Abstracts of the Scientific Posters, 2012 AACC Annual Meeting

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SCIENTIFIC POSTER SESSION SCHEDULE

Posters of the accepted abstracts can be viewed in the Exhibit Hall of the Los Angeles Convention Center, on Tuesday, July 17, Wednesday, July 18 and Thursday, July 19. All posters will be posted for two and one half hours. The presenting author will be in attendance during the final hour. Please refer to the Program Guide for a complete schedule of posters.

Below are the topics and their scheduled times.

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Factors Affecting Test Results. . . . . A40 – A75 14
Lipids/Lipoproteins . . . . A78 – A119 26
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2:00pm – 4:30pm

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Tuesday AM, July 17, 2012

Poster Session: 10:00 AM - 12:30 PM

Cardiac Markers

A-01

Immediate effects of radiofrequency catheter ablation for paroxysmal/persistent atrial fibrillation

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Background: Structural remodeling plays an important role in the genesis and maintenance of atrial fibrillation (AF). The precise mechanism and signaling pathways involved in structural remodeling and atrial fibrosis are still unknown. The transforming growth factor-β1 (TGF-β1) pathway and inflammation are involved in the development of atrial fibrosis. Profibrotic signals act on the balance between matrix metalloproteinases and their local tissue inhibitors. Radiofrequency (RF) catheter ablation of pulmonary veins (PVs) has become an effective treatment for patients with AF. Unfortunately, recurrence of AF still remains a prevalent issue after PVs isolation. We aimed to investigate the immediate effects of RF catheter ablation on the serum levels of some cytokines (IL-1β, IL-6, TNF-α and TGF-β1), matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinases-1 (TIMP-1) in patients with paroxysmal/persistent AF and to find predictors for a good outcome in AF post-ablation patients.

Methods: 8 patients (6M/2F, mean age 50.4±19.7 years) with paroxysmal AF and 8 patients (5M/3F, mean age 53.8±9.7 years) with persistent AF were investigated before (baseline) and immediately after RF catheter ablation. The serum levels of IL-1β, IL-6, TNF-α, TGF-β1, MMP-9 and TIMP-1 were measured by ELISA.

Results: IL-6 showed a significant ablation-induced growth (4.18±0.1-fold for paroxysmal AF patients and 5.64±0.4-fold for persistent AF patients). Myocardial inflammatory response correlated with an increase in IL-6 ($p=0.007$). No differences in the IL-1β and TNF-α levels were noted before and immediately after RF catheter ablation between the two groups of patients. In the paroxysmal AF patients, MMP-9, TIMP-1, MMP-9/TIMP-1 and TGF-β1 showed a significant ablation-induced down-regulation (MMP-9: 2.7±0.5-fold, TIMP-1: 1.78±0.2-fold, MMP-9/TIMP-1: 1.27±0.1-fold, TGF-β1: 1.23±0.1-fold). In the persistent group, RF catheter ablation decreased the serum levels of MMP-9, TIMP-1 (1.4±0.1-fold, 1.85±0.2-fold, respectively) and increased MMP-9/TIMP-1 ratio and TGF-β1 serum concentration with a similar percentage (1.5±0.1-fold, 1.41±0.1-fold, respectively). The changes in MMP-9/TIMP-1 ratio significantly correlated with those in the serum TGF-β1 concentration in all patients after RF ablation of AF ($r=0.73$, $p<0.05$).

Conclusions: RF ablation generates a localized myocardial necrosis that might result in a release of inflammatory mediators. RF ablation does not lead immediately to high serum TNF-α suggesting a better tolerance. Nevertheless, elevated IL-6 levels after RF ablation suggest that inflammatory components have a role in the pathogenesis of post-ablation AF. RF ablation of AF creates left atrial tissue damage with a subsequent healing process. MMP-9 and TGF-β1 are key regulators of tissue repair. The post-ablation changes of these markers were significantly different between paroxysmal and persistent AF patients, suggesting that the intensity of the extracellular matrix synthesis and degradation may be related to the burden or type of AF. Our results suggest that the immediate reduction in the MMP-9/TIMP-1 ratio and TGF-β1 serum level after RF ablation seemed to be a marker for a good outcome in AF post-ablation patients. These findings may contribute to the understanding of possible mechanisms for the high recurrence rates observed in patients after RF ablation of AF.

A-02

Comparison of Automated BNP Assays with the Alere TRIAGE BNP POC Device

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Introduction: B-type natriuretic peptide (BNP) is used in the emergency room for differential diagnosis of heart failure. The first clinical assay for BNP (TRIAGE, from Alere [formerly Bio-Site]) was approved by the FDA in 2000 and has been used in most clinical studies for BNP. Several manufacturers have developed automated assays to be equivalent to the TRIAGE device, and all recommend the same 100 pg/mL clinical cutoff established using the TRIAGE method. We performed studies comparing the BNP assay* on the Dimension Vista® system from Siemens with BNP assays from Alere and other manufacturers.

Methods: Patient sample comparison testing was performed on one lot of Vista (Siemens), ADVIA Centaur® (Siemens), TRIAGE (Alere), ACCESS 2 (Beckman), and ARCHITECT (Abbott) BNP reagents for approximately 120 patient samples spanning the range of the various assays. A 5-day precision study in accordance with CLSI EP15-A2 was performed on two lots of Vista, TRIAGE, ADVIA Centaur, and ACCESS 2 BNP reagents using three EDTA plasma pools.

Results: The 95% confidence interval (CI) for slope using Passing-Bablok regression included 1.00 for the comparison of Vista BNP, ADVIA Centaur BNP, and ARCHITECT BNP vs. TRIAGE BNP. The 95% CI for slope for ACCESS 2 BNP vs. TRIAGE BNP was 1.37-1.53. The intercept was <10 pg/mL for all comparisons. At the cutoff, Vista BNP, ADVIA Centaur BNP, and ARCHITECT BNP had ≥98% negative clinical concordance and 91% positive concordance with TRIAGE BNP. ACCESS BNP had 67% negative concordance and 100% positive concordance with TRIAGE BNP. Precision results are shown in the table.

Conclusions: There are BNP analyte recovery differences between manufacturers. These differences affect clinical concordance at the cutoff.

	Vista		TRIAGE		ACCESS		Centaur		
	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	Lot 1	Lot 2	
Pool 1	N	15	15	15	15	15	15	15	15
	Mean	38	42	48	62	69	72	50	47
	Total %CV	3.4	2.3	9.1	14.4	3.8	4.0	3.5	5.6
Pool 2	N	15	15	14*	15	15	15	15	15
	Mean	103	113	119	146	161	167	119	110
	Total %CV	1.7	2.4	7.2	7.9	2.8	4.0	2.7	3.7
Pool 3	N	15	15	15	15	15	15	15	15
	Mean	461	475	519	606	600	622	448	410
	Total %CV	1.8	2.3	11.4	9.2	3.1	2.2	3.7	3.1

*One result (>10 SD) was removed; when included, mean recovery was 124 pg/mL, with 18.8% total CV.

* Not available for sale in the U.S.

A-04

Receiver Operating Characteristic (ROC) Curve Analysis Of A Novel Index For Coronary Heart Disease Risk Prediction And Diagnosis Of The Metabolic Syndrome

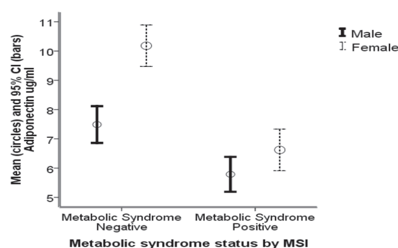
O. A. Mojiminiyi, N. A. Abdella. *Faculty of Medicine, Kuwait University, Kuwait, Kuwait*

Background: There is need to simplify screening tests for metabolic syndrome (MetS) and coronary heart disease (CHD) risk so that high risk patients can be identified early. In routine practice, components of MetS - increased waist circumference (WC), high fasting glucose, low high-density lipoprotein cholesterol (HDL-C), high triglycerides (Tg) and hypertension are assessed separately with presence of three components being diagnostic. We hypothesized that an index (metabolic syndrome index - MSI), computed as WC x Tg/HDL-C would offer a single diagnostic number with high sensitivity and specificity for CHD risk assessment and the diagnosis of MetS.

Methods: Fasting insulin, glucose, adiponectin, high-sensitivity CRP (hs-CRP) and lipid profile were determined in 98 Type 2 diabetic (T2DM) subjects and 521 healthy first degree relatives of T2DM subjects. Clinical and anthropometric data were recorded; subjects were classified with MetS criteria of the International Diabetes Federation. Insulin resistance was assessed with the HOMA-IR. Linear and multivariate (with inclusion of age and gender as confounders) regression analyses were used to determine the associations of MSI with other risk factors, CHD and MetS.

Results: MSI cut off values for MetS were 155 for males and 105 for females. MSI showed significant correlations (r) with age (0.2), WC (0.6), glucose (0.2), Tg (0.9), HDL-C (-0.6), Systolic BP (0.2), Diastolic BP (0.2), adiponectin (-0.4), HOMA-IR (0.4) and hs-CRP (0.4). MSI showed stepwise increase with increasing number of MetS criteria. Binary logistic regression showed that the odds ratio (OR) of MetS and CHD as predicted by MSI were 2.7 (95% CI: 2.1-3.55) and 3.6 (95% CI: 1.9 -5.3) respectively. ROC analysis showed that MSI had significantly higher area under the curve (0.986) compared with WC (0.682), HDL-C (0.790) and Tg (0.955) for detection of MetS.

Conclusions: The MSI offers a single, easily computable risk estimate for CHD and an excellent diagnostic test for MetS.



A-05

Are Advanced Protein Oxidation Products and Total Sulfhydryl Levels Markers of Ischemia as Ischemia-Modified Albumin?

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Background: Acute coronary syndrome (ACS) is an emergency situation caused by destabilization of atheroma plague in patients with atherosclerotic disease. It is characterized by short term total-subtotal occlusion and resolution of coronary artery with resultant disruption of myocardial perfusion with the risk of myocardial infarction and cardiac

death. Pathogenesis of atherosclerotic process is multifactorial and the role of oxidative stress is prominent. In the present study we evaluated the roles of cardiac troponin T (cTnT), myoglobin, mass CK-MB tests with advanced protein oxidation products (AOPP), total sulfhydryl (t-SH) and an ischemia marker, ischemia-modified albumin (IMA) in patients who applied to emergency department with chest pain due to suspected ACS.

Methods: A total of 214 patients (96 women and 118 male) who applied to emergency department of our University hospital with chest pain due to suspected ACS were included in the study. Mean age of the patients was 60.0 ± 17.8 years. Cardiac markers were studied with the method of ECLIA in Roche Elecsys 2010. Serum IMA, AOPP and t-SH levels were

measured with spectrophotometric methods.

Results: Serum cTnT, myoglobin and mass CK-MB levels of the patients were 0.30 ± 1.77 ng/ml, 153.09 ± 294.05 ng/ml and 6.79 ± 16.62 ng/ml; and IMA, AOPP and t-SH levels were 103.07 ± 66.61 U/ml, 38.53 ± 31.96 µmol/l and 902.02 ± 631.89 µmol/l, respectively. Levels of cardiac markers were not significant related with IMA, AOPP and t-SH levels. However, IMA levels correlated significantly with AOPP and t-SH levels (r=0.82, p<0.00001, r=0.78, p<0.00001, respectively). Furthermore, there was a significant correlation between AOPP and t-SH levels (r=0.74, p<0.00001).

Conclusions: Ischemia-modified albumin (IMA) is a marker that starts to rise at the initial 5-10 min of ischemia development and keeps on rising during myocardial ischemia, skeletal muscle ischemia, pulmonary emboli and stroke. The finding of a significant relation of IMA levels with t-SH and AOPP in the present study suggested that AOPP and t-SH

measurements, might also be helpful in evaluation of presence of ischemia in ACS.

A-06

Analysis of plasmatic Lp(a) levels in general population groups of the British Isles

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Background. Cardiovascular disease (CVD), and more specifically myocardial infarction (MI), remains a leading cause of morbidity and mortality despite the targeting of LDL cholesterol via statin therapy. There is a need to identify causal risk factors beyond traditional LDL measurement. Lipoprotein (a) (Lp(a)) is a low density lipoprotein (LDL) particle containing apolipoprotein (a). Apolipoprotein (a) may contribute to blood clot formation, and can help bind LDL particles to artery walls (increasing plaque formation and the narrowing and hardening of arteries). This dual action may explain the role of Lp(a) in the promotion of cardiovascular disease. Lp(a) levels are genetically determined and will remain fairly constant throughout life. Unlike other lipoproteins, the level of Lp(a) is not affected by diet, exercise, and other lifestyle modifications used to lower lipid levels. Large scale studies and international guidelines published recently, have proven that Lp(a) is a major independent genetic risk factor for premature CVD and should be screened in all patients at moderate to high risk. There is a robust and specific association between elevated Lp(a) levels and increased risk of CVD/coronary heart disease.

Relevance. This study reports the evaluation of the levels of Lp(a) in plasma samples from normal population groups in the British Isles to investigate the proportion of individuals presenting levels that could indicate a possible risk factor in the development of CVD/coronary heart disease.

Methods. The levels of Lp(a) were measured in a total of 2,926 plasma samples from general population groups from Ireland (n= 1005), Scotland (n=940) and Wales (n=981). The measurements were performed with a liquid ready-to-use immunoturbidimetric assay (Randox Laboratories Ltd., Crumlin, UK) on a Beckman AU640 analyser (Beckman Coulter Ltd., High Wycombe, UK).

Results. Concentrations between 50 and 100mg/dl were found in 7.86% of the samples from Ireland, 8.83% from Scotland and 9.38% from Wales. Values exceeding 100mg/dl occurred in 1.09% of the samples from Ireland, 1.49% from Scotland and 1.33% from Wales. In the three groups a fifth of individuals had Lp(a) levels above 30mg/dl: 21% in Ireland, 21.81% in Scotland and 22.32% in Wales.

Conclusion. The data shows that approximately 10% of the general population groups from Ireland, Scotland and Wales of this study present Lp(a) levels that could indicate a possible risk of CVD/coronary heart disease, which could be beneficial for clinical awareness.

A-07

Serum Methylmalonic Acid and Holotranscobalamin-II as Markers for Vitamin B12 Deficiency in End-Stage Renal Disease Patients

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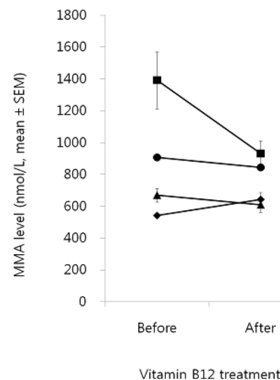
Background: Vitamin replacement, particularly B vitamins, remains an important concern in end-stage renal disease (ESRD) patients undergoing chronic hemodialysis. Serum markers such as methylmalonic acid (MMA) and holoTranscobalamin (holoTC) used to detect vitamin B12 deficiency are affected by impaired renal function making interpretation of these biomarkers difficult in ESRD patients. We investigated the role renal failure has on MMA and holoTC concentrations, and developed a model using MMA and/or holoTC to identify B12 deficient patients.

Materials and Methods: We evaluated the utility of serum MMA and holoTC for detection of vitamin B12 deficiency in dialysis patients (n=17), using the reduction of MMA concentrations as a marker of the response to vitamin B12 treatment (1 mg, intramuscular injections once per month for 3 months). Nerve conduction studies (NCS) were done before and after vitamin B12 treatments to evaluate for any alteration in peripheral sensorimotor nerve function within the cohort.

Results: Receiver operating characteristic curves for detection of vitamin B12 deficiency in dialysis patients showed serum MMA concentrations had the greatest predictive potential (area under the curve = 0.792, p = 0.043) with an optimal cutoff of 750 nmol/L. Dialysis patients with pre-MMA > 750 nmol/L and pre-HoloTC < 260 pmol/L showed a significant response to the vitamin B12 treatment (a mean MMA reduction of 461 nmol/L before and after B12 supplementation; p = 0.006).

Conclusions: MMA and holoTC are viable markers of B12 deficiency in ESRD patients. Future studies on MMA and B12 should be done to confirm these findings in larger cohorts, and to identify individuals who may benefit from vitamin B12 supplementation.

