulin autoantibodies (TgAb), thyroid peroxidase autoantibodies (TPOAb), and T-Uptake were measured using an Abbott ARCHITECT $i2000_{SR}$ analyzer. The free thyroxine index (FTI) was calculated as instructed in the package insert: TT4/ T-Uptake. TSH RI was calculated for all subjects and by ethnic group using TgAb and TPOAb negative subjects. Using the central 95% of TSH RI, RIs were then calculated for FT4, TT4, FTI, FT3, and TT3 for all subjects and by ethnic group. TSH and FT4 RIs are summarized (Table). For TgAb and TPOAb, positivity rates were 13.3 % and 16.1%, respectively (Asian), 3.6% and 7.1% (black), 6.8% and 12.65% (Hispanic), 8.5% and 13.8% (white), and 8.1% and 12.6% (combined). Numerous ethnic differences for first trimester thyroid RIs were observed. For the following, the lower and/or upper reference limit did not fall within the 95% confidence intervals (CI) for all subjects combined: Asian, TSH, FT4, TT4, FTI, TT3; black, TSH, TT4, FT3, TT3; Hispanic, TSH, FT4, TT4, FT3, TT3; white, TSH, FT4, TT4, FTI, FT3, TT3. For non-pregnant subjects, all reference limits were different from the combined group except the lower limit for FT3.

Analyte/Ethnicity	**n**	**2.5th percentile (lower)**	**90% CI (2.5th)**	**50th percentile (median)**	**97.5th percentile (upper)**	**90% CI (97.5th)**
TSH (mIU/L)						
Combined	2172	0.02	0.02 - 0.04	0.94	2.69	2.59 - 2.80
Asian	424	0.02	0.01 - 0.04	0.89	2.81	2.71 - 3.47
Black	506	0.01	0.00 - 0.02	0.82	2.40	2.16 - 2.56
Hispanic	511	0.01	0.01 - 0.05	1.02	2.69	2.36 - 3.06
White	731	0.11	0.06 - 0.16	1.02	2.69	2.59 - 2.88
Non-pregnant	129	0.59	0.00 - 0.77	1.55	3.27	2.94 - 3.76
FT4 (pmol/L)						
Combined	2069	11.35	11.09 - 11.61	14.71	18.58	18.32 - 18.83
Asian	394	12.38	11.61 - 12.64	15.61	19.75	18.96 - 20.64
Black	482	11.10	10.71 - 11.74	14.58	18.56	17.93 - 19.09
Hispanic	485	11.37	10.96 - 11.87	14.71	18.30	17.67 - 18.83
White	708	11.09	10.96 - 11.48	14.19	17.80	17.29 - 18.32
Non-pregnant	123	10.71	10.06 - 11.61	14.84	17.66	17.03 - 19.35

E-131

The value of the detection of fetal SRY gene in maternal plasma at different pregnant states by real-time fluorescence quantitative PCR

D. L. Tang. *Department of clinical laboratory, Renmin Hospital of Wuhan University, Wuhan 430060, China., Wuhan, China,*

***Objective*:** To develop a [[Unsupported Character - ﬂ]]uorescent polymerase chain reaction (PCR) assay for the detection of circulating fetal DNA in maternal plasma and use the established multiplex in noninvasive prenatal genetic diagnosis

***Study design*:** Fetal DNA in maternal plasma was isolated from 28 cases bearing male fetuses in the first trimester, 20 in the mid-trimester of pregnancy and 32 in the late-trimester of pregnancy, respectively.. Real-time Fluorescence Quantitative Polymerase Chain-reaction (FQ-PCR) was used to determine sex-determining region Y (SRY) gene on Y chromosome.

***Results*:** It shows that cell-free fetal DNA is present in high concentration in maternal plasma, reaching a mean and 95% confidence interval for parameter: 58.82 copys/ml, 33.7-126.2 copys/ml; 152.08 copys/ml, 37.7-390.2 copys/ml and 212.34 copys/ml, 87.90-493.56 copys/ml in early normal pregnancy, in middle normal pregnancy and in late normal pregnancy, respectively. Differences were observed among the three groups(P<0.01).

Conclusion: The results show that fetal SRY gene can be found as early as 62 days of gestation in maternal plasma by the use of FQ-PCR. The number of fetal DNA increases with gestational age, so the high sensitivity of PCR-based detection, together with quantification provided by real-time DNA analysis, has clear potential for clinical application in non-invasive prenatal diagnosis.

E-132

Prospective Serologic Screening of a Korean Pregnant Women for Toxoplasmosis

Y. Ko[1], K. Kwon[1], S. koo[1], J. Park[2]. *[1]Chungnam National University Hospital, Daejeon, Korea, Republic of, [2]Abbott diagnostics, Seoul, Korea, Republic of,*

Background: *Toxoplasma gondii* can cause devastating disease in the fetus and newborn infant and yet remain unrecognized in women who acquired the infection during pregnancy. Serologic testing of pregnant women by *Toxoplasma*-specific antibody tests can be used to identify those women at risk for transmission of infection. These tests can be used at the diagnostic level to offer prenatal diagnosis and treatment. In Korea, despite a few reports of the seroprevalence of *Toxoplasma* (Toxo) antibody, the incidence of acute or chronic Toxo infection during pregnancy was not established. Against this background, prospective screening of pregnant women in Korea was performed to establish basic epidemiological data.

Methods: During a six-month period, 789 pregnant women (30.9±7.2 years) at various weeks of gestation were enrolled in the prospective study. Toxo-specific IgG and IgM antibodies were determined by the Abbott AxSYM Toxo IgG and IgM assays. Serum specimens with IgG and IgM positive results were also tested by the Abbott ARCHITECT Toxo IgG Avidity test. All assay test results were interpreted per manufacturer's instructions.

Results: The seroprevalence of Toxo-specific IgG antibody in this cohort was calculated to be 2.3% (18/786), excluding three grayzone results. Median concentration of Toxo-specific IgG antibody in specimens below the assay cutoff was 0.1 IU/mL. The range of Toxo-specific IgG concentration in specimens with grayzone results or specimens above the assay cutoff was 2.4~300.0 IU/mL. The seroprevalence of Toxo-specific IgM in this cohort was calculated to be 0.1% (1/784). Median index value in specimens with negative results was 0.16. Among the specimens with Toxo IgM grayzone results, none were Toxo IgG reactive. Only one specimen was found to have both IgG (300 IU/mL) and IgM (0.66 Index) positive results. This specimen was subsequently tested by Toxo IgG avidity test and a low avidity test result (9%) was obtained, warranting further Toxo serologic diagnostic testing. Unfortunately, further testing was not offered to this woman as her pregnancy was subsequently terminated for an unrevealed reason.

Conclusion: The seroprevalence of Toxo IgG in our pregnant Korean cohort was estimated to be 2.3%, which is lower than most countries. The incidence of acute or chronic toxoplasmosis was estimated to be less than 0.1%. Most Toxo positive IgG and IgM results could be clearly separated from the negative results leaving few grayzone results (IgG, 0.4%; IgM, 0.6%). Through the use of a Toxo IgG avidity test, a woman with an acute or chronic toxoplasmosis was identified.

E-133

Clinical Characteristics and Antibodies to Diabetes Specific Antigens in Children Diagnosed with Type 1 Diabetes before 5 Years of Age

R. S. Berardo[1], M. F. M. C. Pinheiro[2], F. C. C. Silveira[2], J. L. Luersher[1], J. E. P. Oliveira[3]. *[1]Instituto de Puericultura e Pediatria Martagão Gesteira, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2]Sérgio Franco Medicina Diagnóstica, Rio de Janeiro, Brazil, [3]Division of Nutrology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,*

Background: Type 1 diabetes is a chronic autoimmune disease caused by the destruction of insulin-secreting islet cells of the pancreas by several islet cell-specific autoantibodies, such as anti-glutamic acid descarboxylase (anti-GAD) and anti-tyrosine phosphatase-like protein ICA-512 (anti-IA2). Knowing the frequency of these autoantibodies in a population is an important step for a better understanding and diagnosis of type 1 diabetes. The objective of this study was to evaluate clinical characteristics and antibodies to diabetes specific antigens in children with type 1 diabetes mellitus diagnosed before 5 years of age.
Methods: We have studied 38 children with type 1 diabetes diagnosed before 5 years of age, followed at Pediatric Diabetes Outpatient Clinic. Clinical and auxologic data were obtained by retrospective analysis of records and interview. Blood was obtained for anti-GAD (RIA, RSR, UK), anti-IA2 (RIA, RSR, UK), random C-peptide (ICMA, Immulite 2000, Siemens, USA) and HbA1C (HPLC, Variant II, Biorad, USA) measurements.
Results: Study population comprised 17 girls and 21 boys. Their mean age was 7.6 ± 3.7 (2.1 to 18.7) years and their mean disease duration was 4.6 ± 3.7 (0.6 to 17.7) years. The mean age at diagnosis was 3.0 ± 1.2 years with a minimum of 0.6 year. Ketoacidosis was the initial presentation in 56.4% (22 patients). Anti-GAD was positive in 52.6% (20 patients) and anti-IA2 was positive in 10.5% (4 patients) of the children. 57.9% (24 patients) were positive for either antibodies and only 5.3% (2 patients) were positive for both antibodies. There were no clinical differences between antibody positive and negative patients regarding metabolic control. There was no relation of disease duration and positivity to either anti-GAD or anti-IA2, but titers of anti-GAD were significant lower in children with more than 5 years of disease (p=0.032).
Conclusions: Comparing with other populations, in this study, we have found lower prevalence of the antibodies, specially anti-IA2. We could not find any relation to clinical characteristics or control between the antibody positive and negative patients. The dropping of anti-GAD titers with longer disease duration could point to an influence of disease duration in antibody positivity.

E-134

Evaluation of Maternal Thyroid Function Tests during the First and Second Trimesters of Pregnancy

L. F. A. O. Castro[1], M. F. M. C. Pinheiro[2], L. S. Netto[1], C. P. M. Paiva[2], S. C. N. Costa[2], C. M. Coeli[3], A. Buescu[1], M. Vaisman[1]. *[1]Division of Endocrinology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [2]Sérgio Franco Medicina Diagnóstica, Rio de Janeiro, Brazil, [3]Division of Preventive Medicine, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil,*

Background: Maternal thyroid dysfunction has been associated with a variety of adverse pregnancy outcomes. Laboratory measurement of thyroid function plays an important role in the assessment of maternal thyroid health. However, occult thyroid disease and physiologic changes associated with pregnancy can complicate interpretation of maternal thyroid function tests. The objective of this study was to evaluate maternal thyroid function tests (TSH, Free T4 and Total T4) during the first and second trimesters of pregnancy.
Methods: Exclusion criteria were personal and family history of thyroid dysfunction, hyperemesis gravidarum, use of medication that might alter thyroid function and positive thyroid peroxidase antibody (TPO-Ab). Serum TSH, FT4 and TT4 were measured by chemiluminescence method using the Immulite 2000 (Siemens, USA). Reference ranges were 0.4 to 4.5 mcUI/mL for TSH; 0.8 to 1.9 ng/dL for FT4 and 4.5 to 12.5 mcg/dL for TT4. A two-tailed p value < 0.05 was judged to be statistically significant.
Results: We studied 35 pregnants in the first trimester (5 to 13 weeks) and in the second trimester (20 to 24 weeks) of pregnancy. The median age was 23 (13 to 42) years. The mean,SD and range of TSH, FT4 e TT4 from the first and second trimesters pregnants are shown in Table 1:

Table 1: TSH, FT4 and TT4 levels in 35 pregnants in the first and second trimesters of pregnancy (mean ± SD, range):

	TSH (mcUI/mL)	Free T4 (ng/dL)	Total T4 (mcg/dL)
First Trimester	1.50 ± 1.05 (0.03 - 4.33)	1.26 ± 0.18 (0.95 - 1.90)	9.84 ± 1.74 (5.66 - 13.80)
Second Trimester	1.74 ± 1.00 (0.54 - 4.23)	1.08 ± 0.15 (0.78 - 1.60)	10.23 ± 1.75 (6.73 - 15.10)
p value	p = 0.007	p <0.001	p = 0.21

Conclusions: Our results showed lower levels of TSH in the first trimester and lower levels of FT4 in the second trimester of pregnancy. These results reinforce the use of trimester-specific reference values when reporting thyroid test values for pregnant patients. Application of non-pregnant reference intervals to the interpretation of thyroid tests in pregnant women has the potential to result in misclassification of a significant percentage of results.

E-135

Plasma B-type natiuremic peptide (BNP)and Serum N-terminal -pro-BNP(NTpBNP) levels with heart failure children.

Y. Uji, M. Takiwaki, T. Ueno, Y. Taga, N. Shiba, C. Yoshida, T. Kuwabara, H. Niimi, I. Kitajima. *Toyama University Hospital, Toyama, Japan,*

Background: B-type natiuremic peptide(BNP) and N-terminal -pro-BNP (NTpBNP) have a major role in screening and diagnosis of cardiac disease and monitoring of the treatment response. Clinical studies have shown excellent correlation between BNP and NT pBNP levels in adults. However, there are not many reports with neonates and children. Therefore, this study, we evaluated serum NTpBNP levels in serum and BNP levels in plasma for heart failure neonates and children.
Materials and Methods: Serum and plasma samples of heart failure patients (N=142, Age 0~ 20 , 88 males and 54 females) were obtained from the Toyama University Hospital after receiving the patients and /or parents had given their informed consent for this study. NTpBNP levels in serum were analyzed using an Elecsys E170 and NTpBNP II reagent (Roche Diagnostics K.K., Tokyo, Japan) and BNP levels in plasma were analyzed an automated chemiluminescence immuno assay analyzer MIO2 and MIO2- SIONOGI -BNP reagent (Sionogi Co.Ltd,, Osaka,Japan). Left ventricular ejection fraction (LVEF) which is an index of left ventricle function were analyzed an iE-33 ultrasonograph (Philips Electronics Japan, Tokyo, Japan).
Results: Basic analytical performance showed as follows: for NT-proBNP measurement: linearity 5~25000 g/mL, within and day to day precision(CVs) 1.1 ~1.5%(188 ~ 555 pg/mL,N=15); for BNP measurement: linearity 4 ~ 2000 pg/mL, within and day to day precision(CVs) 4.7 % ~ 5.8% (19 ~ 466 pg /mL,N=15). Both serum NTpBNP and plasma BNP levels for less than 4 years old heart failure patients were higher (mean : NTpBNP 970 pg/mL ,BNP 78 pg/mL,N=61)than up to 4 years old heart failure patients(mean : NTpBNP 126 pg/mL and BNP 28 pg/mL,N=81,p<0.0001).
Especially, neonate(less than one years old) heart failure patients of serum NTpBNP and plasma BNP levels were very high (2076 pg/mL and 88 pg/mL, N=8). Furthermore, no significant difference were observed between LVEF , serum NTpBNP and plasma BNP levels in less than 4 years old heart failure patients, respectively. Other hand, excellent correlations were observed between serum NTpBNP, plasma BNP levels and LVEF with up to 4 years old heart failure patients (> r=0.77, p< 0.0001), respectively .
Conclusions: Guidelines for the use of BNP and NTpBNP levels in adult patients are not generalizable to children, because the type of ventricular impairment, underlying cardiac morphology, age, gender, and assay method may affect the reference values for these markers. The role of BNP and NTpBNP in the diagnosis and management of children with heart failure remains controversial.
In this study, proposed data suggested that does not apply in judgment basis of adults plasma BNP and serum NTpBNP levels for heart failure with neonates and less than 4 years old children.