Figure 1. Effect of the intramuscular vitamin B12 injection in the serum MMA (mean ± SEM) of dialysis patients (n=17). The MMA concentrations before vitamin B12 supplementation manifested a significant decrease (a mean MMA reduction of 461 nmol/L; $p = 0.006$, paired t-test) in the dialysis patients with initial MMA > 750 nmol/L and holoTC < 260 pmol/L (■, n = 10). In the dialysis patients with the other range [initial MMA > 750 nmol/L and holoTC > 260 pmol/L (●, n=2) , MMA < 750 nmol/L and holoTC < 260 pmol/L (▲, n=3) and MMA < 750 nmol/L and holoTC > 260 pmol/L (♦, n=2)], the MMA levels between before and after vitamin B12 administration did not showed a significant change.
The cutoff value of MMA = 750 nmol/L is obtained from the best point to predict the vitamin B12 treatment response (more than 18% of MMA level decreased) in the ROC curve.



A-08

Brain natriuretic peptide does not improve the diagnostic performance of highly-sensitive troponin for the early diagnosis of myocardial infarction.

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Background: Recent epidemiological data attests that acute myocardial infarction (AMI) is still the leading cause of death and disability in Western Countries. The huge number of patients admitted with chest pain to the emergency department (ED) is hence causing serious problems of overcrowding, which are also associated with increasing consumption of human and economical resources, so that the introduction of early, sensitive and specific biomarkers of myocardial injury might be globally valuable for decreasing healthcare expenditure and contextually improving patient outcomes. The recent introduction of highly-sensitive (HS) troponin immunoassays has been a major breakthrough in AMI diagnostics, but their assessment does not allow to achieve optimal specificity in the diagnostic approach of chest pain patients acutely admitted to the ED. Several biomarkers have been thereby proposed in combination with HS-troponin testing to improve its diagnostic effectiveness including brain natriuretic peptide (BNP), which is a reliable marker of myocardial stress.

Methods: The aim of this study was to assess whether the measurement of BNP might increase the diagnostic performance of Beckman Coulter prototype HS-Troponin I (HS-AccuTnI, Beckman Coulter Inc., Brea CA, USA) at ED admission. HS-AccuTnI and BNP (Triage BNP reagents, ACCESS Immunoassay Systems, Beckman Coulter Inc) were assessed on 57 consecutive patients presenting at the ED of the Academic Hospital of Parma with chest pain, within 3 h from onset of symptoms. The 99th percentile reference limit and limit of detection of HS-AccuTnI are 8.6 ng/L and 2.1 ng/L, respectively. The diagnostic performance of the two tests, as well as that of their combination, was assessed by Receiver Operating Characteristic (ROC) curve analysis. Statistical analysis was performed with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK).

Results: Nine out of the fifty seven patients (i.e., 16%) had a final diagnosis of AMI at ED admission. The area under the curve (AUC) in ROC curve analysis was 0.89 (95% CI, 0.78-1.00; p<0.01) for HS-AccuTnI and 0.73 (95%CI, 0.53-0.92; p=0.01) for BNP, respectively. The most efficient diagnostic cut-off were identified at 18 ng/L (100 sensitivity and 65% specificity) for HS-AccuTnI and 213 ng/L for BNP (56% sensitivity and 94% specificity), respectively. The combination of the two biomarkers using the optimal cut-offs previously identified yielded an AUC of 0.89 (95% CI, 0.81-0.97; p<0.01), which was not different from that of HS-AccuTnI alone (p=0.95).

Conclusions: The results of this study show that HS-AccuTnI has a good diagnostic performance and a diagnostic algorithm based on the combination of HS-troponin and BNP is not superior to the assessment of the former biomarker alone. These findings, however, does not rule out the hypothesis that BNP testing may still be helpful for gathering useful information about the short and medium term prognosis of patients with AMI.

A-09

Kinetic study of new biomarkers and their usefulness in monitoring and development of heart failure.

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Background and objectives: There are different neurohormones and mediators activated in heart failure (HF). B-type natriuretic peptides are used in clinical practice, but have drawbacks as their variability intra-and inter-individual. The aim of this study is to determine the concentration of new cardiovascular biomarkers (MR-proANP, CT-endothelin-1 and MR-proADM) in patients with unstable and stable HF, compared to a control group. Also, compare their kinetic variability.

Patients and methods

Group I: patients admitted to the cardiology department for unstable HF after attending emergency, Group II: outpatient diagnosed with stable HF (no previous admission) and control group of patients without heart disease, excluded by doppler echocardiography.

Results: Baseline and final mean values of biomarkers in the three groups and p (Wilcoxon test): 1) MR-proANP: unstable HF(425.79 to 228 pmol / L) (p = 0.018), stable HF(269, 71 to 256.72 pmol / L) (p = 0.612) and control group (71.82 to 69.24 pmol / L) (p = 0.401), 2) CT pro-ET1: unstable HF(142.27-81 , 11 pmol / L) (p = 0.018), stable HF (89.18 -74.86 pmol / L) (p = 0.735) and control group (59.08 to 55.38 pmol / L) (p = 0.889) 3) MR-proADM: unstable HF (1.38 to 0.96 nmol / L) (p = 0.028), stable HF (0.96 to 0.74 pmol / L) (p = 0.176) and control group (from 0.56 to 0.56 nmol / L) (p = 0.779).

Conclusions: The analysis showed differences between baseline and final mean values of biomarkers only in unstable HF groups. We observed a correlation between functional class of HF and levels of MR-proANP, CT-pro-ET-1 and MR-pro-ADM with low intra-inter individual variability within each group. Therefore all of them could be used as good markers in monitoring and evolution of heart failure.

A-10

What is the diagnostic accuracy of highly sensitive troponin assays in the emergency room population?

P. O. Collinson[1], D. Gaze[1], P. Thokala[2], S. Goodacre[2]. *[1]St George's Hospital, London, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom*

Objective: To examine the diagnostic accuracy of highly sensitive troponin assays in the unselected emergency room population.

Methods: The study was a sub study of the point of care arm of the RATPAC trial (Randomised Assessment of Treatment using Panel Assay of Cardiac markers), set in the emergency departments of six hospitals. Prospective admissions with chest pain and a non-diagnostic electrocardiogram were randomised to point of care assessment or conventional management. Blood samples were taken on admission and 90 minutes from admission and the serum stored frozen until subsequent analysis. Samples were analysed for high sensitivity cardiac troponin I (cTnI) by 3 methods, by the Stratus CS (CS) (Siemens Healthcare Diagnostics), range 0.03 to 50 µg/L, 10% CV 0.06µg/L, 99th percentile 0.07 µg/L. the Beckman AccuTnI enhanced (B) (Access 2 , Beckman-Coulter) range 1 - 100,000 ng/L, 10% CV 30 ng/L, 99th percentile 40 ng/L, the Siemens Ultra (S) (ADVIA Centaur, Siemens Healthcare Diagnostics), range.6 - 50,000 ng/L, 10% CV 30 ng/L 99th percentile 40 ng/L and cardiac troponin T (cTnT) by the Roche high sensitivity cardiac troponin T assay hs-cTnT (Elecsys 2010, Roche diagnostics), range 3 - 10,000ng/L, 10% CV 13ng/L, 99th percentile 14 ng/L. Diagnosis was based on the universal definition of myocardial infarction utilising laboratory measurements of cardiac troponin performed at the participating sites together with measurements performed in a core laboratory Diagnostic accuracy was compared by construction of receiver operator characteristic curves and by calculation of sensitivity and specificity using the 99th percentile for each assay as diagnostic discriminant.

Results: 66 patients had a final diagnosis of myocardial infarction Admission samples were available from 838/1132 patients enrolled in the study. Areas under the curve were as follows (confidence intervals in parentheses) cTnI CS 0.94 (0.90 - 0.98), cTnI B 0.92 (0.88 - 0.96), cTnI S 0.90 (0.85 - 0.95), cTnT 0.92 (0.88 - 0.96). There were no statistically significant differences between the areas under the curve or diagnostic categorisation. Admission sensitivity was from 0.667 (0.540-0.778) to 0.803 (0.687-0.891). Measurement at admission and 90 minutes had a sensitivity of 0.779 (0.662-0.871) to 0.955 (0.875-0.991). Elevation of troponin in the non-ACS population

occurred in 7 /782 (CS) 15/782 (B) 31/782 (S) and 43/782 (cTnT).

Conclusions: The measurement of high sensitivity cardiac troponin is the best single marker in patients presenting with chest pain in the emergency department. All methods performed with equivalent diagnostic accuracy but differed in the rate of troponin elevation in the non ACS population. Admission measurement alone is insufficiently sensitive for rule out, as is 90 minute measurement. The optimal timing for measurement of cardiac troponin remains to be defined.

A-11

The diagnostic accuracy of novel biomarkers of myocardial injury in the unselected emergency room population.

P. O. Collinson[1], D. Gaze[1], P. Thokala[2], S. Goodacre[2]. *[1]St George's Hospital, London, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom*

Objective: Assessment of the diagnostic accuracy of novel biomarkers of myocardial injury compared to highly sensitive troponin assays for the diagnosis of myocardial infarction using the universal definition of myocardial infarction.

Methods: The study was a sub study of the point of care arm of the RATPAC trial (Randomised Assessment of Treatment using Panel Assay of Cardiac markers), set in the emergency departments of six hospitals. Prospective admissions with chest pain and a non-diagnostic electrocardiogram were randomised to point of care assessment or conventional management. Blood samples were taken on admission and 90 minutes from admission and the serum stored frozen until subsequent analysis.

Samples were analysed for high sensitivity cardiac troponin I (cTnI) by the Stratus CS (Siemens Healthcare Diagnostics), range 0.03 to 50 µg/L, 10% CV 0.06µg/L, 99th percentile 0.07 µg/L for cardiac troponin T (cTnT) by the Roche high sensitivity cardiac troponin T assay, hs-cTnT (Elecsys 2010, Roche diagnostics), range 3 - 10,000ng/L, 10% CV 13ng/L , 99th percentile 14 ng/L, for heart fatty acid binding protein (hFABP) by the Evidence investigator (Randox laboratories), range 1.5 - 100 mg/L CV 7.5-9.8 % 2.5 g/L. 95th percentile 2.5 ng/L 99th percentile 3.0 mg/L and copeptin using the Brahms Kryptor compact system (B·R·A·H·M·S ThermoFisher) range 4.8-500 pmol[[Unsupported Character - ∕]]L CV of 12-17% at 12-20 pmol/L,6-12%, 97.5th percentile 17.4 pmol/L.

The universal definition of myocardial infarction utilising laboratory measurements of cardiac troponin performed at the participating sites together with measurements performed in a core laboratory was used for diagnosis. Test accuracy was compared by construction of receiver operator characteristic curves and the use of pre-specified diagnostic thresholds.

Results: 66 patients had a final diagnosis of myocardial infarction. Admission samples were available from 838/1132 patients enrolled in the study. Areas under the curve were as follows (confidence intervals in parentheses) cTnI CS 0.94 (0.90 - 0.98), cTnT 0.92 (0.88 - 0.96), FABP 1 0.84 (0.77 - 0.90) copeptin 0.62 (0.57 - 0.68).

Both hFABP and copeptin were diagnostically inferior to troponin. The combination of hFABP(at the 95th percentile) and either troponin (at the 99th percentile) increased diagnostic sensitivity, cTnI CS 0.794 (0.673-0.885) to cTnI CS + hFABP 0.921 (0.824-0.974), cTnT 0.778 (0.655-0.873) to cTnT + hFABP 0.857 (0.746-0.933) with a small loss in specificity, cTnI CS from 0.980 (0.967-0.989) to 0.923 (0.901-0.941) cTnT from 0.962 (0.946-0.975) to 0.916 (0.894-0.935). Addition of Copeptin (from an optimised decision level) increased sensitivity for cTnI CS to 0.905 (0.804-0.964) but reduced specificity to 0.591 (0.555-0.626) and for cTnT to 0.841 (0.727-0.921) but reduced specificity to 0.596 (0.561-0.631).

Conclusions: Additional measurement of copeptin is not useful in the chest pain population. Simultaneous measurement of hFABP improves sensitivity.

A-12

Development of a Highly Sensitive Immunoassay for Cardiac Troponin I for the ARCHITECT® i2000SR and i1000SR Analyzers

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Introduction: Troponin I is a 24 Kd modulatory protein that is part of the troponin I-C-T complex which serves to regulate actin and myosin filament interactions. These Ca++ dependent interactions are responsible for the regulation of cardiac muscle contractions. Cardiac troponin I is approximately 60% homologous with the skeletal muscle troponin I isoform, differing at the N-terminus where the cardiac isoform has additional amino acids. Troponin I is measured in the blood as an indication of cardiac or microvascular damage. The ARCHITECT *STAT* High Sensitive Troponin-I (hsTnI) assay is a two-step, double-monoclonal CMIA immunoassay in development that can determine the presence of cTnI in human serum and plasma at concentrations a magnitude lower than the previous generation assay. Two significant design differences between the new ARCHITECT *STAT* hsTnI assay and the current ARCHITECT *STAT* TnI (List #2K41 - predicate device) assay are sample volume and antibody clone construction. The ARCHITECT *STAT* hsTnI assay is designed to have a calibration curve spanning 0 - 50,000 pg/mL (ng/L). It was previously demonstrated in the research phase that the ARCHITECT *STAT* hsTnI assay had good precision (< 10% total CV) and sensitivity (< 10 pg/mL LoQ) and that it was robust to human anti-mouse antibodies (HAMA) and rheumatoid factor (RF) interference.

Objective: In the development phase, factory performance of a number of key performance measurements was confirmed and a 99th percentile cutoff was established from a healthy population.

Methods: Total precision, limit of blank (LoB), limit of detection (LoD), limit of quantitation (LoQ) and linearity were determined following guidance from CLSI documents EP5-A2 , EP17-A, and EP6-A, respectively. Additionally, a large expected values (healthy population) study was conducted on 1402 patients that exhibited normal levels of blood creatinine, BNP, and HbA1C. A 20-day precision study was performed including panels and controls ranging from ~10 pg/mL (near the claimed LoQ) to ~ 45,000 pg/mL (within 10% of the highest calibrator). A robust statistical evaluation of linearity was performed including samples ranging from below the LoQ to above the highest calibrator. A multi-reagent lot correlation was performed versus the predicate device using 198 clinical specimens. Additionally, interference from HAMA and RF was assessed.

Results: Precision CVs ranged from 2.4% to 5.5 %, HAMA interference ranged from -5.7% to 2.3 %, RF interference ranged from -4.4% to10.8 %, and the range of linearity was 7 to 63,000 pg/mL. Correlation with the predicate device had a Passing-Bablok slope which ranged from 0.96 to 0.99. Within-platform LoQ ranged from 4.1 to 7.5 pg/mL.

Conclusions: These results demonstrate that the ARCHITECT *STAT* High Sensitive Troponin-I assay is a precise and highly sensitive method for measuring troponin I in human plasma or serum on a high throughput analyzer.

A-13

Post-exercise levels of biomarkers of cardiomyocyte stress improve prediction of adverse outcomes in patients with advanced heart failure

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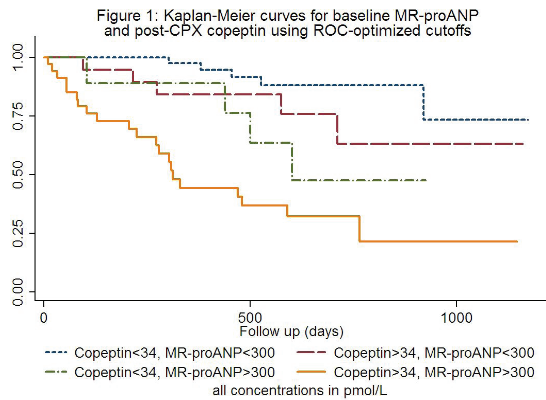
Background: Circulating neurohormones reflect cardiac pressure and volume overload and their baseline levels provide predictive information in heart failure (HF) patients. We hypothesized that their predictive value may be further enhanced by cardiopulmonary exercise (CPX).

Methods: We measured plasma levels of the mid-regional pro-atrial natriuretic peptide (MR-proANP), MR-proadrenomedullin (MR-proADM) and copeptin (all BRAHMS) and of NT-proBNP (Roche) in 108 advanced HF patients (NYHA average 2.73±0.60, 47% ischemic, age 53.7±8.4) undergoing symptom-limited CPX stress testing. Blood samples were collected before and immediately after CPX. We used Cox proportional hazard models to examine the association between biomarker levels and adverse outcomes (death, urgent heart transplant or VAD implantation).