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The Cord Blood and Maternal Serum Neopterin Levels in Patients with Pre-Eclampsia

T. Cayci[1], E. O. Akgul[1], Y. Gulcan Kurt[1], I. Aydin[1], H. Alacam[2], E. Ozkan[1], H. Yaman[1], E. Cakir[1], O. Deren[2], P. Dogan[2], C. Bilgi[1], K. M. Erbil[1]. *[1]Gulhane Military Medical Academy, Ankara, Turkey, [2]Hacettepe University, School of Medicine, Ankara, Turkey,*

Background: Pre-Eclampsia (PE) is a syndrome, characterized by hypertension and proteinuria. The aim of this study was to investigate neoptein levels of cord blood and maternal serum, in the PE and control groups.

Methods: Cord blood and maternal serum neopterin levels were assessed in 21 patients with PE, and in 27 control subjects. Neopterin concentrations were measured by high performance liquid chromatography.
Results: Cord blood neopterin concentrations were significantly increased in patients with PE compared to controls (54.3 ± 16.8 vs. 43.4 ± 8.5 nmol/L, p = 0.011, respectively). Maternal serum neopterin levels (257.3 ± 36.8 vs. 150.9 ± 33.8 nmol/L, p < 0.001) were also higher in patients with PE.
Conclusion: Cord blood and maternal serum neopterin levels are higher in patients with PE. Maternal serum neopterin concentrations used may be used as a marker in the early diagnosis of PE.

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Assessment of status in pregnant women and newborns in South Spain.
T. Arrobas Velilla[1], C. Miguel Angel[2], M. Sánchez Mateos[2], A. Barco Sanchez[1], R. Perea[2], E. Pascual Salvador[2], M. Perea Brenes[2], E. Marina Tascón[3], J. Limon Padilla[2], R. Albusac Aguilar[2], C. Gonzalez Martin[1], F. Fabiani Romero[1], C. Gonzalez Martin[1]. *[1]Hospital Universitario Virgen Macarena, Sevilla, Spain, [2]Hospital Rio Tinto, Huelva, Spain, [3]Hospital Rio Tinto, Hielva, Spain,*

Iodine is an essential trace element required by humans. These requirements vary throughout life, being especially important during childhood. Major consequences of iodine impairment are observed on mental development in newborns. According to the WHO, urine iodine measurement (ioduria) can be used to assess iodine nutritional impairment. Studies carried out in Spain and Europe about iodine impairment in pregnant women show iodine deficiency in most of them with secondary hypothyroxinemia
Aim: To assess iodine nutritional status in the Huelva-Andevalo sanitary district,thus, Ioduria and thyroid function biochemical markers in pregnant women at the moment of birth and TSH concentration in their newborns were determined.
Patients and Methods: This study was carried out by both the Congenital Hypothyrodism Unit of the Clinical Biochemistry Department of the Virgen Macarena University Hospital (Seville) and the Gynecology and Clinical Analysis Units of the Rio Tinto hospital (Huelva) during 2 years. Finally, 314 patients and their newborns were studied, including those with normal pregnancy, with or without iodine supplement during pregnancy and absence of thyroid pathologies. With previous informed consent, blood was withdrawn for TSH and free T4 determination and urine samples were obtained. Ioduria was analysed by high resolution liquid chromatography. Neonatal TSH was measured by time-resolved immunofluorometry with an Autodelfia (Perkin Elmer, MA, USA) analyzer in dry blood samples on filter paper. TSH and free T4 determination were done by electrochemoluminiscence. Results were analyzed with SPSS v13.0 for Windows. Variable adjustment analysis for normal distribution, T-Student or U-Mann Whitney univariate analysis (parametric or not) and Spearman correlation tests were carried out.
Results: Ioduria values didn't show a normal distribution and most of them were lower than 200µg/L. Median value was 137,51µg/L, meaning that more than 50% of pregnant women have an insufficient iodine intake, according to the WHO guidelines. TSH and free T4 median values in pregnant women and TSH concentration in newborns are within ranges of normality.
Significant correlation was not found between neonatal TSH levels and ioduria (p=0,024), although a trend towards negative correlation was observed between TSH values > 5mUI/L and low urine iodine concentration, neither between ioduria and maternal TSH and free T4 concentration. Prevalence of children with TSH > 5mUI/L was 19%, which corresponds to a slight iodine deficiency and slight endemia. Moreover, 25% of pregnant women showed TSH concentration > 3mUI/L which exceed pathologic values during pregnancy, according to the Endocrine Society's Clinical Guidelines.
Conclusions: Pregnant women in South Spain show slight Iodine impairment measured in urine, neonatal TSH concentration and maternal TSH levels.

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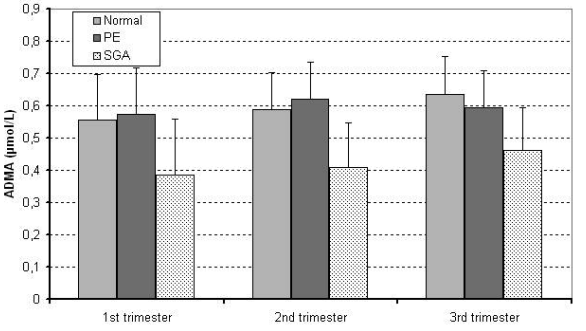
Measurement of asymmetric dimethylarginine (ADMA), throughout normal pregnancy and in pregnancies that developed preeclampsia or had a small for gestational age baby
D. Rizos[1], E. Batakis[2], M. Rizou[2], A. Haliassos[3], M. Eleftheriades[4], D. Hassiakos[1], D. Botsis[1]. *[1]2nd Dept of Obstetrics & Gynecology, Medical School, University of Athens "Aretaieion" Hospital, Athens, Greece, [2]Hormone Laboratory "Aretaieion" University Hospital, Athens, Greece, [3]"Diamedica", Athens, Greece, [4]"Embryo Care", Athens, Greece,*

Introduction-Aim: Preeclampsia is a systematic disease of pregnancy characterized by hypertension and proteinouria developing after 20th week of pregnancy. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthase. In many studies, elevated ADMA levels have reported in preeclampsia compared to normal pregnancies, while other studies failed to show such difference. The aim of our study was to compare ADMA concentrations in all trimesters of pregnancy between normal pregnancies and pregnancies that developed preeclampsia (hypertension and proteinouria) after 24th week (PE) or had a small for gestational age (SGA) baby.
Patients and Methods: In stored serum samples that have drawn in the first (11.57 to 13.57 weeks), second (20.29 - 23.43 weeks) and third (28.0 - 34.71 weeks) trimester of pregnancy, we retrospectively determined the concentrations of ADMA in 23 normal pregnancies, 11 pregnancies with PE and 7 pregnancies that delivered a SGA baby. The measurement of ADMA was done by the commercial kit: ADMA-ELISA (DLD, Hambugr, Germany).
Results: Mean(±SD) concentrations of ADMA (mirco-mol/L) in normal pregnancies were: 0.55±0.14; 0.58±0.12; 0.63±0.17 in the three trimesters respectively. ADMA concentrations in PE pregnancies (0.57±0.12; 0.62±0.15; 0.59±0.14, in the three trimesters respectively) didn't differ significantly from normal pregnancies in any trimester. On the contrary, women with SGA infants had significantly lower ADMA concentrations in each trimester compared to normal pregnancies. (0.39±0.12, P=0,029 1st trim; 0.41±0.11, P=0,002 2nd trim; 0.46±0.13, P=0,016 3rd trim).
Conclusion: ADMA concentration does not change throughout normal pregnancy despite the fact that there is an upward trend as the pregnancy develops. No statistically significant difference in ADMA concentration was noticed in any of the three trimesters between normal pregnancies and PE pregnancies. The most important finding of our study is that pregnancies with SGA infants had significantly lower ADMA levels in all trimesters of pregnancy.