Results: The average follow-up was 17.0±9.8 months; 35 patients experienced adverse outcomes. After adjustment for important clinical covariates including age, gender, BMI, history of PCI, CABG, hypertension, eGFR, LVEF, diabetes, smoking and common medications, the most predictive baseline tests were MR-proADM (HR 3.74, 95% CI 1.49-9.41, p=0.005) and MR-proANP (HR 3.21, 95% CI 1.31-7.84, p=0.01). With the exception of MR-proADM, CPX increased predictive value of the other markers. Post-CPX MR-proANP was associated with highest HR (4.82, 95%CI 1.52-15.28, p=0.007), followed by copeptin (HR 3.74, 95%CI 1.46-9.59, p=0.006). Among combinations of pre- and post-exercise tests, baseline MR-proANP and post-CPX copeptin provided the best prediction of adverse events (Figure 1).

Conclusions: Immediate post-CPX testing improves outcome prediction in HF patients.



A-14

First trimester PlGF and sFlt-1 are significantly related to PAPPA levels

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Background: Placental growth factor (PlGF) is a pro-angiogenic factor involved in the arterial growth during pregnancy. The biological activity of PlGF can be inhibited by binding to the soluble fms-like tyrosine kinase-1 (sFlt-1). In preeclamptic pregnancies the concentrations of sFlt-1 are increasing, blunting the action of PlGF. PlGF and sFlt-1 represent therefore two biomarkers for an early identification of pregnant women at risk for preeclampsia. The pregnancy-associated plasma protein A (PAPPA) has also recently been related to preeclampsia. The objectives of our study were to evaluate the analytical performances of electrochemiluminescent immunoassays for measurement of PlGF and sFlt-1, to measure their levels in first trimester pregnant women and to assess their relationship with PAPPA and β-HCG.

Methods: PlGF and sFlt-1 were measured with electrochemiluminescent immunoassays on Cobas e411 analyzer (Roche Diagnostics). Circulating levels of PAPPA and β-HCG with the Kryptor immunoassays (ThermoScientific). Assay imprecision was evaluated with quality control materials and functional sensitivity was determined with serum pools. PlGF, sFlt-1, PAPPA and β-HCG concentrations were measured in 306 pregnant women (mean age: 32 years) coming to the hospital for a first trimester work-up.

Results: The between-run (n=5) coefficients of variation were 0.5 and 1.3% for PlGF concentrations of 102 and 1002 pg/mL and 1.9 and 1.1% for sFlt-1 concentrations of 97 and 990 pg/mL. The functional sensitivities for a 10% CV were 8 pg/mL for the PlGF assay and 30 pg/mL for the sFlt-1 assay. In the 306 pregnant women the PlGF levels were ranging from 8.3 to 136.5 pg/mL (median: 48.9 pg/mL), sFlt-1 from 362.4 to 446.0 pg/mL (median: 1352.0 pg/mL), PAPPA from 0.2 to 16.1 mU/mL (median: 3.8 mU/mL) and β-HCG from 8.02 to 194.0 ng/mL (median: 36.2 ng/mL). A significant correlation was observed between PlGF and sFlT-1 levels (r = 0.23, p<0.001). No significant correlation was observed between women age and PlGF and sFlt-1 levels (r = 0.04 and r = -0.06; p>0.05). A significant correlation was observed between sFlt-1 and β-HCG (r = 0.31; p<0.001) but not between PlGF and β-HCG (r = 0.07; p = 0.25). Interestingly, strong relations were observed between PlGF, sFlt-1 levels and PAPPA concentrations (r = 0.50 and r = 0.68, respectively; p<0.0001).

Conclusions: Our results showed that PlGF and sFlt-1 levels are stronlgy related to PAPPA in frist trimester pregnant women. Such a relation might have to be considered for a fisrt trimester risk estimation of preeclampsia.

A-15

Performance Evaluation of a New Myoglobin Assay on the High-Throughput ADVIA Chemistry Systems

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Background: Myoglobin (17,800 Da) is an intracellular, oxygen-binding heme protein found in cardiac and skeletal muscle. During myocardial infarction, myoglobin is released into the circulation as early as 2 to 4 hours after cell damage and peaks at between 9 and 12 hours, returning to normal within 24 to 36 hours.[1] When used in combination with other cardiac markers such as CK-MB or troponin I, myoglobin is a valuable diagnostic tool in the early evaluation of the potential acute myocardial infarction patient.[2] A new assay* for myoglobin on the automated ADVIA® Clinical Chemistry Systems is under development. The objective of this study was to evaluate the performance of this new Myoglobin (MYO) assay on the ADVIA Chemistry Systems.

Methods: In the ADVIA Chemistry MYO assay, diluted sample is reacted with latex particles coated with myoglobin-specific antibodies. The formation of the antibody-antigen complex during the reaction results in the agglutination of the latex particles, with an increase in turbidity that is measured at 571 nm. The myoglobin concentration in a sample is determined from a five-level standard curve using assay-specific serum-based calibrators. The performance evaluation in this study included precision, interference, linearity, and correlation with the Siemens ADVIA Centaur® Myoglobin assay. Data were collected for all ADVIA Chemistry Systems (ADVIA 1200, ADVIA 1650, ADVIA 1800, and ADVIA 2400), which use the same ADVIA Chemistry MYO reagents, calibrators, and controls.

Results: The imprecision (%CV) of the ADVIA MYO assay with three-level commercial controls ranging from 80.0 to 418.0 ng/mL (n = 40), each measured over 10 days on all systems, was less than 2.4% (within-run) and 2.7% (total). The analytical range of the new assay was from 10.0 to 750 ng/mL (the myoglobin concentration of the highest calibrator). The assay correlated well with the ADVIA Centaur Myoglobin assay: ADVIA Chemistry = 0.98x (ADVIA Centaur) + 6.4 (r = 0.99; n = 72; sample range: 23.8-712.0 ng/mL). The new assay also showed no interference at a myoglobin level of ~60 ng/mL with unconjugated or conjugated bilirubin (up to 60 mg/dL), hemoglobin (up to 1000 mg/dL), lipids (INTRALIPID, Fresenius Kabi AB; up to 500 mg/dL) and rheumatoid factor (up to 800 IU/mL). No prozone effect was observed at the highest myoglobin concentration (320,000 ng/mL) tested. Minimum on-system stability and calibration frequency were both 30 days.

Conclusions: The data demonstrate good performance of the myoglobin assay on the high-throughput ADVIA Chemistry Systems from Siemens Healthcare Diagnostics.

* Under development. Not available for sale.

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A-16

Performance Evaluation of the BGM Galectin-3™ Enzyme-Linked Immunosorbent Assay

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Background: Galectin-3 is one of several known beta-galactoside-binding lectins involved in the regulatory function of immunity, inflammation and cancer. Studies suggest that galectin-3 participates in cardiac inflammation, fibrosis and remodeling of heart muscle, contributing to the development and progression of heart failure. As a result, serum/plasma galectin-3 concentrations are potentially useful in the assessment and prognosis of patients with chronic heart failure, predicting the risk of short- and long-term adverse outcomes. The BGM Galectin-3™ ELISA (BG Medicine, Inc., Waltham, MA) is an FDA cleared ELISA for the quantification of galectin-3 in serum or plasma. The assay utilizes two monoclonal antibodies. A rat anti-mouse antibody for capture of the galectin-3 protein and a horseradish peroxidase-labeled mouse anti-human antibody for detection. Quantification of unknown samples is achieved by comparing the measured absorbance against a standard curve prepared from seven calibrators of known (assigned) galectin-3 concentrations. The purpose of this study was to assess the performance characteristics of the BGM Galectin-3 ELISA and verify the manufacturer's suggested reference interval.

Methods: Serum galectin-3 was measured according to the assay manufacturer's instructions. Deidentified, residual serum specimens sent to ARUP Laboratories were used in this study as were serum specimens provided by BG Medicine, Inc. The performance characteristics evaluated included analytical sensitivity, linearity, precision, accuracy, verification of the manufacturer's suggested reference interval and lot-to-lot comparison. The project was approved by the University of Utah's Institutional Review Board.

Results: The limit of detection determined by parametric analysis was 1.1 ng/mL using the assay buffer (blank, n = 28) and a serum sample with a low galectin-3 concentration (1.2 ng/mL, n = 28). Linearity was determined by combining two serum pools with low (0.8 ng.mL) and high (96.0 ng/mL) galectin-3 concentrations in different ratios

to create a set of 11 samples ranging 1.0 - 95.5 ng/mL. Linear regression of the means of duplicate measurements generated a slope of 0.981, y-intercept of -1.017 and r^2 of 0.992. For imprecision, repeatability studies produced CVs of 2.9, 1.8 and 1.4% at mean concentrations of 9.5, 21.4 and 52.3 ng/mL, respectively (n = 20 per concentration). Within-laboratory CVs were 7.8, 3.6 and 6.0% at the same respective concentrations. Accuracy was evaluated using 21 serum samples with known galectin-3 concentrations. Deming regression of the means of duplicate measurements generated a slope of 0.97, a y-intercept of 0.79 and r^2 of 0.995. The reference interval was verified to be ≤17.8 ng/mL using 32 serum specimens collected from healthy adults. To evaluate lot-to-lot consistency, 22 samples were tested in duplicate with two reagent lots. Deming regression of the means generated a slope of 1.1, a y-intercept of -2.4 and r^2 of 0.981.

Conclusions: The BGM Galectin-3 ELISA demonstrates acceptable performance characteristics for quantifying galectin-3 in human serum. The reference interval established in previous studies is confirmed at 0.0 - 17.8 ng/mL.

A-17

Should both laboratorians and physicians be concerned about the implementation of high sensitivity troponin?

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Background: The introduction of the high sensitivity cardiac troponin T (hs-cTnT) assays has the advantage of detecting myocardial infarction (MI) earlier, identifying more acute myocardial infarction (AMI) and less unstable anginas (UA). Both clinicians and laboratorians feel concerned about the clinical impact of higher rates of hs-cTnT positivity since it is now clear that clinical specificity for AMI decreases as assay sensitivity increases.

Aims: To describe the impact of the implementation of hs-cTnT (Roche): test volumes, positivity rates and clinicians interpretation of results; and to evaluate the agreement between hs-cTnT results and what electrocardiogram (ECG), coronary angiogram (CA) and computed tomography (CT) revealed in comparison with fourth-generation assay (4G-cTnT).

Methods: We collected statistics on test volumes and positivity rates of 2 equivalent semesters before and after the implementation of the hs-cTnT assay. We correlated the results of both 4G and hs-cTnT with ECG, CA and CT findings and the following outcomes: hospital admission (HA), invasive anticoagulant therapy (IAT) and antiplatelet agents (APA) using Kappa: K (values -1.0 indicating perfect disagreement below chance, 0.0 indicating agreement equal to chance, and 1.0 perfect agreement). We also evaluated the correlation between the positivity rate and the discharge diagnosis.

Results: The positivity rate of hs-cTnT (>0.014 ng/ml) vs 4G-cTnT (≥0.01 ng/ml) increased by 11% with a significantly decreasing in test volume (-8%). We correlated 500 tests corresponding to 336 patients of 4G and hs-cTnT: 27% were over percentile 99 (p99) of both methods; 14% were only positive according hs-cTnT and 34% were between the functional sensibility and p99 of hs-cTnT and 4G-cTnT negative. They showed no difference in the low agreement with ECG, CA and HA (K between 0.3 and 0.5); and the almost disagreement with the anticoagulant therapy (K between -0.04 and -0.01). We assessed the positivity rates of 4G and hs-cTnT with the discharge diagnosis: AMI: 100% for both methods, UA: 51 vs 69% and cardiac failure: 83 vs 100%.

Conclusions: We consider significative the rise of patients with hs-cTnT higher than 99 percentile (11%) which could be considered as burden for the ED department. It´s surprising that the number of patients in ED with a troponin request was reduced. We cannot judge the clinical performance of hs-cTnT is better than 4G-cTnT because the rate of agreement with imaging and invasive studies and hospital admission showed no difference. Besides there was almost no correlation between the instauration of anticoagulant therapy and the positivity rate of both assays. Regards the discharge diagnosis, hs-cTnT had higher positivity rate with UA and cardiac failure.

A-19

Small Dense LDL and Cystatin-C: Searching for “Non - Traditional” Risk Biomarkers In Young Indians With Coronary Artery Disease

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Background: Coronary artery disease (CAD) has assumed epidemic proportions in India. CAD affects Indians 5-6 years earlier and is diffuse and malignant. Since the affected population is in the working age group, this poses a heavy burden on a developing economy like India. Traditional risk factors have failed to explain the high incidence of premature CAD in Indians and hence there is an urgent need to identify newer “non-traditional”risk biomarkers. LDL heterogeneity is an important determinant of lipoprotein composition, size and metabolism and remains the primary therapeutic target. Hence, small dense (sd) LDL is viewed as an important CAD risk biomarker. Recently, Cystatin-C has also emerged as a potential predictor of impaired cardiovascular outcome.

Methods: In our study, we evaluated the role of two “non-traditional” CAD risk markers - sdLDL (by linear, polyacrylamide gel electrophoresis) and Cystatin-C (latex enhanced immunoturbidimetry) in 92 CAD patients ≤ 45 years of age and compared with 62 age matched healthy controls. The traditional lipid profile parameters i.e.cholesterol, LDL, HDL, and triglycerides as well as Lp(a) were also studied in the control and CAD groups. Statistical analysis was done using unpaired t tests for continuous variables and chi-square or fisher’s exact test for categorical variables as appropriate.

Results: Mean LDL particle size was significantly smaller in CAD patients as compared to controls (p=0.017). 69.6% of CAD patients showed pattern B (LDL particle size <268 A°) while 30.4% patients showed pattern A (LDL particle size > 268 A°). In the control group, 54.8% showed pattern B and 45.2% showed pattern A. A significant number of CAD patients with triple vessel disease showed pattern B. However no significant change was observed in Cystatin-C levels in the control and CAD groups. Of the traditional lipid tests, only HDL showed a significant decrease in the CAD group (P=0.001). Lp(a) showed no significant change, though the mean Lp(a) in the control group was higher than the global reported mean. In addition, the incidence of CAD was significantly higher in males as compared to females (p<0.001).

Conclusions: This study shows a preponderance of sdLDL in young Indian CAD patients ≤ 45 years, and that this dyslipidemia may not be conclusively detected by the traditional lipid profile tests. High levels of Lp(a) in the normal population may partially explain the increased predisposition of young Indians to CAD. As far as Cystatin-C is concerned, further studies in a larger sample size are required to fully elucidate its role in CAD. Hence, we stress the need for effective, newer CAD risk biomarkers to identify “at risk individuals” at an earlier age to help limit the epidemic in Indians.

A-20

Preliminary reference interval estimates for the high-sensitivity cardiac troponin T assay in a non-cardiac emergency department population

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Background: The 99th percentile for the high-sensitivity cardiac troponin T (hs-cTnT) assay in a younger, non-acute, healthy reference population has been reported to be ≥14 ng/L. Using the ≥14 ng/L cut off for the hs-cTnT assay the clinical sensitivity for the diagnosis of myocardial infarction (MI) is superior to the 4th generation cTnT assay, albeit at a lower clinical specificity (N Engl J Med 2009;361:858-67). Recent studies have suggested that a higher cutoff for elderly patients may improve the clinical performance for the hs-cTnT assay for the diagnosis of MI (Eur Heart J 2011;32:1379-89). As the mechanism for an increase in cTnT concentration measured by the hs cTnT assay could be due to other conditions besides MI, we sought to determine what an upper limit of normal would be in a non-cardiac emergency department (ED) population. We also wanted to assess if there would be differences in concentrations between those <65 years (y) and those ≥ 65y of age in this ED population.

Methods: Over 5-weeks at a general hospital, consecutive ED patients with a lithium heparin sample collected at presentation for which an ED physician did not clinically order cTnT comprised the study population. After confirmation that a cTnT was not resulted in the 24 hours after presentation, the lithium heparin sample was measured with the hs-cTnT assay on the Roche Modular Analytics E170 [CV=3.5% with Roche level 1 quality control (mean=24 ng/L; n=43) and CV=1.6% with Roche level 2 quality control (mean=2001 ng/L; n=43)]. Patients were excluded from the reference interval analysis if: i) their emergency admission could result in

elevated cTnT concentrations as per the 2007 Universal MI definition; ii)

had cTnT ≥14 ng/L and were admitted to the hospital within 30 days after their presentation; iii) had an eGFR <30 (ml/min/1.73m^2); iv) the samples were haemolysed. Non-parametric testing was performed to determine the upper 99th percentile in the overall population, as well as those <65y and those ≥65y (as well as differences in cTnT concentrations between these 2 groups via Mann Whitney test) using Analyse-it software.