E-139

Multiplex Enzyme Assay in Dried Blood Spots for Newborn Screening of Galactosemia Using UPLC-Tandem Mass Spectrometry
D. Ko[1], S. Jun[1], M. Han[1], S. Song[1], J. Kim[2], J. Song[1]. *[1]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam-si, Korea, Republic of, [2]Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea, Republic of,*

BACKGROUND: Galactosemia is an important inherited metabolic disorder detected by newborn screening tests. Abnormal screening results should be followed by confirmatory tests for the three responsible enzymes - galactokinase (GALK), galactose-1-phosphate uridyltransferase (GALT) and uridine diphosphate galactose-4-epimerase (GALE). We had developed a multiplex enzyme assay for galactosemia using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). In this study, we applied this method for enzyme activity assay using dried blood spots (DBS).
METHODS: Supernatant of rehydrated DBS was incubated with reaction mixture containing [13C2]galactose-1-phosphate and UDP-glucose as substrates for GALT and GALE assay, respectively. GALK activity was measured using DBS directly immersed in reaction mixture and [13C6]galactose was used as a substrate. After overnight incubation, the end products - UDP-[13C2]galactose , UDP-galactose, and [13C6]galactose-1-phosphate - were simultaneously measured using UPLC-MS/MS.
RESULTS: Substrates, products, and internal standards were clearly separated in UPLC-MS/MS system within 10 min. Within-run imprecision were 4.0-13.3%. DBS from 2 patients with reduced GALK activities and 1 patient with reduced GALT activity were also analyzed. Patients' samples showed low enzyme activities compared to those of normal individuals, as expected.
CONCLUSIONS: Multiplex enzyme assay utilizing UPLC-MS/MS can be successfully applied to DBS. Our study provides the possibility of confirmatory tests for galactosemia without additional venipuncture, which is difficult and painful for neonates.

E-140

Prevalence Of Cardiovascular Risk Factors And Insuline Resistance In A Population Of Overweight Children

T. Arrobas Velilla, F. Sanchez Jimenez, A. Barco Sanchez, J. Bermudez Castro, C. Gonzalez Martin, C. Cruz Mengibar, I. Orive de Diego, F. Fabiani Romero. *Hospital Universitario Virgen Macarena, Sevilla, Spain,*

INTRODUCTION: Obesity is a high prevalent public health issue that affects increasingly more young people, including children and adolescents. An elevated body-mass index (BMI) is associated with metabolic disorders that show alteration in biochemical dyslipidemia markers such as increased total cholesterol, LDL cholesterol, triglycerides or decreased HDL cholesterol. The alteration of some others parameters related to diabetes, atherosclerosis or hypertension is also usual. These evidences represent several risk factors for cardiovascular and coronary heart disease that become specially important in young people due to the necessary role of prevention.
OBJECTIVES: To analize the cardiovascular risk associated to a population of children that show overweight.
METHODS: We studied a population of 73 children (46.6% girls, 53.4% boys) with an average age of 12 ±1.5 years old. These patients were going to the pediatrician for obesity, showing an average BMI of 30.87±4.77. None of them had diabetes or hypertension, although 14.4% had familiar history of cardiovascular disease and 11.6% were previously diagnosed of dyslipemia. We measured lipids and cardiovascular risk parameters: Total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), tryglicerides (TG), Lipoprotein(a) (Lp(a), Apolipoprotein B (Apo B), Fibrinogen (Fibr), C-Reactive Protein (CRP) and Homocystein (Hcys). We calculated the Homeostasis Model Assessment (HOMA) index by using the glucose and insulin serum levels.
RESULTS: We found a high prevalency of altered parameters; more significantly,CT> 170 mg/dL = 39.72% LDL-C>100 mg/dL (43.83%), HDL-C <45 mg/dL (53,42%), TG>75mg/dL (61.64%), Lp(a) >30 mg/dL (27%), Fibr>350 mg/dL (19%); CRP>3 mg/L (41.09%). The average HOMA index was 3.96 ±2,13, that showed an increased insulin resistance in our population, which is for values higher than 3; 16,44% had HOMA<2; 24.65% had HOMA between 2 and 3.99; and 38.5% had HOMA>4.
CONCLUSION: Our population under study shows a high prevalence in emerging cardiovascular risk factors and dyslipemia, in addition to an increased insulin resistance. This state, if prolongated, could cause the patients to develop metabolic syndrome and increased morbimortality in the future.

E-141

Evaluation of the measurement of nucleated red blood cells in neonatal blood with ADVIA 2120

M. Park[1], W. Song[1], T. Sung[2], K. Lee[3]. *[1]Department of Laboratory Medicine, Seoul, Korea, Republic of, [2]Department of Pediatrics, Seoul, Korea, Republic of, [3]Department of Obstetrics and Gynecology, Hallym University College of Medicine, Seoul, Korea, Republic of,*

Background: Nucleated red blood cells (NRBCs) are immature erythrocytes. The current line of thought is that increased NRBC production in the immediate neonatal state primarily reflects hypoxic injury and a direct response to mediators of inflammation in newborns with early-onset neonatal sepsis. So, the accurate, reproducible, and timely reporting of NRBC is an important function of the clinical laboratory. The aim of this study is to evaluate the performances of the ADVIA 2120 Hematology System (Siemens, Deerfield, IL, USA) in the NRBCs measurement and compare them with the conventional microscopic method.
Methods: The study was performed in Kangnam Sacred Heart Hospital from March to September, 2009. 120 neonatal samples were submitted to measure NRBC count. For comparison, 225 blood samplesbut for except neonates were also included. The specimens were analyzed for the NRBC counts both with ADVIA 2120 analyzer and by microscopic method.
Results : Correlations of NRBC counts form the ADVIA 2120 compared with microscopic results were excellent in neonatal and in all specimens, r=0.8630 and r=0.9012, respectively. Sensitivity and specificity for the presence of NRBC was 98.2% and 94.2%, respectively. Concordance rate was 95.5%. There were some discordant results in cases of chronic lymphocytic leukemia and iron deficient anemia.
Conclusions : The ADVIA 2120 NRBC method correlated well with the manual microscopic method. The automatic correction of the WBC count and differential, the shorter turnaround time compared to microscopic counting are additional advantages of automatic method. The automatic NRBC counting is considered to be a useful method in neonatal hematology.
Key Words: NRBC, ADVIA 2120, neonates

E-142

Simultaneous detection of trisomy 21, 18, and 13 using multiplex ligation-dependent probe amplification-based real-time PCR

Q. Guo[1], Y. Zhou[2], X. Wang[1], Q. LI[1]. *[1]XMU, Xiamen, China, [2]Molecular Diagnostics Laboratory, Department of Genetics, Maternal and Children's Health Hospital of Xiamen, Xiamen, China,*

BACKGROUND: Trisomy 21, 18, and 13 account for the majority of chromosomal aneuploidy that needs prenatal diagnosis. Diagnoses of these trisomies mainly rely on karyotype analysis and few molecular methods. However, performance or throughput limitations of these methods currently constrain routine testing.
METHODS: We evaluated a novel copy number detection technology with DNA templates from 144 blinded clinical samples. The three trisomy statuses were simultaneously determined by a multiplex ligation-dependent probe amplification-based multi-color real-time PCR (MLPA/rtPCR), and results were compared with those for karyotype analysis with the same samples.
RESULTS: The MLPA/rtPCR accurately identified all categories of trisomies detected by karyotype analysis, including 32 cases of trisomy 21, 11 cases of trisomy 18, 1 case of trisomy 13 and no false-positive was detected with the 100 normal control samples. This method also detected at least 1.2 times change in chromosome dosage, implying its potential use for noninvasive detection of fetal trisomy status with maternal blood.
CONCLUSIONS: This novel technology can simultaneously categorize three trisomy statuses in a single reaction; accurately detect trisomy including those with mosaicism; and greatly reduce time and cost in comparison to its counterpart methods.