Results: There were 565 patients with lithium heparin samples measured with the

hs-cTnT assay. Of these patients, 78 were excluded either due to a condition that could result in an elevated cTnT concentration or were admitted to the hospital within 30 days of the presentation. A further 6 patients were subsequently removed due to low eGFR with another 67 samples removed due to haemolysis. The calculated 99th percentile in the final overall population [n=414 patients, median (interquartile range) age=56y (41-71)] was 64 ng/L (90%CI:39-105). In patients <65y (n=263) the 99th cTnT percentile was 45 ng/L, whereas in those ≥65y (n=151) the 99th was 71 ng/L (<65y group median cTnT <3 ng/L vs. ≥65y group median cTnT =11 ng/L; p < 0.0001).

Conclusions: In a non-cardiac emergency department population the 99th percentile is higher than the reported healthy population, and is in agreement with a pre-operative non-cardiac surgical population reported reference interval (Clin Biochem 2011;44:1021-4).

A-21

High Sensitive Troponin I. A Useful Tool For Identifying Non-St Segment Elevation Myocardial Infarction

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Background: High sensitivity troponin (hsTn) assays are able to measure cardiac Troponin (cTn) concentrations corresponding to the 99th reference percentile (99th) with the recommended imprecision (<10%) and detect cTn in most healthy subjects. Characterizing these assays in coronary and non-coronary patients before their introduction to routine clinical practice is critical.

Methods: We included 256 patients (66.4% men, mean age: 68.1 years) with chest pain suggestive of acute coronary syndrome (ACS); patients with ST elevation via ECG were excluded. Samples were obtained at admission (T0) and 2 (T1), 4 (T2) and 6-10 (T3) hours later with patient consent. Myocardial infarction (MI) was diagnosed in the appropriate clinical context using TnI concentrations (contemporary method, Abbott Diagnostics) exceeding the 99th (0.028 ug/L) and an increase/decrease≥20% using serial measurements. Troponin was also measured with a new highly-sensitive assay (ARCHITECT *STAT* hsTnI, Abbott Diagnostics). This assay has a limit of detection (LoD) of 1.2 ng/L, measuring range of 0-50 ng/L and 99th of 24 ng/L. In our laboratory, a hsTnI concentration of 25 ng/L had a total imprecision of 3.3% coefficient of variation.

Results: When TnI was included in the criteria for diagnosing MI, 104 patients were classified as non-ACS, 64 as unstable angina (UA) and 84 as non-ST segment elevation MI (NSTEMI); in 4 cases, diagnosis could not be adjudicated. Median time from symptoms to admission was 180 minutes for UA and NSTEMI, and 193 minutes for non-ACS patients. The TnI (ug/L) ranges at admission were 0.01-17.7 in non-ACS, 0.01- 0.032 in UA and 0.01-9.88 in NSTEMI; the same ranges for hsTnI (ng/L) were <1.2-19666, 1.6-2470 and 1.9-10057. All NSTEMI patients had an admission hsTnI measurement above the LoD. Median increases between admission and serial samples were 65% (T1), 160% (T2), and 177% (T3). When the MI diagnosis was assessed with hsTnI using the same criteria used for TnI (99th of 24 ng/L and a serial increase/decrease≥20%), 9 (8.6%) non-ACS (most of them with diseases known to show a kinetic pattern) and 11 (17%) UA fulfilled MI criteria. Surprisingly, 10 NSTEMI (according to TnI) did not fulfill the MI criteria; most of these patients had TnI concentrations only slightly higher than the 99th of TnI. This suggests that analytical imprecision at the TnI 99th percentile could confound true MI diagnosis and/or that using a 20% delta for hsTnI is not ideal as suggested by the median hs-TnI increases found in our NSTEMI patients. By ROC analysis, we found that the best combination of sensitivity (90.2%) and specificity (87.9%) for NSTEMI diagnosis was at 4h after admission (T2); i.e. 7h after symptoms.

Conclusions: 1.The imprecision (3.3%) of this hsTnI assay is appropriate for measuring the 99th; 2. NSTEMI patients had admission hsTnI above the LoD, supporting a negative predictive value of 100%; 3. hsTnI did not identify NSTEMI in some patients with TnI values only slightly higher than the 99th, suggesting that the improved precision of hsTnI methods may improve confidence in diagnosis and, 4. The best sensitivity and specificity for NSTEMI diagnosis was found 7h after onset of symptoms.

A-22

99th Percentile Value Determinations for 18 Cardiac Troponin Assays Within Same Healthy Population

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Introduction: The primary goal of the study was to determine 99th percentile values for cardiac troponin (cTn) measured by a large number of sensitive and high-sensitivity cTnI and cTnT assays using the same specimens from a large normal reference population. The secondary goal was to compare and contrast the findings regarding male and female subjects.

Materials and Methods: Lithium heparin plasma specimens were obtained and banked from 524 subjects with sufficient volume and measured on 17 cTnI and 1 cTnT assays. These included 272 males and 252 females. The following manufacturers and instruments participated in this study. Abbott Diagnostics-ARCHITECT i2000$_{SR}$, ARCHITECT i2000$_{SR}$ high sensitivity (hs), AxSYM Troponin-ADV, i-STAT; Alere Triage; Beckman Coulter-Access hs and modified sensitive; bioMerieux Vidas cTnI-Ultra; Ortho Clinical Diagnostics Vitros ECi ES; Singulex ERENNA hs; Siemens Healthcare Diagnostics Centaur TnI-Ultra, Dimension ExL, Dimension VISTA, Immulite 2000 XPi, Stratus CS, Dimension VISTA hs-cTnI; Roche Diagnostic Cobas e601 cTnI and hs-cTnT. 99th Percentile values (ng/L) were determined by nonparametric statistics following the Clinical and Laboratory Standards Institute guideline C28-A3.

Results: For the high-sensitivity cTnI assays, there was a 2-fold difference in 99th percentile values, ranging from 23.5 to 57.6 ng/L. All hs-cTnI assays measured concentrations in >85% of all the normal subjects compared to 25.4% for the hs-cTnT assay. All 5 hs-cTn assays demonstrated higher 99th percentile values for males compared to females (range 1.2 to 2.3-fold).

For the 13 sensitive cTnI assays, 99th percentile values ranged 43.3-fold (range 9 to 390 ng/L). There were three assays that showed substantial greater 99th percentile, the Roche cTnI assay at 86 ng/L, the Beckman Access modified sensitive cTnI at 284 ng/L and the Siemens Immulite XPi assay at 390 ng/L. The remaining 10 assays were all <39 ng/L. Besides the Roche and Beckman Access modified sensitive cTnI assays that measured 91.6% and 32.4% of normal subjects respectively, all the other assays measured only 0.0 to 7.3% of normal subjects. Seven of thirteen assays demonstrated substantially higher 99th percentiles for males compared to females.

Conclusions: Our findings underscore the importance of knowing the individual cTn assay used in the clinical setting as well as in research. Different cTn assays have different 99th percentiles. High sensitivity cTnT and cTnI assays will provide the ability to measure cTn in a substantial greater number of normal subjects, providing a baseline that may assist in defining true normality, patient management, as well as a potential for primary prevention for improving long-term outcomes.

A-23

Defining the 99th percentile upper reference limits using a new high-sensitive cardiac troponin I (cTnI) assay in healthy Asian subjects

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Objective: This study determined the 99th percentile reference limit for healthy Asian subjects using a pre-market prototype of the ARCHITECT high-sensitive (hs) cTnI assay (Abbott Diagnostics).

Design and Methods: TnI was measured on serum obtained from 1091 healthy ambulatory individuals, comprising 582 men (35-65 years) and 509 women (40-65). Exclusion criteria: clinical parameters (pregnancy, history of diabetes, heart or renal disease) and B-type natriuretic peptide (BNP) above the gender-specific 95th centile. Both BNP and hs-cTnI were measured on an ARCHITECT i2000$_{SR}$ (Abbott Diagnostics) analyzer. The cTnI assay is a 2-step chemiluminescent immunoassay. In the first step TnI-Ab coated microparticles are added to the sample and incubated. Following a wash step, TnI-Ab-acridinium labeled conjugate is added in a second incubation step. Following another wash cycle, pre-trigger and trigger solutions are added to the mixture. The resultant chemiluminescent signal is proportional to the TnI concentration in the sample. Statistical analyses were performed on MedCalc v 12 (MedCalc Software, Mariakerke, Belgium) and IBM SPSS v 19 (SPSS Inc., Chicago, USA). The limit of detection (LoD) for this assay as previously described (Clin.Chem 2012;58:59) is 1.2 ng/L. The inter-assay imprecision was determined as per CLSI EP17A guidelines.

Results: In the subjects studied, cTnI concentrations ranged from 0-49.3 ng/L with

a positive skew. The TnI concentration corresponding to an inter-assay coefficient of variation (CV) of 20% was 2.0 ng/L, 15% was 3.5 ng/L and 10% was 6.0 ng/L. The overall 99th centile for cTnI was 21.0 ng/L (median 2.60 ng/L). Gender differences in TnI values ($p < 0.001$) were noted with higher concentrations in men. The median and 99th centile TnI values for men were 2.80 and 30.74 ng/L; for women - 2.30 and 17.65 ng/L. Age-related differences (<50 years vs. > 50 years) were seen in both sexes, but were more marked in men ($p < 0.001$). Except for one subject (female 48 y), all other individuals had measurable cTnI values - 93.6% above the assay LoD (1.2 ng/L).

Conclusions: This ARCHITECT hs-cTnI assay is able to measure more than 95% of the values below the 99th percentile reference limit of 21.0 ng/L with an assay precision (CV) at this cut-point of less than 10%. Gender differences were noted with higher hs-cTnI in men.

A-24

Circulating levels of MR-proANP in healthy and heart failure subjects.

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Background: Natriuretic peptides are important biomarkers for the diagnosis, monitoring and prognosis of heart failure (HF). The circulating levels of several N-terminal fragments of the Atrial Natriutic Peptide (ANP) can be measured with different manual immunoassays. An automated immunoassay using Time-Resolved Amplified Cryptate Emission (TRACE™) allows now the measurement of Mid-Regional proANP (MR-proANP). TRACE™ assay is a homogeneous chemiluminescent immunoassay offering a higher sensitivity and a reduced turn around time of analysis in comparison to the manual immunoassays. The aim of this study was to measure the circulating concentrations of MR-proANP in healthy individuals as well as in patients with severe heart failure (HF). Furthermore, the relationships between MRproANP and Nt-proANP$_{68-98}$, Nt-proBNP and BNP were also determined in HF patients.

Methods: Imprecision of the MR-proANP Kryptor® TRACE™ assay (Thermo Scientific) was evaluated with the quality control materials and references values obtained from 49 healthy Caucasian individuals (females n=15; males n=34; mean age: 37.5 ± 9.1 years). MR-proANP concentrations were also measured in 72 patients with severe HF (females n=15; males n=57; NYHA II-IV; mean age: 68.3 ± 12.2 years; mean ejection fraction: 22.1 ± 6.2 %). Circulating levels of Nt-proANP$_{68-98}$, Nt-proBNP and BNP were also analyzed using immunoassays.

Results: Between run (n=40) coefficients of variation were 4.7% and 3.4% for MR-proANP concentrations of 92.3 pmol/l and 474 pmol/l, respectively. Upper limit of MR-proANP in healthy individuals was fixed at 68.7 pmol/l (95% reference interval: 17.1 - 68.7, median 41.1 pmol/l [range: 22.9 - 75.5]), gender difference was observed with a slight trend of higher MR-proANP mean values in females. As expected, HF patients have significantly higher MR-proANP values (517 pmol/l vs 42.9 in the healthy subjects; $p<0.001$). MR-proANP was significantly correlated with Nt-proANP ($r = 0.875$, $p<0.001$) as well as with Nt-proBNP ($r = 0.863$, $p<0.001$) and BNP ($r = 0.882$, $p<0.001$).

Conclusions: Our study demonstrated a good reproducibility for automated MR-proANP testing and provided a reference interval in healthy Caucasian subjects for this biomarker. Moreover, in HF patients, MR-proANP levels are significantly increased and strongly related to Nt-proANP and other natriuretic peptides.

A-25

Urinary Liver-Type Fatty Acid-Binding Protein on Admission and Risk of Acute Kidney Injury in Patients Hospitalized for Worsening Heart Failure

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Acute kidney injury (AKI) remarkably increases the mortality of critically ill patients. Urinary liver-type fatty acid-binding protein (L-FABP) is a promising indicator of tubular but not glomerular damage. We prospectively investigated the association of urinary level of L-FABP on admission with the development of AKI in 176 consecutive patients hospitalized for worsening heart failure. AKI was defined as a greater than 50% increase in creatinine from the baseline or an absolute increase of ≥0.3 mg/dl within 48 hours after admission.

Results: Patients with AKI (n = 20) had a higher in-hospital mortality rate than those without (25% vs. 9%, P = 0.03). Clinical and biochemical findings according to quartiles of L-FABP were shown in Table. Age, B-type natriuretic peptide (BNP), urinary albumin and prevalence of AKI were positively correlated with quartiles of urinary L-FABP. Also, estimated glomerular filtration rate (GFR) was negatively correlated with quartiles of urinary L-FABP. In a stepwise logistic analysis, L-FABP (odds ratio 3.03 per 10-fold increment, P = 0.001) was independently associated with AKI.

Conclusions: Admission measurements of urinary level of L-FABP may be useful for evaluating the risk of AKI in this population.

Quartile of urinary L-FABP (μg/gCr)	1st <8.5 (n=43)	2nd 8.5-24.7 (n=44)	3rd 24.8-94.7 (n=46)	4th 94.7< (n=43)	P value
Age (years)	71	79	78	75	0.02
Diabetes (%)	28	39	48	44	0.08
Previous history of old myocardial infarction (%)	23	32	41	26	0.6
Estimated GFR (ml/min/1.74m^2)	57	47	49	38	<0.0001
BNP (pg/ml)	386	623	746	1230	<0.0001
Urinary albumin (mg/gCr)	20	60	210	411	<0.0001
AKI (%)	10	4.7	4.5	27.9	0.01

Data are expressed as median or %.

A-26

Urinary Concentration of Liver-Type Fatty Acid-Binding Protein and Cardiac Risk in Outpatients with Type-2 Diabetes Mellitus

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Urinary levels of liver-type fatty acid-binding protein (L-FABP) reflect tubulointerstitial damage. We investigated the relationship between urinary L-FABP levels and cardiac risk in 383 outpatients (median age of 66 years) with type-2 diabetes mellitus. A previous history of old myocardial infarction was present in 38 patients, chronic heart failure in 15 patients, and stroke in 47 patients.

Results: Urinary L-FABP levels were positively correlated with urinary albumin levels ($r = 0.65$, $P < 0.0001$). On a stepwise logistic analysis including age, male gender, history of myocardial infarction, heart failure or stroke, and biochemical variables, both high-sensitivity troponin T (odds ratio, 3.1 per 10-fold increment, P = 0.0003) and increased levels of urinary albumin (>30 mg/gCr; 6.2, P <0.0001) was independently associated with increased levels of urinary L-FABP (> 8.4 μg/gCr of upper reference limit). Clinical and biochemical findings in patients with and without increased levels of urinary L-FABP were shown in Table. Patients with increased urinary L-FABP were older, had higher levels of high-sensitivity troponin T and B-type natriuretic peptide (BNP), and had a high prevalence of electrocardiographic abnormality compared with those without increased urinary L-FABP.

Conclusions: Urinary L-FABP measurements may be useful for evaluating cardiac risk in this population.

L-FABP (>8.4μg/gCr)	+ (n=187)	− (n=196)	P value
Age(years)	69	64	<0.0001
Medical history			
Heart failure(%)	3.7	4.1	0.9
Old myocardial infarction(%)	11.2	8.7	0.4
Stroke(%)	12.3	12.2	0.9
Hemoglobin A1c(%)	7.0	6.7	0.1
Estimated GFR(ml/min/1.73m^2)	70	76	0.0002
Urinary albumin(mg/gCr)	103	12	<0.0001
Urinary albumin(>30mg/gCr)	72	25	<0.0001
High-sensitivity troponin T (pg/ml)	9	5	<0.0001
BNP(pg/ml)	30	19	<0.0001
Electrocardiographic abnormality(%)	35	15	0.0001

Data are expressed as median or %

A-27

The effect of a new generation of troponin assay in patients' management: a real world emergency department experience

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Background: Thanks to the improved analytical characteristics, the new generations of troponin assays have substantially increased the diagnostic sensitivity for the early detection of acute myocardial infarction. Thus, troponin elevations measured using these new and more sensitive assays, are frequently found in patients without acute coronary syndrome (ACS).