E-143

Rapid and ultra-sensitive time-resolved immunofluorometric assay of human thyroid stimulating hormone using Eu(III) chelate-bonded silica nanoparticles

X. Xia[1], Y. Xu[1], W. Ke[1], Y. Zhou[2], Q. LI[1]. *[1]XMU, Xiamen, China, [2]Xiamen Branch of Fujian Newborn Screening Centre and Xiamen Prenatal Diagnosis Centre, Xiamen Maternal & Children's Health Care Hospital, Xiamen, China,*

Introduction: Screening programs to detect congenital hypothyroidism were initiated about 30 years ago in North America and Europe, and over 10 years ago in China. Recently, measurement of human thyroid stimulating hormone (TSH) has replaced thyroxine as the analyte of choice in most countries because of sufficient sensitivity and fewer false positive test results. However, the currently widely used dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) method is vulnerable to ambient Eu(III) contamination and becomes problematic in detecting analytes of low concentrations.
Methods: We used silica nanoparticle covalently bonded with Eu(III) chelates of bright fluorescence as new type of labels and applied them to a time-resolved immunofluorescence assay of TSH.
Results: The detection limit of the assay calculated according to the 2SD method was 0.0007mU/l, which is lower than the TSH assays of fourth generation. The linear range was from 0.005 to 100 mU/l within which the coefficient of variation was between 1.9% and 8.3%. A head-to-head comparison between this new method and the commercial dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) system by using 204 blood spot samples from newborns resulted in a complete agreement with the correlation coefficient of 0.938. Because no signal development step was needed, the whole procedure of this new assay involved only two steps and could be finished within 2 hours.
Conclusion: The rapid, simple, sensitive, and quantitative nature of this nanoparticle label-based TrIFA makes it a strong candidate for routine use in TSH screening of neonatal hypothyroidism.

E-144

Pediatric Haematological Reference Values for Central Ghana

D. K. Dosoo[1], K. Osei-Kwakye[1], S. Amego-Etego[1], P. Bilson[1], S. Agra[1], H. Abdul[2], S. Apanga[1], E. Kwara[1], R. Owusu[1], K. Asante[1], E. Mahama[1], K. Kayan[1], K. Tchum[1], J. Ocran[2], P. Ayamba[1], K. Koram[2], S. Owusu-Agyei[1]. *[1]Kintampo Health Research Centre, Kintampo, Ghana, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana,*

Reference values, when used with clinical history and physical examination provide useful information for the diagnosis and management of diseases. The process of establishing reference values can be expensive and time consuming. As a result many laboratories use reference values from literature which were established with different populations and methods. The International Federation for Clinical Chemistry (IFCC) recommends that each laboratory establish reference values using the population it intends to serve.

This study was aimed at establishing pediatric hematological reference values for central Ghana.
Methods: Four hundred and seventy-six (476)males and 414 female reference individuals up to the age of 14 years were selected randomly from a Demographic Health Surveillance System database using Visual FoxPro software. Following parental consent, eligibility was determined by a clinician using clinical history and physical examination. Two (2) ml of venous blood was collected into EDTA tubes. Samples were analyzed using the ABX Micros 60 Hematology analyzer (ABX-Horiba, France) for Hemoglobin (Hb), White Cell count (WBC), Platelet (PLT) and Red blood cell count (RBC). The 2.5th and 97.5th percentiles for each parameter was determined using the Visual FoxPro software.
Results: Reference values obtained are shown in the table below:

Gender-specific pediatric hematological reference values

Parameter	Male Reference Value	Female Reverence Value
Hgb, g/dl	8.1 - 13.5	7.8 - 13.3
WBC, x 10^9	4.2 - 15.8	4.1 - 17.1
PLT, x 10^9	109 - 560	115 - 587
RBC, x 10^12	3.23 - 5.46	3.15 - 5.45

Conclusions: Significantly lower values for hemoglobin and higher values for white blood cell counts were obtained for children in central Ghana. It will, therefore, be necessary to establish reference values for other parameters for the area.

E-145

Establishing Pediatric Reference Intervals For 25 Common Analytes On The Vitros® 5600 Integrated System

I. M. Blasutig[1], B. Jung[2], V. Kulasingam[1], S. Baradaran[1], Y. Chen[3], M. Chan[4], D. Colantonio[4], K. Adeli[4]. *[1]University of Toronto, Toronto, ON, Canada, [2]Childre's and Women's Health Centre of British Columbia, Vancouver, BC, Canada, [3]Dr. Everett Chalmers Regional Hospital, Fredericton, NB, Canada, [4]The Hospital for Sick Children, Toronto, ON, Canada,*

Objectives: The aim of the study was to determine age- and sex-specific pediatric reference intervals for 25 chemistries and immunoassays on the VITROS® 5600 Integrated System, as part of the CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) initiative.
Design and Methods: A total of 770 serum/plasma samples from ethnically diverse children attending select outpatient clinics were collected and analyzed for 25 analytes. The results were partitioned based on age and gender into one of ten subgroups (Male/Female; <1 year; ≥1 year to ≤ 5 years; >5 years to ≤ 10 years; >10 years to ≤ 15 years; and >15 years to ≤ 20 years) and used to determine age- and gender-specific reference intervals.
Results: Over 15,000 immuno and chemistry assays were performed on collected samples. Samples were assessed for common interferants and samples deemed to be unacceptable were eliminated. Results were also analyzed for outliers by the Dixon method, and all outliers were removed. The remaining data were used to determine reference intervals according to the CLSI/IFCC C28-A3 guidelines. In total, 250 pediatric reference intervals were determined. The reference intervals for four assays utilizing Microtip technology are shown in Figure 1.
Conclusion: These pediatric reference intervals are valuable to all VITROS users as well as all laboratories measuring these analytes, once they validate the acceptability of transference to their laboratory.

Figure 1. Reference intervals for MicroTip™ assays

	AAT (g/L)				PALB (mg/L)			
Age (yrs)	Female	*n*	Male	*n*	Age (yrs)	Female	*n*	Male	*n*
<1	0.73-1.56	45	0.79-1.70	45	<1	108-276	45	101-243	47
≥1 – ≤5	0.92-2.25	54	0.94-1.75	61	≥1 – ≤5	87-267	54	99-271	69
>5 – ≤10	0.95-1.83	63	1.02-1.78	51	>5 – ≤10	95-300	71	95-291	63
>10 – ≤15	0.91-1.60	70	0.98-2.15	71	>10 – ≤15	127-321	101	127-348	78
>15 – ≤20	0.98-1.97	35	1.04-1.52	29	>15 – ≤20	136-363	72	101-328	35
	hsCRP (mg/L)				TRFRN (g/L)			
Age (yrs)	Female	*n*	Male	*n*	Age (yrs)	Female	*n*	Male	*n*
<1	<0.1*-3.8	45	<0.1*-0.7	44	<1	1.34-2.98	46	1.43-3.24	47
≥1 – ≤5	<0.1*-3.7	55	<0.1*-5.1	64	≥1 – ≤5	1.47-3.47	56	1.57-3.48	71
>5 – ≤10	<0.1*-1.5	61	<0.1*-4.4	58	>5 – ≤10	1.55-3.07	68	1.64-3.05	62
>10 – ≤15	<0.1*-2.9	102	<0.1*-9.0	72	>10 – ≤15	1.90-3.34	97	1.70-3.11	78
>15 – ≤20	<0.1*-1.6	54	<0.1*-4.7	34	>15 – ≤20	1.91-3.58	70	1.37-2.79	35