Objective: The aim of this study was to verify whether the use of a new generation of troponin I (cTnI) assay changes the clinical management of patients (pts) presenting to the Emergency Department (ED) and leads to more hospital admissions in comparison to the previous assay.

Methods: We retrospectively evaluated 3886 pts who presented, from September 2010 to January 2011, to the ED of our hospital and who underwent to cTnI measurement with a new cTnI assay (LOCI, Dimension Vista, Siemens Health Care Diagnostics) that shows improved analytical performance in comparison to the previous one (Dimension RxL, Siemens) and in particular: the analytical sensitivity = 0.015 vs 0.04 µg/L; the 10% CV < 0.040 vs 0.15 µg/L; the 99th percentile = 0.045 vs 0.07 µg/L; the cut off value for myocardial injury = 0.045 vs 0.15 µg/L. From a previous linear regression study, carried out in 65 pts, we found that using the "new" cTnI assay, the range of concentrations between 0.045 to 0.400 µg/L (values higher than cut-off) corresponds to the range 0.04-0.15 µg/L using the"old" cTnI (values lower than cut-off). We specifically focused on patients showing cTnI elevations detected only using the "new" assay.

Results: During the observation period in the ED and/or hospital floor, 839 out of 3886 pts (21.6%) (males n=440, females n=399; aged 22-102 years) shows cTnI elevations only with the "new" sensitive assay; 609 out of 3886 pts (15.7%) evidenced the elevation already in ED. Final discharge diagnoses were: acute myocardial infarction (n=69, 8.2%), cardiac disease other than ACS such as heart failure (n=314, 37.4%), non cardiac chest pain (n=42, 5.0%), pulmonary disease (n=159, 18.9%), gastroenteric disease (n=52, 6.2%), neurologic disorders (n=48, 5.7%), kidney disease (n=30, 3.6%), infection-sepsis (n=30, 3.6%), oncological disease (n=25, 3.0%), metabolic diseases (n=20, 2.4%), syncope (n=15, 1.8%), ortopedic disease (n=11, 1.3%), psychiatric disease (n=4, 0.5%), gynaecological disease (n=1, 0.1%), others (n=19, 2.3%). A pool of skilled ED physicians, blinded to the cTnI data, retrospectively reviewed the electronic records of the 609 pts. They confirmed the need for hospital admission in 581 out of 609 pts (95.4%) based exclusively on the clinical risk stratification; 28 pts (4.6%) instead have been hospitalized on the basis of cTnI values obtained with the new assay.

Conclusions: The introduction of a more sensitive cTnI method in the ED was associated with an increased percentage of troponin elevations that, however, not substantially increase the hospital admission rate. Noteworthy, 69 out of 839 pts (8.2%) were discharged with a diagnosis of acute myocardial infarction instead of unstable angina based on the values obtained with the new generation assay.

A-28

Clinical performance of two last generation of cardiac troponin assays in a population of elderly subjects

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Background: The introduction of cardiac troponin (cTn) assays with improved analytical sensitivity allows reliable detection of minor cTn elevations in patients (pts) with non-ischaemic cardiac conditions. Purpose: This study evaluated the clinical performance of two last generation assays recently introduced in routine practice, in a group of elderly subjects: cardiac troponin I (new cTnI) and high sensitive cardiac troponin T (hs-cTnT).

Methods: From March to October 2011, 384 pts attending the Division of Geriatrics have been enrolled. GROUP 1: 30 pts (6 males, 24 females; aged 69-92 years) attending the outpatients's ambulatory; GROUP 2: 354 consecutively hospitalized pts (114 males, 240 females; aged 64-105 years). New cTnI and hs-cTnT were measured using respectively: Dimension Vista (Siemens), Modular Analytics E 170 (Roche); cut off for myocardial damage=0.045, 0.014 ng/mL. 1 blood sample was collected for GROUP 1 pts whereas serial samples (min 2) have been collected for GROUP 2 pts. GROUP 2 pts have been classified according to new cTnI concentration and the main disease (D) in the discharge diagnosis: pts showing cTnI ≤ 0.045 in all sample (negative, NEG), pts showing cTnI > 0.045 in at least 1 sample (positive, POS); Acute Coronary Syndrome (ACS), Heart Failure (HF), Pleuro-Pulmonar D (PPD), Gastroenteric D (GD), Neurological D (ND), Kidney D (KD), Metabolic D (MD), Anaemia (AN), Other Diagnosis (OD). In a comparison study, 235 plasma samples collected from GROUP 2 pts were tested using the new cTnI, hs-cTnT and the previous cTnI assay (old cTnI, Dimension RxL, Siemens; cut off for myocardial damage=0.15 ng/mL); concentration range, respectively: 0.046-0.437, 0.010-0.550, 0.00-0.88.

Results: GROUP 1: new cTnI was negative in 100% of pts. GROUP 2: 242 pts (68%) were NEG and 112 pts (32%) were POS. Main discharge D in GROUP 2 pts: ACS, 9 (2.5%; 9 POS); HF, 46 (13.0%; 15 POS); PPD, 64 (18.1%; 17 POS); GD, 55 (15.5%; 15 POS); ND, 60 (16.9%; 18 POS); KD, 35 (10.0%; 18 POS); MD, 6 (1.7%; 3 POS); AN, 19 (5.4%; 3 POS); OD, 60 (16.9%; 14 POS). In GROUP 2, 28 pts (7.9%) died during hospital stay: 12 (42.9%) POS, 16 (57.1%) NEG. In the comparison study, 32 (13.6%) and 232 (98.7%) out of 235 samples resulted positive using the old cTnI and hs-cTnT assay respectively; in particular, in ACS pts, the old cTnI and hs-cTnT were positive in 28.6% and in 100% of samples tested.

Conclusions: cTnI was negative in 100% of outpatients (GROUP 1) confirming the expected cTnI specificity. The most frequent diagnosis in hospitalized pts (GROUP 2) was PPD (18.1%) and cTnI concentrations were negative in 68% and positive in 32% of pts. The highest percentages of positive cTnI were observed in ACS pts (100.0%) and KD pts (51.4%). In particular, the increased rate of positive values in ACS pts, highlights the improved diagnostic sensitivity for early detection of myocardial damage of the last generation cardiac troponin assays. Finally, deaths resulted more frequent in pts showing positive values with new cTnI (10.7% *vs* 6.6%), confirming the expected prognostic usefulness of the biomarker.

A-29

Preliminary investigation of a sensitive and two high-sensitivity cardiac troponin assays for detecting a short-term serious outcome in an emergency department chest pain population

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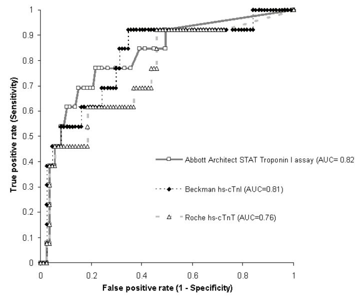
Background: Studies indicate superior diagnostic performance with high-sensitivity cardiac troponin (hs-cTn) or sensitive cTnI assays versus the 4th-generation cTnT assay for myocardial infarction (MI). However, few data exist comparing a sensitive cTnI with hs-cTn assays in an emergency department (ED) chest pain population for MI diagnosis or other acute cardiovascular events. In this pilot study we assessed one sensitive cTnI and two hs-cTn assays for their ability to predict a short-term serious cardiac outcome.

Methods: After ethics approval, ED nurses collected blood from patients being evaluated for chest pain (n=102, 1-month convenience sampling). The presentation sample with sufficient volume for analytical analyses (n=100 patients) was thawed (serum stored -70°C) and measured for the following immunoassays (cTn/platform/company): cTnI/ARCHITECT $i1000_{SR}$/Abbott, hs-cTnI/Access II/Beckman Coulter and hs-cTnT/Elecsys 2010/Roche Diagnostics. Follow-up for each patient enrolled occurred up to 72-hours after presentation to determine if a serious cardiac outcome occurred. The serious cardiac outcomes were admissions to hospital due to: significant arrhythmia, refractory ischemic symptoms, heart failure, MI (4th-generation cTnT used clinically), PCI, CABG, stroke, cardiac arrest, or death. These outcomes were adjudicated by an emergency physician blinded to the hs-cTn and cTnI results. Diagnostic performance was assessed by ROC analyses (Analyse-it software).

Results: In this ED chest pain cohort (52% female) the median (interquartile range) age was 65y (57-76) with the median (IQR) cTnT<0.01ug/L (<0.01-0.01) and eGFR=77mL/min/1.73m^2 (55-91) at presentation. The median (IQR) for the sensitive cTnI was <0.01ug/L (<0.01-0.02), whereas the median concentrations of the hs-cTn assays were at the published 99th percentiles (hs-cTnI=10ng/L (5-19); hs-cTnT=14ng/L (<3-26)). The area under the curve was similar for all 3 assays for the composite outcome (Figure).

Conclusions: Additional studies comparing sensitive cTnI and hs-cTn assays assessing important acute cardiovascular outcomes, in addition to MI, are needed to establish if differences exist between these assays.



A-30

A comparison of high-sensitivity cardiac troponin assays for the diagnosis of acute coronary syndrome in an early presenting chest pain population

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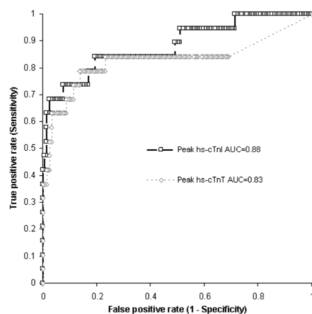
Background: Data are emerging to suggest the increased analytical sensitivity of high-sensitivity cardiac troponin (hs-cTn) assays may be used for ruling-out emergency department (ED) chest pain patients for myocardial infarction (MI). To date, the cutoff used has been the limit of the blank (LoB); however, this may not be applicable to all high-sensitivity assays. Moreover, the utility of hs-cTn assays for acute coronary syndrome (ACS) diagnosis has not been thoroughly investigated. We assessed two hs-cTn assays for diagnostic performance and the ability to rule-out ACS using cutoffs below the 99th percentile.

Methods: ED patients presenting within 6 hours (h) of chest pain onset had blood collected at presentation, 3, 6h (Clin Chem 2012;58:298-302) with serum stored below -70^{0}C. Patients were followed for 72h for hospital admission of ACS (i.e., either: a) MI defined by elevated cTnI (Abbott AxSym) along with either ischemic symptoms, development of ECG changes, coronary artery intervention or pathologic findings, or b) refractory ischemic cardiac pain defined as recurrent ischemic symptoms lasting 15 minutes or more, along with documented ischemic ECG changes or a referral for an additional intervention). The serum samples were measured with the hs-cTnI assay (research-use only, Beckman Coulter) and hs-cTnT assay (Roche) with the peak concentration used in receiver operator characteristic (ROC) curve analyses (n=180 subjects) (Analyse-it software).

Results: ROC analysis suggested different diagnostic performance between the hs-cTn assays (p=0.042; Figure). The percentage of patients with cTn>LoB was also different between assays (71%(95%CI:63-77%) for hs-cTnT vs. 100%(95%CI:97-100%) for hs-cTnI). The sensitivity/specificity for ACS using the LoB (3 ng/L) for hs-cTnT was 84%(95%CI:62-95%)/31%(95%CI:24-39%) and 100% (95%CI:80-100%)/28%(95%CI:22-35%) using 6 ng/L for the hs-cTnI assay (6 ng/L is the ROC-derived cutoff for the hs-cTnI assay from a stable high-risk population; Clin Chem 2011;57:1146-53).

Conclusions: Analytical sensitivity is an important variable for clinical application of hs-cTn assays.



A-31

Analytical Evaluation of the Abbott ARCHITECT Galectin-3 Assay on the Abbott ARCHITECT $i2000_{SR}$ Analyzer

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Purpose: The purpose of this study was to evaluate the analytical characteristics of Abbott's ARCHITECT Galectin-3 Assay. Galectin-3 has been associated with physiological processes seen in heart failure, including inflammation, myofibroblast proliferation, fibrogenesis, tissue repair and cardiac remodeling.

Methods: The Galectin-3 assay analytical performance was evaluated using CLSI guided protocols with EDTA plasma specimens tested on the ARCHITECT $i2000_{SR}$ analyzer for verification of the LoB, LoD and LoQ, within-run and total imprecision (%CV), linearity and reference value determination in 120 apparently healthy individuals (60 men, 60 women).

Results: LoB of 0.8 ng/mL, LoD of 1.0 ng/mL and LoQ equal to the LoD of 1.0 ng/mL were all verified. Imprecision in 3 plasma pools with means of 9.4, 19.8 and 74.1 ng/mL run 2 times a day for 10 test days showed within-run imprecisions of 4.6, 2.7 and 1.6% CV, respectively, and total imprecisions of 6.6, 3.1 and 2.4% CV, respectively. The linear range was evaluated using 4 sets of dilution pools and verified to be linear: range tested 17 to 99 ng/mL; slopes 0.96 to 1.00; intercepts -3.78 to 0.09; r-values 0.998 to 1.000; p<0.001 for all. The 90th, 95th, and 99th percentiles of the normal reference range were not normally distributed and were log-transformed and back transformed. For all subjects (n=120) the 90th, 95th and 99th percentiles were non-parametrically determined to be 16.5, 17.8 and 23.1 ng/mL; 15.0, 17.1, 21.0 ng/mL for men; and 17.3, 19.9 and 23.7 ng/mL for women.

Conclusions: We have shown that the Abbott ARCHITECT $i2000_{SR}$ Galectin-3 assay provides an analytically acceptable measurement of Galectin-3 in EDTA plasma specimens.

A-32

Analytical Evaluation of Cardiac Troponin I on the Radiometer AQT90 FLEX, a Random Access Point-of-Care Testing (POCT) Analyzer

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Introduction: Cardiac troponin I (cTnI) is a critical test that must be available for emergency departments on a 24/7 basis. The goal of this study was to evaluate the analytical performance of Radiometer's AQT90 FLEX random access POCT analyzer for cTnI. The AQT90 FLEX TnI Test was labeled for investigational use only and is subject to U.S. FDA 510(k) regulations.

Methods: cTnI analytical performance was evaluated for EDTA and lithium heparin plasma, in comparison to EDTA and lithium heparin whole blood, for: *a*) matrix comparison; *b*) sample stability (room temperature (18-25 ^{o}C), refrigerated (2-8 ^{o}C) and frozen (<-18 ^{o}C)); *c*) within-run and total imprecision (%CV); and *d*) 99th percentile reference value determination in 969 apparently healthy individuals (522 women, 447 men).

Results: The cTnI limit of detection (LoD) is 7 ng/L, and the linear range is 10 to 2500 ng/L. Results from the three matrix comparisons showed that heparin plasma and EDTA plasma could be used interchangeably with whole blood (n = 94 samples, range 12 to 23000 ng/L, slopes 0.98 to 1.04, intercept <2 ng/L, r > 0.99). Samples (n = 88) were stable for 2 hours at room temperature for whole blood, and up to 24 hours at 2-8 ^{o}C or frozen with one freeze thaw cycle for plasma. Imprecision for spiked plasma at five cTnI concentrations ranging from 20 to 2400 ng/L over 20 test days showed a within-run imprecision of 1.2 to 9.5% CV and a total imprecision of 2.8 to 10.4% CV. Using ten whole blood samples at cTnI concentrations ranging from 243 to 18000 ng/L, within-run imprecision ranged from 2.0 to 10.7% CV, and total imprecision ranged from 2.0 to 10.7% CV. 4% of the normal subjects had a measurable concentration. The 99th percentile reference value for all subjects was 21 ng/L, with no differences between whole blood, EDTA plasma and lithium heparin plasma.

Conclusions: The Radiometer AQT90 FLEX random access analyzer provides an analytically acceptable POCT measurement of cTnI in EDTA and lithium heparin whole blood, and in EDTA and lithium heparin plasma.

A-33

Validation and harmonization of high sensitivity cardiac troponin I assays by Centaur CP and XP

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Background: As part of the upcoming 2013 CMS changes on hospital outpatient prospective payment system, one of the changes is OP-16, defined as troponin results to be reported, within 60 minutes of patient arrival in emergency department(ED), to physician for treating acute myocardial infarction patients or chest pain patients. Monitoring of this change commenced on January 1, 2012, with data guiding 2013 payments. In preparing for this change, Centaur CP (CP) is being installed in a newly developed ED laboratory. This would eliminate sample transport time. The objective of this study is to validate a high sensitivity troponin I assay by CP, and to harmonize with the established troponin I monitoring with the Centaur XP (XP) in the clinical chemistry core laboratory.