* These values represent the Limit of Detection for this assay

AAT (α1-antitrypsin), hsCRP (high sensitivity C-reactive protein), PALB (prealbumin/transthyretin), and TRFRN (Transferrin)

E-146

Evaluation of an In-Line Monitor for Electrolyte and Blood Gas Measurements in Neonates in the Intensive Care Nursery

A. M. Ferguson, D. Burford, U. Garg. *Children's Mercy Hospitals and Clinics, Kansas Ctiy, MO,*

Background: Frequent blood draws for laboratory testing is one of the major causes of anemia in critically ill neonates, often resulting in the need for transfusion. The VIA LVM ® (VIA Medical, San Diego, CA) is an in-line ex vivo monitor capable of measuring pH, blood gases, electrolytes and hematocrit at the patient's bedside and requires a sample volume of 1.5 mL. After analysis, the sample is infused back into the patient. Not only does the use of this monitor reduce the amount of blood loss in the patient, it can potentially improve patient care by decreasing the turnaround time of laboratory results and reducing preanalytic errors and opportunities for infection. An extensive analysis and method comparison of the VIA monitor was done prior to its introduction to the intensive care nursery. This study is a follow up to examine the clinical performance of the monitor in use.
Objective: To assess the bias and precision of the VIA LVM monitor in the intensive care nursery in comparison to laboratory analyzers.
Method: Paired samples (n=43) from 5 patients were analyzed for pH, PCO_2, PO_2, sodium, potassium, and hematocrit using the VIA LVM monitor and ABL 825 blood gas analyzer (Radiometer America, Inc, Westlake, OH). In six samples, sodium and potassium was measured using a Vitros 5,1 FS (Ortho Clinical Diagnostics, Rochester, NY). 18 samples had no sodium or potassium measurement for comparison, and 9 samples had no hematocrit comparison.
Results: Bias (the mean difference between the VIA and laboratory analyzers) and ranges of measurements were as follows:

	Bias ± SD	Range
pH	0.02 ± 0.02	7.08-7.44
PCO_2	3.8 ± 3.6 mm Hg	35.9-80 mm Hg
PO_2	8.8 ± 11.8 mm Hg	34-171 mm Hg
Sodium	4 ± 2 mmol/L	133-154 mmol/L
Potassium	0.2 ± 0.2 mmol/L	2.9-7 mmol/L
Hematocrit	1.5 ± 2.4%	30-50%

Conclusions: The VIA monitor compared quite well with the ABL, with all parameters examined except sodium and PO_2 within acceptable allowances. The VIA monitor exhibited a positive bias in sodium measurements when compared to both the ABL and Vitros.

E-147

Validation of lamellar body counts (LBC) using three hematology analyzers

C. M. Lockwood[1], J. C. Crompton[2], D. J. Dietzen[1], D. G. Grenache[3], A. M. Gronowski[1]. *[1]Washington University School of Medicine, St. Louis, MO, [2]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [3]University of Utah Health Sciences Center, Salt Lake City, UT,*

Background: Respiratory distress syndrome of the newborn is the seventh cause of newborn mortality in the US. Decisions regarding obstetric management rely partially on results of fetal lung maturity (FLM) tests. The lamellar body count (LBC) allows the rapid enumeration of lamellar bodies in amniotic fluid using the platelet channel of an automated hematology analyzer. Numerous outcome studies have established the clinical utility of LBCs for predicting FLM. The LBC represents an alternative method to the widely used TDx-FLM II fluorescence polarization assay that is planned to be discontinued. However, the LBC must be thoroughly validated prior to implementation.
Objective: Our objective was to validate the LBC on three hematology analyzers.
Methods: Residual amniotic fluid samples submitted for physician-ordered FLM testing were utilized. IRB approval was obtained for this study. Samples were stored at 4°C for up to 12 days before performing the LBC. Lamellar body stability was assessed in 4 samples with immature or mature TDx-FLM II results that were stored at 4°C for 20-33 days and tested on 6-9 separate occasions. TDx-FLM II analysis was performed on a TDx-FLx analyzer (Abbott Laboratories; Barnes-Jewish Hospital, St. Louis, MO). The LBC was performed using the platelet channel of a Coulter LH750 (Beckman Coulter; Barnes-Jewish Hospital, St. Louis, MO), Coulter AcT diff2 (Beckman Coulter; ARUP Laboratories, Salt Lake City, UT), and Sysmex XE-2100 (Sysmex; St. Louis Children's Hospital, St. Louis, MO).
Results: Intra-assay CVs for all methods ranged from 1.7 - 21.8% with inter-assay CVs of 1.3 - 7.1%. All analyzers demonstrated excellent linearity (linear regression equations: Coulter LH750, y = 1.027x - 0.821, r^2 = 0.997; Sysmex XE-2100, y = 0.981x - 0.446,

$r^2 = 0.999$; Coulter AcTdiff2, y = 1.061x - 1.628, $r^2 = 0.996$) in the concentration range from 1,000 to 80,000 counts/µL. In stability studies, the Coulter AcT diff2 analyzer had the largest deviation from initial LBC with a CV of 15% at an LBC of 20,000 counts/µL. Across all instruments, a single freeze-thaw cycle caused a mean (SD) percent LBC decrease of 31 (12.6%) with a range of 3-57%. The addition of whole blood to amniotic fluid pools had no effect on the LBC until the mean red blood cell count was $\geq 0.05 \times 10^{12}$/L (56,000/uL), at which point the LBC was decreased. The addition of meconium to amniotic fluid pools caused the LBC to increase dramatically from baseline values. Using a TDx-FLM II cutoff of ≥55 mg/g and LBC cutoff of ≥50,000/µL to indicate lung maturity, concordance between the TDx-FLM II and the LBC on all instruments was, expectedly, poor (<80%). Concordance between the three hematology analyzers was excellent (≥94% in all cases).

Conclusions: The LBC demonstrates acceptable imprecision and linearity. Only never-frozen amniotic fluid should be used to determine the LBC. The presence of blood or meconium in amniotic fluid can interfere with the LBC and should be avoided. When laboratories are performing in-house validations, they should not correlate LBC with TDx FLM II results without outcome data. Correlation with another validated LBC method is preferred.

E-148

Clinical utility of amniotic fluid "shake test", lecithin/sphingomyelin ratio and phosphatidylglycerol for predicting fetal lung maturity

Q. Wu, Y. Zhang, E. K. Torgbe, I. A. Hashim. *University of Texas Southwestern Medical Center, Dallas, TX,*

Background: Laboratory analysis of amniotic fluid for predicting fetal lung maturity include: the "shake test", lecithin/sphingomyelin (L/S) ratio, phosphatidylglycerol (PG), as well as lamellar body count. Although such assays are widely used, some are cumbersome, lack standardization and clear recommendations on their utility.

Objective: The aim of this study is to analyze the clinical utility and accuracy of the "shake test", L/S ratio and PG assays for predicting fetal lung maturity.

Methods: Amniotic fluid samples from 30 patients being investigated for fetal lung maturity were analyzed using the "shake test". Using this test, an amniotic fluid specimen is considered positive for lung maturity if an uninterrupted ring of bubbles were present around the entire meniscus of duplicate test tubes at the 15-minutes read time. A specimen is considered negative if there is an incomplete ring of bubbles or no bubbles at the meniscus. Negative samples were sent to a reference laboratory for L/S ratio and PG determination by thin-layer chromatography. The results of these three assays were compared with clinical review of outcome [i.e. healthy baby with no evidence of respiratory distress (RDS)].

Results: In this study, all newborns showed no evidence of RDS (mature lung) on retrospective clinical review. Using the "shake test", sixteen patients were reported positive (mature lung) compared with fourteen patients reported negative (immature lung). All "shake test" negative samples were positive (mature lung) when analyzed for L/S ratio with the cutoff ratio for maturity set at 2.0. Furthermore, of the fourteen "shake test" negative specimens, six specimens were positive for PG (mature lung) compared with eight specimens negative for PG (immature lung). The specificities of the "shake test" and PG assays are 53.3% and 42.9% respectively. The L/S ratio assay showed a specificity of 100%.

Conclusions: Our findings suggest that confirmation of "shake test" negative specimens by L/S ratio is recommended. The value of additional testing using other biochemical markers for lung maturity such as Amniostat-FLM assay (Irvine Scientific, Santa Ana, CA) and lamellar body counts (Sysmex XE-2100) as well as their comparison with the described "shake test", L/S ratio and PG assays are under investigation.

Acknowledgment: The authors thank Patricia Kutscher, Barbara Slocum, William Beaupre, and our other medical, nursing and laboratory colleagues for their assistance with this study.

E-149

Analytical Validation of an Improved, 35-Minute Automated Chemiluminescent Immunoassay, Standardized to GC-MS, for Unconjugated Estriol on the IMMULITE® 2000 and IMMULITE® 2500 Analyzers from Siemens Healthcare Diagnostics

S. Phagoo, R. Jalakian, B. Bachani, P. Durham, S. Thompson, J. Lei, N. Sahakian, D. Sustarsic. *Siemens Healthcare Diagnostics Inc., Los Angeles, CA,*

Background: Unconjugated estriol present in the maternal circulation is due to secretion of estriol by the fetus and placenta. Maternal serum levels provide a sensitive indicator of fetal maturity and well-being. This study is aimed at validating an improved assay for the quantitative measurement of unconjugated estriol in serum on the IMMULITE 2000 and IMMULITE 2500 Analyzers.

Method: The improved unconjugated estriol assay is designed as a one-step, solid-phase competitive chemiluminescent enzyme immunoassay with a 30-minute incubation time. Estriol in the sample competes with an estriol-alkaline phosphatase conjugate for limited binding sites to a polyclonal antibody highly specific for unconjugated estriol and coated on a polystyrene bead. After incubation, unbound material is removed during a centrifugal wash procedure, substrate is added, and the chemiluminescent signal is measured. The test requires 40 µL of sample and has a reportable range of 0.07-12 ng/mL. The assay calibration was determined by measuring unconjugated estriol in pregnancy serum samples by a gas chromatography-mass spectroscopy (GC-MS) method. Time-to-first-result is 35 minutes. For both the IMMULITE 2000 and the IMMULITE 2500 assays, throughput is up to 200 tests per hour.

Results: Weighted Deming regression of a method comparison to GC-MS results for 58 pregnancy serum samples yielded a slope = 0.98, intercept = 0.02 ng/mL, and correlation coefficient (r) = 0.98. The IMMULITE 2000 unconjugated estriol assay was also compared to the ACCESS Unconjugated Estriol assay, commercially available from Beckman Coulter, on 150 pregnancy serum samples. This produced a slope = 1.01, intercept = -0.01 ng/mL, and r = 0.95 (for the IMMULITE 2500: slope = 0.97, intercept = 0 ng/mL, and r = 0.94). The LoB of the assay was 0.01 ng/mL, the LoD was 0.04 ng/mL, and the functional sensitivity at 20% CV was 0.15 ng/mL. Precision was assessed over 20 days on unconjugated estriol-spiked normal female serum pools using three IMMULITE 2000 kit lots comprising three different reagent and bead lots and one IMMULITE 2500 kit. At 0.3, 1.2, and 10.4 ng/mL, the within-run imprecision for IMMULITE 2000 was 10.2%, 7.2%, and 5.2%, respectively (IMMULITE 2500: 9.7%, 7.5%, and 6.3%). The total imprecision was 13.3%, 9.8%, and 7.3%, respectively (IMMULITE 2500: 12.8%, 9.7%, and 8.0%). Dilutional linearity of six pregnancy serum pools diluted with the zero calibrator matrix yielded mean recoveries of 94%-110%, whereas mean unconjugated estriol recoveries for six spiked normal female serum pools were 81%-102%. The presence of bilirubin, hemoglobin or triglycerides had no effect on the results within the precision of the assay. The assay is highly specific for unconjugated estriol. At 50 ng/mL of each cross-reactant, the cross-reactivity for estriol-3-glucuronide was not detectable; for estriol-3-sulfate, 0.1%; and for estriol-16-glucuronide, 1.3%.

Conclusions: The data demonstrate that the improved IMMULITE 2000 and IMMULITE 2500 unconjugated estriol assays provide standardization to GC-MS, faster time-to-first-result, improved precision at the low end, and a clinically reliable automated method for the routine measurement of unconjugated estriol in pregnancy serum samples.

E-150

Gestational diabetic patients have increased circulating leptin levels correlating with insulin levels

G. Cisneros[1], A. Perez-Perez[1], P. Fernandez-Riejos[1], C. Varone[2], M. Ramirez-Pineda[1], J. Dueñas[1], V. Sanchez-Margalet[1]. *[1]Virgen Macarena University Hospital, Seville, Spain, [2]University of Buenos Aires, Seville, Argentina,*

Objectives: Leptin is a regulatory hormone in many cellular systems, including trophoblastic cells, where it is produced and may function as a trophic factor. In the present work we aimed to study circulating leptin and insulin levels in blood samples from gestational diabetic patients at 24-28 weeks of pregnancy with control blood samples from normal pregnancies .

Materials and Methods: We have studied serum levels of glucose, insulin and leptin in blood samples obtained from 40 pregnant women with gestational diabetes, and 40 control pregnant women obtained at 24-28 weeks of pregnancy. Leptin and insulin levels were determined by ELISA method. Student´s t test was employed to assess statistical significance of differences between both groups. Lineal correlation between insulin and leptin levels was analysed.

Results: We have found that glucose, insulin and leptin levels are significantly increased in gestational diabetic patient. Mean blood glucose levels were 80.7±7.0 mg/dL in control group and 111.0±5.5 mg/dL in gestational diabetic group ($p<0.05$). Mean blodd insulin levels were 5.4±2.0 mU/ml in control group and 12.3±10 mU/ml in gestational diabetic group ($p<0.05$). Mean blood leptin levels were 25.0±14 ng/ml in control group and 54.0±31 ng/ml in gestational diabetic group. Leptin and insulin levels showed a significant correlation ($p<0.05$), r= 0.4.

Conclusions: Circulating leptin levels in gestational diabetic patients are increased. Insulin levels are also increased in these patients as a result of their insulin resistance state. Moreover, circulating leptin levels correlate with insulin levels in pregnant women. Leptin may be a useful marker in the diagnosis and follow-up of gestational diabetes.

E-151

A Panel of Cervicovaginal Fluid Biomarkers Efficiently Predicts Gestational Age at Delivery.