Methods: Troponin I was calibrated simultaneously in both instruments. For two weeks, troponin patient samples assayed by the core laboratory XP were saved after routine testing. These samples, n=100, were simultaneously analyzed by both instruments. Typical run time for CP troponin I was about 18 min.

Results: Calibration was linear from 0.006 to 50.000 ng/mL. Precision studies showed acceptable day-to-day (n = 10) CVs of 7.79%, 2.34% and 1.86 % for control concentrations of 0.027, 0.891 and 23.848 ng/mL respectively. Linear regression analysis of 100 patient paired samples yielded slope of 0.9564, intercept of 0.0022 and correlation coefficient of 0.9992. Paired t-test did not show significant difference. Compared to XP with a 99 percentile concentration of 0.04 ng/mL, CP sensitivity and specificity were 94% and 96% respectively.

Conclusions: In conclusion, high sensitivity cardiac troponin I monitoring by CP yielded comparable and clinically efficacious results when compared to XP. The smaller CP would fit within the small ED lab, ensuring ED patient troponin I monitoring and result reporting to be completed within 60 minutes. Further, this would enhance tracking troponin I of admitted patients whose subsequent troponin monitoring would be performed by XP.

A-34

High Sensitivity Troponin and 5 Year Mortality in Hemodialysis Patients

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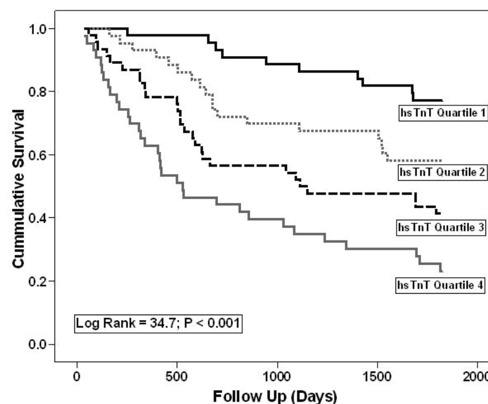
Background. The cardiac troponins are now well recognized as biomarkers with prognostic significance in patients with end stage renal disease. More recently, higher sensitivity troponin assays have been introduced into routine clinical use. We examined whether the use of such assays would provide better risk stratification in patients on maintenance hemodialysis.

Methods. A cohort of 175 patients undergoing maintenance hemodialysis had blood samples taken immediately prior to dialysis which were subsequently analysed using higher sensitivity troponin assays: Troponin I (hsTnI; Siemens Advia Centaur TnI-Ultra) and Troponin T (hsTnT; Roche Elecsys). Baseline hemodynamic and demographic parameters were recorded. The cohort was followed up for 5 years for the outcome of mortality (all cause).

Results. 87 patients died during the follow up period. Both hsTnT (77 vs 37 pg/L; $p < 0.001$) and hsTnI (38 vs 16 pg/L; $p < 0.001$) were significantly higher in those who died compared to those who were still alive at 5 years. Quartile analysis based on troponin levels demonstrated a stepwise increase in mortality through the quartiles for both hsTnT (log rank 34.7; $p < 0.001$) and hsTnI (log rank 34.4; $p < 0.001$). In addition, both hsTnT ($p = 0.03$) and hsTnI ($p = 0.043$) predicted death by 5 years even when adjusted for age, sex, diabetes, ischaemic heart disease, smoking and serum creatinine. Furthermore, having an hsTnT or an hsTnI in the upper quartile (compared to quartile 1) was associated with a 43% or 25% increased chance of death over 5 years respectively.

Conclusions. This study demonstrates that detectable levels of hsTnT and hsTnI are associated with a poor long term prognosis in patients undergoing renal dialysis. Such information may allow patients at increased risk to be targeted with more aggressive therapy or indeed inform transplant priority lists.



A-36

Troponin I Pediatric Decision Limits with Insights form a Healthy Pediatric Population: a CALIPER Study

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Objectives: Troponin I (TnI) is commonly used to assess cardiac injury. The Joint Task Force (2007) recommended the use of assays that demonstrate a total CV <10% at the 99th percentile. As TnI assays become increasingly sensitive the question arises as to how to interpret minutely elevated troponin levels. In this current study, the decision limit for TnI was determined in a healthy paediatric population. Furthermore, routine laboratory tests, along with health survey data, were used to investigate troponin elevations in this healthy cohort.

Methods: This study followed the CLSI C28-A3 guidelines. Samples, along with medical history and current health status measures, from 701 healthy and ethnically diverse children ages birth to 18 years of age were collected. Troponin I was measured on the Abbott ARCHITECT i2000 system; the LoD was 10 ng/L. Non-parametric methods were used to establish the 99th percentile for the decision limit.

Results: The value at the 99th percentile was determined to be 110 ng/L. Interestingly, elevations above this level, which could not be explained by review of their medical histories, were seen in 7 children. Twenty-two children showed detectible levels between 10 and 110 ng/L.

Conclusions: As TnI assays become increasingly sensitive, elevations in TnI levels above the LoD and 99th percentile are seen in a small proportion of healthy children. The question arises on how to interpret these results. This study explored the relationship between elevated troponin and various health indicators and laboratory tests in a healthy paediatric population.

A-37

Analytical Performance of An Assay for Galectin-3 on ARCHITECT *i* System

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Background: Elevated expression of Galectin-3, a multifunctional beta-galactoside-binding lectin, has been associated with inflammation and fibrosis processes which are pivotal to the development of heart failure (HF) (Boer et al, 2009; Dumic et al, 2006). An Assay for Galectin-3 on ARCHITECT *i* System (ARCHITECT Galectin-3) is being developed and the analytical performance of this assay are presented.

Methods: ARCHITECT Galectin-3 is an immunoassay utilizing paramagnetic microparticles coated with monoclonal antibody (mAb) M3/38, and an acridinium-labeled mAb 87B5 conjugates. Sample and microparticles are incubated in the first step. Following a wash, the conjugate is added to the mixture in the second step. The resulting relative light units (RLUs) are directly proportional to the amount of Galectin-3 antigen in the sample and allow a quantitative determination of Galectin-3

in serum and plasma in a sensitive, precise and accurate manner.

Results: The calibration range of the ARCHITECT Galectin-3 assay is 0 - 114 ng/mL. The limit of blank (LoB), detection (LoD) and quantitation (LoQ) were ≤ 0.81, 0.97 and 0.97 ng/mL, respectively. In the onboard sample stability study, the values of 5 runs for each of the freshly loaded controls and panels stored at 2-8°C were compared with the values of those controls and panels stored onboard for 3 and 5 hours. The percent difference of measured levels of Galectin-3 in the freshly loaded samples and those stored onboard were all within the acceptable range of ≤ 10% of the freshly loaded samples. The Freeze/thaw study obtained a mean difference ≤ 10% between the samples which underwent 1, 5, 7 and 10 freeze/thaw cycles and the paired samples with no freeze/thaw (6 neat and 18 Galectin-3-spiked sera and plasma). The High Hook effect study showed that the RLU signals of all of the 3 neat extreme sera and plasma were greater than that for the Cal F. The Spike Recovery study generated an acceptable mean difference of ≤ 15% and 10% for the sera and plasma (N = 5) spiked with > 30 - 114 ng/mL and LoQ - 30 ng/mL, respectively, versus unspiked ones. In the Interference studies, 9 endogenous substances spiked individually into sera and plasma (N ≥ 7) were tested at the following levels (≥): 1000 ng/mL HAMA, 40 mg/dL conjugated bilirubin, 40 mg/dL unconjugated bilirubin, 5 g/dL human gamma globulin, 5 mg/dL creatinine, 3000 mg/dL triglyceride, 500 mg/dL hemoglobin, 500 mg/dL cholesterol and 12 g/dL human serum albumin. The levels of Galectin-3 in the paired control sera and plasma were between Cal B and F. The average percent difference of Galectin-3 in ng/mL between the individual interference substance-spiked samples and the control ones were all within the acceptable range of ≤ 10% of the control samples.

Conclusions: The ARCHITECT Galectin-3 Assay under development is an accurate and sensitive assay for the measurement of Galectin-3 in human serum and plasma.

A-38

Evaluation of high-sensitivity troponin and heart-type fatty acid binding protein in the detection of sub-clinical myotoxic effects of high-dose chemotherapy in patients with malignancy

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Background: Treatment with anthracycline chemotherapy is standard treatment for certain malignancies but is associated with cardiotoxicity. Recognising such cardiotoxicity is critical, in order to prevent further damage to cardiac myocytes and subsequent cardiovascular disease. Cardiomyopathy and heart failure are often a late consequence of these forms of chemotherapy. The objective of the study was to determine if high sensitivity troponin and heart-type fatty acid binding protein are of superior clinical utility in the detection of the sub-clinical myotoxic effects of anthracycline chemotherapy, which would help identify patients at higher risk in order to guide therapeutic intervention.

Methods: 58 patients were included in the cohort of patients receiving chemotherapy. The cohort was divided into two groups; group A defined patients receiving cardiotoxic anthracycline chemotherapy (n=28, 11 with haematological malignancy), group B defined patients receiving routine chemotherapy (n=30).

We also established the 99th centile for hsTnI in a reference population to be 24 pg/ml. These subjects had normal ECG and ECHO and were reviewed by a cardiologist. Each of the 58 oncology patient had levels of high sensitivity troponin (hsTnI), standard troponin, as well as heart-type fatty acid binding protein (h-FABP), measured immediately pre-chemotherapy and post-chemotherapy throughout the cycles of treatment. Samples were analyzed for standard TnI and STAT high sensitive Troponin I (hsTnI) (both Abbott Diagnostics) and h-FABP (Randox Laboratories Ltd.) on the ARCHITECT i1000SR.

Results: There were no significant differences in patients in group A or B pre-chemotherapy as compared with the 99th centile of our reference population. There was a significant difference in hsTnI in patients post cardiotoxic chemotherapy (p=0.011), whereas there was no significant difference (p=0.426) in this patient group using the standard troponin assay. Both troponin assays showed significant differences, hsTnI (p=0.036), TnI (p=0.0001), in patients post-chemotherapy in group A as compared to group B. There was no difference in h-FABP in patients post-treatment in group A (p=0.750) or when comparing patients in group A with those in group B (p= 0.987).

Conclusions: The significant findings in this study suggest that the new generation high sensitivity troponin I has a clinical utility in evaluating patients receiving cardiotoxic chemotherapy. The degree of sensitivity of this new assay is advantageous in evaluating ongoing subtle changes in the myocyte, which should guide the need for specialist cardiac imaging and pharmacological intervention. hsTnI should form part of the ongoing monitoring of patients receiving cardiotoxic chemotherapy.

A-39

Plasma myeloperoxidase and its associations with subclinical atherosclerosis and social deprivation

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Background: The association between social deprivation and cardiovascular disease risk is well recognised, but not adequately explained by classical cardiovascular risk factors. The Psychological, Social and Biological Determinants of Ill Health (pSoBid) study examined participants from the most deprived and least deprived areas of Greater Glasgow, Scotland, United Kingdom. Despite measuring an extensive repertoire of classical and emerging cardiovascular risk factors, deprivation-based differences in ultrasound markers of subclinical atherosclerosis (carotid intima-media thickness (cIMT) and plaque presence) were not fully explained. Myeloperoxidase (MPO) is a pro-inflammatory enzyme that has recognised associations with coronary heart disease (CHD). We studied the associations between myeloperoxidase concentrations, social deprivation and subclinical atherosclerosis. Our hypothesis was that there would be differences in myeloperoxidase concentrations between most deprived and least deprived participants, and that these differences would help explain the previously observed deprivation-based differences in subclinical atherosclerosis.

Methods: MPO was measured in plasma from participants (n=641) in the pSoBid study using a chemiluminescent microparticle two-step MPO immunoassay on the ARCHITECT system (Abbott Diagnostics).

Results: Plasma MPO concentrations were significantly higher in the most deprived (median; IQR, 97.4 pmol/L; 74.5 - 127.4) compared to the least deprived (85.2 pmol/L; 63.3 - 107.8) study participants (p < 0.001). On univariate analysis, MPO was positively associated with interleukin-6 (p = 0.001) and C-reactive protein (p < 0.001). However, MPO concentration was not associated with cIMT (p = 0.123) or plaque presence (p = 0.309).

Conclusions: Despite a strong association between social deprivation and MPO concentration, MPO is not associated with subclinical atherosclerosis in this study population and does not help explain the previously observed deprivation-based differences in ultrasound markers of subclinical atherosclerosis.

Tuesday AM, July 17, 2012

Poster Session: 10:00 AM - 12:30 PM

Factors Affecting Test Results

A-40

Effects of artificial light and storage temperature on the stability of bilirubin in serum

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Background: Blood samples collected in the pediatric emergency room are assessed in the central Laboratory with delay and exposed to light at various temperatures for different periods of times during storage and transport. Although it is known that Bilirubin is photo-sensitive, detailed effects of both temperature and artificial light have not been well studied. The objective of this study was to determine the effects of artificial light and storage temperature on bilirubin stability in serum samples.

Methods: Serum samples with elevated total bilirubin from pediatrics were selected for this study. Total and direct bilirubin were analyzed on ATAC 8000 Chemistry Analyzer (USA) using a timed endpoint diazo method. To determine the impact of light exposure on the stability of bilirubin, 20 samples were exposed to artificial light for 2 , 8 , 24 , and 48 hours at 22°c , and then total and direct bilirubin were measured. To determine the influence of temperature on the stability of bilirubin, after the baseline measurement of 20 samples, each was aliquoted into six tubes and three of them were stored at 3°c , while another three at 22°c , protected from light. Total bilirubin was analyzed after 2, 4, 8, and 24 h respectively. The differences between the baselines and all subsequent measurements were evaluated using analysis of variance (ANOVA) with a post hoc Dunnett's test and p < 0.05 was considered statistically significant. The allowable total error for bilirubin was less than 20 .

Results: The average baseline total and direct bilirubin concentrations were 174.4 ± 29.1 mmol/L and 85.5± 32.5mmol/L , (mean±SD) respectively . A 2, 4, 8, and 24 h light exposure at 22 , the average total bilirubin concentrations were 172.7± 30.9, 171.0 ± 30.8 ,171.0± 30.8 and 159.0± 34.2 mmol/L and direct bilirubin concentrations were 83.8 ± 30.8, 82.1± 30.8 , 82.1± 30.8 ,and 71.8± 27.4 mmol/L. In the second set of samples, the average baseline total bilirubin concentration was 164.2± 128.3 mmol/L and the average concentrations after 2 , 4, 8 , and 24 h were 164.2± 140.2 , 153.9 ± 120.6 , 153.9 ± 120.6 and 150.5± 128.3 mmol/L at 3°c and 162.5 136.8mmol/L at 22°c . There was no statistical significant difference between any of the study groups and the baseline (p<0.05 , n = 20). No statistical significant difference was found between any of the study groups and the baseline (p> =0.05 , n= 20) . As we extended the light exposure to 48 h , total and direct bilirubin concentration decreased to 143.6 ± 39.3 mmol/L(p= 0.01) and 59.9± 25.7 mmol/L(p= 0.01) compared to the baselines .

Conclusions: Bilirubin is relative stable in refrigerator and at room temperature for at least 24 h in a dark environment. Artificial light exposure can cause gradual decrease in both total and direct bilirubin , but the differences are neither statistically nor clinically significant for at least 24 h.

A-42

Status of serum-calcium, -magnesium, -albumin, and -total-protein measurement in Norway assessed with 20 fresh frozen single donation sera

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Background: External quality assessment (EQA) schemes traditionally use a limited number of processed samples. However, the samples' potential non-commutability prevents investigation of between assay biases. The observed systematic differences indeed may not be genuine but an artifact caused by matrix effects. To overcome this limitation, two Norwegian EQA organizers collaborated with Ghent University and STT Consulting to perform a pilot EQA study with a panel of 20 native fresh frozen single-donation sera. The study focused on serum calcium, magnesium, albumin and total protein. It aimed at creating reliable documentation of the quality and standardization status of assays, also with attention to performance characteristics, such as imprecision, accuracy, and sample-related effects.