A. Woodworth, E. O'Keeffe, T. A. Scott, E. Bishop, C. Moffett, M. L. McPheeters. *Vanderbilt University School of Medicine, Nashville, TN,*

Background: Preterm birth (PTB) is a syndrome with several etiologies and is thus difficult to predict. Single cervicovaginal fluid (CVF) biomarkers cannot predict PTB (delivery <37 weeks gestation). A biomarker panel representing different etiologies of disease may better predict women who will deliver preterm.
Objective: To determine the diagnostic utility of a CVF biomarker panel to predict PTB.
Methods: 143 residual CVF specimens (127 women) collected for physician-ordered fetal Fibronectin (fFN) analysis were utilized. Alpha-fetoprotein (AFP), DHEAS, Inhibin-A (DIA), Interleukin-6 (IL-6), and human chorionic gonadotropin (hCG) were quantified using the UniCel DxI 800 (Beckman Coulter). Each analyte was validated for use in CVF. Optimal cut-offs, determined by ROC analysis, were used to evaluate each marker's ability to predict PTB, gestational age (GA) at delivery, and time between collection and birth. Biomarker combinations were compared for their ability to predict GA at delivery and time to delivery using adjusted r^2 values. **Results**: Twenty-eight of 143 CVF samples were associated with PTB (24/127 women). The table shows cut-offs and measures of diagnostic utility for each biomarker. The biomarker combination that best predicts GA at delivery and time to delivery contained fFN, IL-6, hCG, and DIA (all dichotomized at optimal cut-offs), adjusted for GA at collection (adjusted r^2=0.25). At the average GA at collection (31.1 weeks), women positive for all 4 biomarkers delivered 5.4 weeks earlier than women negative for all markers.
Conclusions: The diagnostic utilities to predict PTB were similar among DIA, hCG, fFN and IL-6. After adjustment for GA at collection, patients with positive results for any of these delivered sooner than patients negative for the same marker. Combined, the biomarkers more efficiently predicted GA at delivery and time to delivery than any marker alone. A biomarker panel associated with PTB etiologies may efficiently predict delivery in women with labor symptoms.

Diagnostic Utilities for Prediction of Preterm Birth

Statistical Parameter	fFN	IL-6	DIA	HCG	AFP	DHEAS
Cut-Off	>50 ng/mL	>444 pg/mL	>6.15 pg/mL	>63 mIU/mL	>15 ng/mL	< 10.5 ng/mL
Sensitivity [95%CI]	39% [22;59]	25% [11;45]	27% [12;48]	41% [22;61]	25% [11;45]	89% [71;98]
Specificity [95%CI]	92% [86;96]	98% [93;100]	94% [88;98]	88% [80;93]	87% [80;93]	23% [15;31]
Positive Predictive Value [95%CI]	55% [32;77]	78% [40;97]	50% [23;77]	44% [24;65]	32% [14;55]	21% [14;30]
Negative Predictive Value [95%CI]	86% [79;92]	84% [76;90]	85% [77;91]	86% [79;92]	83% [75;89]	23% [15;31]
ΔGest Age(wks) at Birth[a,b] [95% CI]	2.4* [1.5;3.4]	3.7* [2.2;5.1]	2.4* [1.1;3.5]	1.8* [0.8;2.9]	1.0 [0;2.1]	0.6 [0;1.5]
ΔTime to birth(d)[a,b,c] [95%CI]	17* [10;24]	26* [15;36]	16* [7;21]	13* [6;20]	7 [0;15]	3.9 [-3;11]
Likelihood Ratio+	5.0	13.9	4.4	3.4	1.9	1.15
Odds Ratio [95%CI]	7.3* [2.5;21.2]	39.6* [4.5;345]	4.53* [1.2;16.7]	3.81* [1.2;16.7]	2.01 [0.7;6.0]	13.0 [0.7;13.8]

*P < 0.05, [a]Adjusted for Gestational Age at Collection, [b]Difference between Average for Negative and Positive results, [c]Time between specimen collection and birth

E-152

Rubella seroepidemiology and estimations of the catch-up immunization rate and waning immunity in pregnant women in Taiwan

C. Lin, Y. Huang, B. Chen. *Kaohsiung Medical University Kaohsiung, Taiwan, Kaohsiung, Taiwan,*

Background: Rubella vaccine has successfully prevented rubella infections and congenital rubella syndromes (CRS). In 1986, Taiwan implemented a rubella vaccination program, which has eliminated rubella infections effectively.
Methods: We reviewed 43 640 prenatal rubella test results for pregnant women during 2001-2008 from four different areas of Taiwan to investigate rubella seroepidemiology and estimate the rates of catch-up immunization and waning immunity in pregnant women in Taiwan after mass immunization.
Results: The rate of seronegativity was 21.0% in foreign-born women; in indigenous women born before September 1971, between September 1971 and August 1976, and after September 1976, the rates were 20.3, 6.3, and 7.4%, respectively. Women in western Taiwan exhibited lower seronegativity (6.6%) than those in other areas. Rates of seropositive conversion in Taiwan- and foreign-born women were 11.5 and 30.7%, respectively. The rubella antibody titers for vaccinated women in the 1971-1976 and after-1976 birth cohorts declined by -1.4 and -0.5% per year, respectively.
Conclusion: This study demonstrates the high seronegativity of older indigenous and immigrant women, the low catch-up immunization rate, and the low antibody decline in women who had been vaccinated at school age in Taiwan.

E-153

Prevalence of bacteria causing bacteraemia in children under five years in a rural African area and their antimicrobial susceptibility patterns.

A. Agyekum[1], B. Nkrumah[1], S. Ekuban Kobina Acquah[1], Y. Adu Sarkodie[2], T. Agbenyega[2], F. Huenger[3]. *[1]Agogo Presbyterian Hospital, Asante Akim, Ghana, [2]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Kumasi Centre For Collaborative Research In Tropical Medicine, Kumasi, Ghana,*

Background Mortality rates in sub-Saharan Africa, particularly children under 5 years old is estimated to be 100-250 per 1,000 compared with 10-30 per 1,000 in developed countries. One in every six African children dies before the age of five due to infectious diseases. The vast majority of these deaths are not fully investigated. Bloodstream infection is a frequent cause of morbidity and associated with mortality in excess of 25%. In Ghana few studies on bacteraemia have been published. We aimed to prospectively determine the prevalence of bacteria causing bacteraemia in children under five years of age in a rural African area and their antimicrobial susceptibility patterns.
Method Between 1st January 2008 and 31stDecember 2008 we studied children under five years of age with medical cases admitted to the Agogo Presbyterian hospital in the Asante Akim North District. 1-3 mls of venous blood were taken from all children admitted in the ward into commercially produced BD BACTEC™ PEDS PLUS™ culture vials. Blood cultures were transferred to the BACTEC 9050 incubator at Agogo Presbyterian Hospital Laboratory. The blood cultures were incubated for a period of five days. Identification of isolates was done by subculturing, cultural morphology, gram staining, biochemical methods, API from biomerieux and several serological testing. Susceptibility testing was by the Kirby-Bauer disk diffusion method and by measuring Minimum Inhibition Concentration (E-test) using the CLSI guidelines. We documented pathogens identified and their susceptibility patterns.
Results 1356 patients were admitted and had a blood culture taken. 304 (22.4%) had a positive result and 207 (15.3% overall) were considered a genuine pathogen and 97 (7.1%) were contaminants (Coagulase negative *Staphylococci* and other skin organisms). Four organisms accounted for 60.9% of bacteraemias: *Salmonella enterica* (sero group B, 35.2%), *Streptococcus pneumoniae* (10.2%), *Staphylococcus aureus (7.9%) and Salmonella Typhi (*7.6%). The majority of *Salmonella* isolates were resistant to chloramphenicol (82%), ampicillin (90%) and co-trimoxazole (84%), however they were all susceptible to ciprofloxacin and ceftriaxone. Antimicrobial pattern of all *Streptococcus pneumoniae* isolates showed 100% susceptibility to penicillin. Most of the *Klebsiella pneumoniae* (60%) and *Escherichia coli* (50%) produced Extended Spectrum Beta lactamases (ESBLs). Four (16.7%) methicillin resistant *Staphylococcus aureus* (MRSA) isolates were identified.
Conclusion Bacteraemia affects 1 in every 6 child admitted to the Agogo Presbyterian hospital. Dependent on the causing pathogen mortality was very high. Ceftriaxone turned out to be the best choice as empiric antibiotic therapy. Our study underlines the significance of bacteraemia in this population. Further surveillance of the incidences and the susceptibility patterns of the bacterial pathogens is necessary.

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