Methods: Norwegian laboratories using homogeneous test systems were invited for participation; 5 manufacturers peer groups with n ≥6 users (except for Abbott magnesium) could be distinguished. Two measurements per sample in different runs were requested. Peer group targets were calculated for each sample as mean of mean of duplicates, overall target values (= ManuMean) as mean of peer group means. System comparability and quality was assessed by correlation and regression analysis of peer group means against the ManuMean, but also based on laboratory quality indicators grouped by system, i.e., (i) run-to-run quality and peer group performance, and (ii) overall performance. To calculate fail percentages (%) the following limits were used: (i) CV (2.5%), mean difference between the two replicates (2.5%), mean bias from the peer group target (3%), trend r (>0.65), outliers; (ii) correlation with the ManuMean (r <0.9), overall bias (3%), bias at range limits of sample concentration (3%, including confidence interval (CI) of regression line), number of results with a total error >5% (≥3 results), slope (<0.9 or >1.1, including CI of slope).

Results: Comparability of systems was excellent for total protein (<1.2%), moderate for calcium (maximum bias -2.6 %, Roche Modular), and problematic for albumin (-7.4 % Abbott Architect, 5.1 % Roche Modular). For magnesium, a high bias was observed for the Abbott Architect assay (11.2 %). The overall fail % (across systems and analytes) for run-to-run quality and peer group performance was 4.7% (range systems: 2.5% - 8.6% [Siemens Advia, due to calcium & magnesium assays]; range analytes: 3.1% - 6.6% [calcium]). The system fail % for overall performance was higher (16%), due to standardization problems (range systems: 6.5% - 33% [Abbott Architect, magnesium & albumin]; range analytes: 3.8% - 30% [albumin]). The laboratory fail % for run-to-run quality and peer group performance amounted to 4.9 % (range indicators: 2.6% - 7% [difference from peer]; range analytes: 3.4% - 6.5% [calcium]). The laboratory fail % for overall performance was higher [17%, mainly due to failures in magnesium & albumin assays], reflecting the dominance of system performance.

Conclusions: The study showed the potential of EQA using native sera. It revealed in particular the need to improve comparability of calcium, magnesium and albumin assays, as well as the basic quality of some calcium and magnesium assays. This will require more stringent control of lot variation (calcium), re-standardization (Abbott magnesium) and international standardization (albumin).

A-43

Interpatient distributions of bloodspot areas for fixed-volume application of blood: comparison between filter paper (FP) cards and non-cellulose dried matrix spotting (DMS) cards, and correlations to hematocrit

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Background: Bloodspot area per unit volume of blood is known to vary across patients in bloodspot preparations using standard bloodspot cellulose-based filter paper (FP). Dried matrix spotting cards (DMS, Bond Elut, Agilent Technologies, Santa Clara, CA) are a non-cellulose alternative to FP for bloodspot collection. Our objectives in this study were to compare the distributions of bloodspot areas across patients for fixed-volume samples using FP and DMS, and to evaluate extent of correlation between bloodspot areas and hematocrits for FP and DMS bloodspots.

Methods: A random selection of EDTA whole blood adult patient samples stored at room temperature were utilized within 8 hours of measurement of hemoglobin and hematocrit (Sysmex XE-5000). Replicate (4x) bloodspots were produced by bolus drop application of whole blood via a fixed-volume 50 µL pipettor to either FP or DMS. Bloodspots were dried at room temperature overnight. Bloodspot areas were determined by image analysis (Visual Basic) of 24-bit color, 300 dpi JPEG images obtained using a desktop scanner (Hewlett-Packard Scanjet G3110). Blood-colored area was distinguished from white background by green reflectance (G: integer values 0-255), counting pixels meeting criterion of G < 1.2 Gmax, where Gmax was the maximum G within a 20-pixel reference area at the bloodspot center. Area calibration was 14432 pixels/cm^2.

Results: Samples were from 50 patients (25 males, 25 females), median age 57 years, range 22-88 years. Hematocrits (H) were normally distributed (H = 30.9±5.3 %, r^2=0.987) with range of 19.7-42.7 %. For both FP and DMS, mean bloodspot areas (A,

cm²) across patients were normally distributed (r^2>0.99). For FP: A = 1.11±0.056 cm², range 0.93-1.25 cm² (coefficient of variation (CV)=5.0%); for DMS: A = 0.378±0.037 cm² (CV=9.9%), range 0.295-0.473 cm². Area ranges across patients for both FP and DMS represented substantial % variations: for FP, % differences in areas across the range were +29% (maximum relative to minimum) or -22% (minimum relative to maximum); for DMS, % differences were +60% (maximum relative to minimum) or -38% (minimum relative to maximum). Average intrapatient CV of mean areas of 4 x 50 µL bloodspot replicates across patients was 3.1±1.4 % (FP) and 5.9±2.7 % (DMS). By linear regression, FP areas were negatively correlated to hematocrit: (r=-0.80). In contrast, DMS areas were positively correlated to hematocrit (r=+0.78). There was essentially no correlation between patients' FP and DMS bloodspot areas (r^2<0.01). Patients' DMS bloodspot areas were on average 34±3.2 % of their FP bloodspot areas.

Conclusions: Interpatient variation of bloodspot areas for manual fixed-volume applications of EDTA whole blood was significant for both FP and DMS, with 5.0 and 9.9% CVs, respectively, and with >20% differences between highest and lowest areas within both of the observed ranges of areas. Hematocrit was a significant variable influencing area distributions for both FP and DMS. Interpatient variation in blood volume per area is thus a preanalytical variable in use of either FP or DMS bloodspots. Such variation could in principle affect certain quantitative analyses, such as drug concentration measurements, when using bloodspot punch samples of fixed area.

A-44

Stability of 1,25-dihydroxyvitamin D at Physiological Concentrations in Human Serum

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Background: 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) is the biologically active metabolite of vitamin D that plays an important role in calcium and phosphate regulation. 1,25-dihydroxyvitamin D exists in two biologically active forms, 1,25(OH)$_2$D$_2$ and 1,25(OH)$_2$D$_3$, with a reference range of 15 to 60 pg/mL. There is limited information available in literature regarding the stability of 1,25(OH)$_2$D in human serum at physiological concentrations. In this study, our goal was to investigate the stability of 1,25(OH)$_2$D$_2$ and 1,25(OH)$_2$D$_3$ in human serum at two different physiological concentrations under two commonly encountered storage conditions in a clinical lab.

Methods: Left-over patient serum was pooled, separated into two batches, and labeled "low" and "high". The "low" was spiked with 1,25(OH)$_2$D$_2$ only, while the "high" was spiked with both 1,25(OH)$_2$D$_2$ and 1,25(OH)$_2$D$_3$. Both "low" and "high" were then aliquoted and 3 baseline samples from each batch were frozen at -80°C, while the remaining aliquots were stored either at room temperature or under refrigeration. Aliquots were then retrieved in duplicate from every batch under each storage condition and frozen at -80°C at different days. Frozen aliquotes were then thawed simultaneously and analyzed by a published LC-MS/MS method in a single batch. **Results:** Mean serum baseline concentrations were 10.3 and 32.7 pg/mL in "low" and 19.1 and 45.6 pg/mL in "high" for 1,25(OH)$_2$D$_2$ and 1,25(OH)$_2$D$_3$, respectively. 1,25(OH)$_2$D$_2$ and 1,25(OH)$_2$D$_3$ in serum were stable under both examined conditions for at least 1 week (Table 1).

Conclusions: 1,25(OH)$_2$D$_2$ and 1,25(OH)$_2$D$_3$ in serum at physiological concentrations are stable for at least a week with no special care when stored at room temperature or under refrigeration.

Stability of 1,25-dihydroxyvitamin D2 and D3 at different temperatures and concentrations

			Room Temperature			2-8 °C		
1,25(OH)$_2$D, pg/mL		Baseline	24 h	5 days	7 days	24 h	5 days	7 days
D2 Low	Mean	10.3	11.1	11.0	9.4	10.3	10.2	10.1
	Range	8.1-12.3	11.0-11.2	9.5-12.4	8.4-10.3	10.2-10.4	9.2-11.2	9.8-10.3
	% of baseline	100	108	107	91	100	99	98
D2 High	Mean	19.1	22.4	20.4	22.9	19.9	22.3	22.0
	Range	17.9-20.2	20.4-24.3	20.2-20.6	21.2-24.6	19.0-20.8	20.5-24.1	20.8-23.2
	% of baseline	100	117	107	120	104	117	115
D3 Low	Mean	32.7	34.7	34.1	30.8	34.4	33.6	32.7
	Range	30.8-33.9	33.4-36.0	32.8-35.3	30.1-31.4	32.5-36.2	32.8-34.4	30.2-35.1
	% of baseline	100	106	104	94	105	103	100
D3 High	Mean	45.6	44.6	43.6	42.4	45.1	43.9	44.8
	Range	42.4-50.5	44.3-44.8	41.7-45.5	40.8-43.9	44.8-45.3	39.3-48.5	44.2-45.4
	% of baseline	100	98	96	93	99	96	98

A-45

Long-term stability of laboratory tests for chronic kidney disease

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Background: Long-term stability of laboratory tests is of vital importance for adequate management of chronic kidney disease. This study investigated the multiyear stability of test results for serum calcium, albumin, phosphate, 25-hydroxyvitamin D (25OHD) and parathyroid hormone (PTH) in 2 university hospital laboratories (Brussels and Ghent).

Methods: Both laboratories provided patient and internal quality control (IQC) data, the former for 14 and 9 years, respectively, the latter covering a 4 to 5 years period. The study considered the patient data 50th- percentiles, however, after exclusion of data from weekends/holidays and outliers (deviation >4 SD). For albumin, 25OHD and PTH, working days with a low number of results (n<5) were excluded. Further stratification was necessary for albumin (only results of outpatients) and PTH (exclusion of days with a high value for the 50th-percentile and/or a high number of results). Moving means of the 50th-percentile were plotted together with the mean of the IQC data to visualize shifts and drifts. The IQC data were normalized for IQC lot changes. The long-term mean of the 50th-percentile and the most representative CV for stable periods was calculated, together with the percentage deviation from the long-term mean for the most negatively and positively biased period lasting ≥4 weeks. This allowed investigation of the effect of the deviation on the percentage of results outside the locally chosen cut-off values (=percentage hypo and hyper) and the ratio thereof. For 25OHD and PTH, we used for that purpose the variation observed in the IQC data.

Results: From the graphical presentation of the moving means of the 50th-percentile of patient data and the mean of the IQC results, we could conclude that for calcium, the IQC data followed the patient data closely. The same was true for phosphate and albumin, apart from an occasional mismatch. For 25OHD and PTH the variation in the patient data was much higher than in the IQC data. Differences between patient long-term means of both laboratories were within 10%, except for 25OHD (24%). The CV of the daily 50th-percentile in stable analytical periods ranged from ~1% for calcium, over ~2-4% for albumin and phosphate to ~20-30% for 25OHD and PTH. Maximum positively or negatively biased periods showed biases in the order of 3-4% (calcium, phosphate-Ghent), 6-10% (albumin, phosphate-Brussels, PTH) and 20% for 25OHD. The ratio of the percentage hypo and hyper in negatively and positively biased periods was negligible (ratio ≤1.2) for phosphate-Ghent, PTH-Ghent; it was pronounced (ratio 1.4 - 2.4) for calcium-Brussels, phosphate-Brussels, 25OHD-Brussels, PTH-Brussels; it was high (ratio 4.8 - 26) for calcium-Ghent, albumin and 25OHD-Ghent.

Conclusions: Patient percentiles make it possible to assess and control the medium- to long-term stability of assays. However, the utility may be restricted to medium and high volume analytes. In other cases (in this study 25OHD and PTH), IQC data must be used as index for analytical stability. As a "surrogate" medical outcome we have defined the frequencies of abnormal results defined by the cut-offs. This shows that analytical differences in the market could significantly affect medical outcomes.

A-46

Oxalate analysis of hemodialysate

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Background: Oxalate is a dicarboxylic acid, an end product of glyoxalate and glycerate metabolism, that is excreted in the urine where it is a common component of kidney stones (up to 85%). Hyperoxaluria can be either genetic (e.g. Primary Hyperoxaluria) or acquired/secondary (e.g. enteric hyperoxaluria), and can lead to nephrocalcinosis and renal failure. Monitoring the adequacy of oxalate removal during hemodialysis can be useful in the management of patients with hyperoxaluria and renal failure, particularly following transplantation.

Methods: An enzymatic oxalate oxidase method for measuring oxalate in plasma and urine was adapted for dialysate fluid. At the time of analysis samples were acidified to pH 2.5 - 3.0, and

250 µL of the sample was incubated with reagents A and B from the Trinity Biotech oxalate kit (Trinity Biotech plc, Bray, Co. Wicklow, Ireland). The reaction yields hydrogen peroxide, which in the presence of peroxidase reacts with a dye to give a colored end point that is measured photometrically at 590 nm (Beckman Coulter DU800). Patient dialysate and plasma samples were used to validate analytic

performance and calculate rates of oxalate removal.

Results: Dialysate oxalate measurements were linear over the range of 2- 66 μmol/L. Precision of the method improved with the level of signal from a CV of 10% at 1.4 μmol/L to <2 % for values above 10 μmol/L. Treatment of dialysate samples after collection was critical, since oxalate levels increased variably among patients dialysate samples over 3 days unless the sample was acidified (pH<3.0) and kept refrigerated (4ºC) or frozen (-20ºC). In a clinical validation study hourly dialysate flow rates together with oxalate levels were used to calculate total oxalate removal for individual patients, and compared to known rates of oxalate production.

Conclusions: Oxalate can be precisely measured in hemodialysate using an oxalate oxidase method adapted from a sensitive plasma method. Pre-analytic acidification is essential to prevent non-enzymatic prediction of oxalate, as has been well-described during measurement of oxalate in plasma. This assay can be used to calculate oxalate removal during dialysis of hyperoxaluric patients, and facilitates individualized dialysis prescription for this select group.

A-47

The bias of routine hemostasis testing due to underfilling of primary blood tubes is not mathematically predictable.

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Background: The activities of the manually intensive preanalytical phase, especially those directly associated with collection of blood specimens, represent the most vulnerable steps throughout the total testing process. This is dramatically amplified in certain diagnostic areas such as hemostasis testing, where high quality standards other than those traditionally applied to clinical chemistry, hematology and immunochemistry testing are required. A peculiar problem in hemostasis testing is underfilling of primary blood tube, which is the third most common preanalytical problem after spurious hemolysis and undue clotting. The objective of this study was to verify whether mathematical correction of values by polynomial equations would help predict the bias in hemostasis testing due to tube underfilling, thus preventing specimen rejection and sample recollection.

Methods: Blood was drawn from 27 subjects (21 healthy individuals recruited among the laboratory staff as well as 6 patients on warfarin therapy) by a plastic syringe and immediately transferred into 3.6 mL primary blood tubes containing 3.2% buffered sodium citrate (Terumo Europe N.V., Leuven, Belgium). The 3.6 mL tubes were filled at 100%, 89%, 78% and 67% of the nominal volume by adding 3.6, 3.2, 2.8 and 2.4 mL of citrate anticoagulated blood. The tubes were properly mixed and centrifuged at 1300 x g for 10 min at room temperature. The plasma was separated and tested for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FBG), factor VIII (FVIII) and activated protein C resistance (APCR) on ACL TOP (Instrumentation Laboratory, Milan, Italy), employing proprietary reagents. The study was carried out in accordance with the Declaration of Helsinki, under the terms of all relevant local legislation and all patients provided informed consent. Statistical analysis was performed with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK).

Results: Although the correlation coefficients (r) were always greater than 0.79, in no case a model based on a polynomial equation including percentage of filling (PF) of primary blood tubes and the corresponding variation of values could reliably predict the bias, i.e., PT = 17.87 - 0.06387xPF + 0.0003444xPF^2 (adjusted r=0.94; p=0.20); APTT = 46.6 - 0.24PF + 0.0009106PF^2 (adjusted r=0.94; p=0.20); FBG = 142.8 + 4.307PF - 0.02158PF^2 (adjusted r=0.98; p=0.11); FVII = 83.31 + 1.718PF - 0.008488PF^2 (adjusted r=0.79; p=0.36); and APCR = 2.11 - 0.02139PF + 0.0001255PF^2 (adjusted r=0.83; p=0.32).

Conclusions: The results of this study clearly show that the bias in hemostasis testing due to underfilling of primary blood tubes cannot be reliably predicted by the use of polynomial equations. As such, primary blood tubes filled at less than 80 to 90% of the nominal volume should not be processed.

A-48

Hemolysis, Icterus and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis

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Background: Prevention of medical errors is an ongoing goal of health care. Medical errors that may arise from preexamination variables such as hemolysis, icterus, and lipemia/turbidity (HIL) in patient specimens may interfere with accurate measurement of analytes. The addition of automation to the specimen inspection process can improve HIL detection by introducing harmonization and uniformity, improving quality and efficiency of laboratory processes, and, most importantly, enhancing the accuracy of reportable patient test results. This information is addressed in the new CLSI document C56, *Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline*

Methods: HIL indices are (semi) quantitative estimates of hemoglobin, bilirubin, and turbidity that can be spectrophotometrically detected in a sample (eg, serum, plasma), and expressed in ordinal values (eg, +1, +2,) or actual concentration units (eg, mg/dL) to assess sample quality. Absorbance readings at several strategically selected wavelengths allow the calculation of the HIL indices. Instruments from different manufacturers vary in how they implement their HIL applications.

Parameters to be considered in establishing HIL indices are source and concentrations of interferents, sample type, volume, diluent, wavelengths, read times, calculation algorithms, and number of indices. An alert index indicates the lowest tested concentration of interferent at which the analyte concentration would be falsely increased or decreased. Example: Bilirubin Interference Testing for Glucose and Assignment of Alert Index and Gray Zone. A dose response test was performed for the glucose assay on the effects of increasing concentrations of bilirubin. Any bias greater than 10% is considered an interference. An interferograph was created and the lowest concentration of bilirubin that caused 10% or greater bias is considered an interference as presented in the CLSI C56 document. This bilirubin concentration is established as the alert level and assigned with an "icterus" index in the glucose analyzer. The gray zone is also assigned. An I index of 4 is the gray zone, while an I index of 5 is the alert index assigned for glucose.

Results: The glucose method was evaluated for interference according to CLSI document EP07, *Interference Testing in Clinical Chemistry; Approved Guideline_ Second Edition.* Bias is the difference in the results between the control (without the interferent) and the test (with the interferent) expressed as a percentage. Bias exceeding 10% is considered interference. In this example,unconjugated bilirubin at 15 mg/dL showed a bias of 15% on a sample containing 100 mg/dL of glucose.

C56 provides procedures so that the laboratory can establish its own guidelines for addressing patient samples that need HIL error flags , such as when reporting interference error flags without the results, rejecting the patient results, redrawing the specimens with considerations given to neonatal and geriatric populations, and notifying the health care provider (physician).

Conclusions: The CLSI document, C56 enhances the continuous education of health care personnel by explaining the mechanisms of HIL interference; the establishment, strengths, and limitations of HIL measurements; and the verification of HIL indices in the clinical laboratory.

A-49

Effect of cholesterol VLDL levels on HDL and LDL cholesterol quantification by two homogeneous methods.

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Background: in the present study we investigated the causes by which 0,18% of the samples analyzed by a homogenous method for the quantification of HDL cholesterol (cHDL) and LDL cholesterol (cLDL) sum more than the total cholesterol (TC), in our analytical platform 3 DIMENSION VISTA 1500 (D-V). We think would be interesting to find out if the cause could be a different composition of the lipoprotein fractions of these samples. For it we decided to compare these samples with an ultracentrifugation method (quantification beta; BQ) and another homogenous one in another platform MINDRAY BS-380 BIOSYSTEMS (M-B).

Methods: during 2 months the samples of our routine were selected (n=968± 105 daily), that met the following condition: TC< cHDL+cLDL. Twice at week we carried out the cHDL and cLDL determinations by other homogenous method and also the BQ for cHDL, cLDL andc VLDL (group1). As group control, were selected 30 samples randomly in 2 different days from our daily routine in which TC> cHDL+cLDL (group2). The maximum time of storage at 4ºC of the samples, was 24 hours. The test t-Student was used for comparison between groups.

Results: the cHDL concentrations were significantly higher in the homogenous methods of group1; this did not happen in group2 and agreed with a significant lower fraction cVLDL (BQ) in group1. The table where it is both reflected, its concentration average and standard deviation (parenthesis) in mg/dL of groups and his statistical significant p (*) is shown below.

Conclusions: the quantifications by homogenous methods of cHDL would

overestimate this fraction when the TC< cHDL+cLDL, due to cVLDL concentration would be lower than general population. This could have consequences in order to evaluate the coronary risk of a patient.

GROUP	N	TC	cHDL(D-V)	cHDL(M-B)	cHDL(BQ)	cVLDL(BQ)
1	33	176.8(37.5)	63.3(16.4)	63.6(14.1)	47.4(12.2)	5.85(6.65)
2	30	187.9(45.5)	51.5(16.5)	51.9(11.6)	44.7(8.94)	16.8(13.0)
p		0.245	0.0018*	0.0009*	0.236	0.00006*

A-50

Evaluation of the Interference by Hemoglobin on selected Roche Cobas 6000 Assays

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Objective: Hemolysis is the most common pre-analytical source of error and is the main cause for rejection of samples. The delay in sample re-collection and processing compromises patient care. Knowledge of possible effects of hemolysis is important for correct interpretation of the results. The aim of this study was to evaluate the effects of hemolysis on ten routinely used biochemical analytes (Figure 1). Findings of this study may help with correct interpretation of patient's results and also prevent unnecessary rejection of samples.

Methods: Pooled lithium heparin plasma samples were spiked with various concentrations of hemoglobin (1 - 8 g/dL) to simulate hemolysis. The selected analytes (Figure 1) were measured on Roche Cobas 6000 analyzer and the change in analyte concentrations were compared and calculated. The Hemolysis index (H-Index) causing 10 % change in the analyte concentration was considered as the cut-off limit of H-index for sample rejection. Each valve represents the average of three separate measurements.

Results: Effect of hemolysis on Potassium (K+), Creatine kinase (CK). N-terminal pro B-type natriuretic peptide (NT-proBNP), Haptoglobin (Hp), and direct bilirubin (D-Bili) were depicted in Figure 1A. Effects on Folate, Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), Parathyroid hormone (PTH) and Troponin T (TnT) were depicted in Figure 1B.

Conclusions: In the present study no significant effect due to hemolysis was observed for TnT, PTH and NT-proBNP while all other studied analytes showed significant bias. Laboratories should use H-Index limit provided in the kit inserts as a study guide rather than as a cut-off limit for rejecting the samples, which may result in unnecessary rejection of some samples, while lack of information on others may lead to reporting of erroneous results. Laboratories should therefore evaluate the manufacturer's recommended H-index before they are put into routine practice.

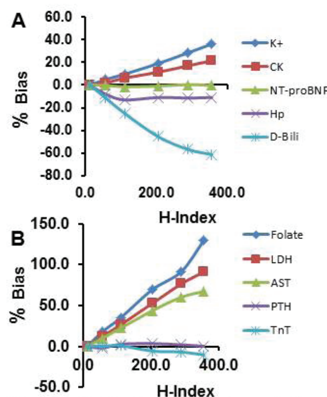

Figure 1. Interferogram for hemoglobin and measured parameters: K+, CK, NT-proBNP, Hp and D-Bili (A). Folate, LDH, AST, PTH and TnT (B)

A-51

Effect of using different quality criteria in an experimental study in order to evaluate the comparability of patient results between two biochemistry analyzers

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Introduction: Nowadays many laboratories have multiple instruments to provide analytical results for an individual patient. To assure comparability of patient results between different analyzers, a periodic verification is needed. Moreover, it is included as an ISO 15189 requirement for accreditation. Otherwise, a unique procedure has not been established and different quality specifications could be applied to determine the allowance criteria. Our emergency laboratory has been accredited by ISO 15189 since 2005. Quality assurance system includes internal quality control monitoring (IQC) and participation in External Quality Assurance programs (EQAP).

Objective: Assess an experimental design to evaluate comparability of patient results between two biochemistry analyzers depending on different quality criteria.

Material and Methods: Following CLSI C54-P recommendations, every fifteen days over one year, a pool was obtained by mixing five lithium heparin patient plasmas that had been collected and analyzed in the previous four hours. A single run was performed in two Dimension Vista (Siemens HD) simultaneously. Throughout the year, IQC and EQAP were monthly monitored and the analytical goals based on biological variation accomplished. The difference between every pair of data was calculated and expressed as a percentage related to mean. This result was compared with four quality criteria: Fraser (1/3 Intraindividual biological variation), Reference Change Value (RCV), desirable Total allowable error (Tea) based on biological variation and CLIA´88.

Results: Twenty-four pairs of data per test were obtained. n represents times that differences were greater than each criteria.

TEST	Measuring Interval	Fraser (%)	n	RCV (%)	n	Tea (%)	n	CLIA (%)	n
ALT (UI/L)	20-72	8.1	3	20.1	1	32.1	0	20	1
Amylase (UI/L)	35-106	2.9	8	7.2	1	14.6	0	30	0
AST(UI/L)	17-73	3.9	17	9.9	11	15.2	2	20	2
Calcium (mg/dL)	7.8-9.2	0.6	19	1.6	14	2.4	10	1 mg/dL	0
Chloride (mmol/L)	101-111	0.4	18	0.9	6	1.4	6	5	0
Creatinine (mg/dL)	0.62-2.59	1.8	20	4.4	15	8.2	8	15 (0.3 mg/dL)	2 (0)
GGT (UI/L)	24-200	4.6	6	11.4	1	22.2	0		
Glucose (mg/dL)	83-233	1.9	15	4.7	8	6.9	3	10 (6 mg/dL)	0 (8)
LDH (UI/L)	221-358	2.9	6	7.1	1	11.3	0	20	0
Magnesium (mg/dL)	1.71-3	1.2	19	2.9	11	4.8	5	25	0
Phosporus (mg/dL)	2.5-4.4	2.8	16	7.0	4	10.2	1		
Potasium (mmol/L)	3.7-4.7	1.6	10	3.9	1	5.8	0	0.5 mmol/L	0
Sodium (mmol/L)	136-146	0.2	23	0.6	21	0.9	18	4 mmol/L	1
Total protein (mg/dL)	6-7.6	0.9	22	2.2	14	3.4	5	10	1
Urea Nitrogen (mg/dL)	27-68	4.1	8	10.2	0	15.7	0	9 (2 mg/dL)	1 (8)
CRP (mg/dL)	11-109	14.1	2	34.9	0	56.6	0		

Conclusions: Quality criteria show many differences in assessing comparability of patient results. Each laboratory should select its own criteria according to the quality assurance specifications and operative characteristics. This feasible approach could be used as a warning strategy, in addition to IQC and EQAP routinely. However, more replicates or an increase in monitoring frequency could be needed for some analytes with more strict control requirements.

A-52

Improved Uniformity in a Serological Assay for Anti-Sm by Treatment of Sm Autoantigen With RNase

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Background: Antibodies to the nucleoplasmic autoantigen Sm, which is composed of RNA and at least seven polypeptides, are associated with certain autoimmune

diseases, notably systemic lupus erythematosus. Commercial lots of Sm exhibit considerable differences in patient results in serological assays for anti-Sm IgG.

Objective: The aim of this study was to investigate whether incubation of commercial Sm antigen lots with RNase would confer improved lot-to-lot consistency as demonstrated in an anti-Sm serology assay.

Methods: Sm antigen lots were acquired from a commercial source. Each of the individual lots were chemically coupled to microparticles and then conditioned with RNase (Agilent Technologies, Santa Clara, CA). These solid-phased antigens, and antigen-bound control microparticles in which RNase was not employed, were used to assay 137 sera with the BioPlex 2200 ANA screen (Bio-Rad Laboratories, Hercules, CA).

Results: Hydrolysis of RNA in commercial Sm antigen lots improved lot-to-lot consistency in the anti-Sm assay. The table below shows pronounced improvement in the correlation of assay results obtained with six Sm lots compared to a reference lot (W707). In every case the slope, y-intercept, and correlation coefficient of the regression graph significantly improved.

	RNase	Regression analysis statistics versus lot W707		
Sm lot	treatment	Slope	y-Intercept	R^2
T304	No	0.72	0.08	0.636
T304	Yes	0.97	0.01	0.943
N111	No	0.67	0.08	0.634
N111	Yes	0.92	0.01	0.963
N206	No	0.73	0.08	0.697
N206	Yes	0.94	0.01	0.992
T010	No	0.84	0.05	0.799
T010	Yes	1.01	-0.02	0.958
T309	No	0.74	0.12	0.682
T309	Yes	0.94	0.03	0.974
C803	No	0.87	0.07	0.839
C803	Yes	0.93	0.02	0.952

Additionally, some assay results shifted from positive to negative in a commercial anti-Sm ELISA when incubated with RNase.

Conclusions: The presence of RNA in affinity purified Sm antigen can serve as a contributor to autoantigenicity, lead to inconsistency between autoantigen lot performances, and possibly produce false positive results. Degradation by enzyme hydrolysis of Sm bound RNA that is sterically available for binding by autoantibodies results in a more uniform assay performance. Serum antibodies that bind to the Sm protein complex when associated with RNA are precluded from binding after removal of RNA sterically available on the complex surface. Sm antigen with degraded RNA allows for more reproducible anti-Sm assays.

A-53

Cross-reactivity of insulin analogs in the Elecsys insulin assay

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Background: Insulin secretagogues are being synthesized for use in diabetes mellitus treatment. Reports have determined the cross-reactivities to these preparations of many automated insulin assays, but not to newly developed ones. We evaluated the cross-reactivity of the Elecsys insulin assay (Roche Diagnostics, Mannheim, Germany) for the Cobas e601 analyzer.

Methods: The cross-reactivity to 7 commercial insulin preparations was assessed on the Cobas e601 analyser. These were as follows: insulin lispro; Humalog® (Eli Lilly and Co., Indianapolis, IN, USA), insulin glargine; Lantus®(Sanofi-Aventis, Paris, France), insulin detemir; Levemir® (Novo Nordisk A/S, Bagsvaerd, Denmark), biphasic insulin lispro; Humalog® Mix 25_{TM} (Eli Lilly and Co.) and Humalog® Mix 50_{TM} (Eli Lilly and Co.), biphasic isophane insulin; Humulin® 70/30 (Eli Lilly and Co.), biphasic insulin aspart; NovoMix® 30 (Novo Nordisk).

The recombinant insulin analogues employed in this study were pharmaceutical preparations, which were obtained at a nominal concentration of 100 IU/mL. All insulin analogues were diluted with the Roche Universal Diluent (Elecsys insulin concentration <0.2 mIU/L). Final insulin concentrations of 0, 50, 100 and 1000 mIU/L were achieved. All dilutions of each insulin preparation was analyzed in duplicate, and the percentage cross-reactivity was calculated from the ratio of the measured and nominal concentration. Measurements were made on cobas e601 analyser using the dedicated reagents according to the instructions.

Results: Most of the insulin preparations showed little cross-reactivity with the Elecsys insulin assay, except 2 insulin preparation. Humulin® 70/30 (biphasic isophane insulin) showed cross-reactivity of 83.6% (81.1-86.7%). Another cross-reactivity was observed with Lantus® (glargine) with a value of 6.8% (5.3-7.7%).

Results: Most of the insulin preparations showed little cross-reactivity with the Elecsys insulin assay, except 2 insulin preparation. Humulin® 70/30 (biphasic isophane insulin) showed cross-reactivity of 83.6% (81.1-86.7%). Another cross-reactivity was observed with Lantus® (glargine) with a value of 6.8% (5.3-7.7%).

Conclusions: This study has evaluated the cross-reactivity of 7 preparations in the Roche cobas e601 insulin assay. We found absence of cross-reactivity with some insulin analogue in Elecsys insulin assay, but Humulin did not. When investigating hypoglycaemia it is important to be aware of the cross- reactivity of the recombinant insulin analogues in Elecsys insulin assays.

A-54

Evaluation of automated serum hemolysis index measurement on Roche Cobas c501

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Objective: The objective of this study were i) to evaluate the linear correlation between serum hemolysis index on Roche Cobas c501 and the actual concentration of hemoglobin, and ii) to determine the range of serum hemolysis index corresponding to different degree of hemolysis. Once the performance is verified, the automated assessment of sample integrity will replace the visual inspection that is often time consuming and provides inconsistent results.

Methods: A hemolysate stock solution of 14 g/dL was prepared using patient's whole blood sample collected in EDTA tube. The whole blood was centrifuged to pack cells, washed with saline, lysed with deionized water and followed by separation of the supernatant (i.e. hemolysate) from pellet. A pooled serum was prepared and spiked with the hemolysate stock solution to give five tubes of different hemoglobin concentration levels (0.1, 0.2, 0.3, 0.4 and 0.8 g/dL). The serum hemolysis index of each tube including a neat sample were measured on Roche Cobas c501 and plotted against the hemoglobin concentrations. The hemoglobin concentrations were measured using Sysmex XE2100.Sixty-one patient samples were visually assessed and graded by twenty-two medical technologists for their hemolysis degree and measured on Roche Cobas for their hemolysis indexes. The visual assessment results were then compared against the hemolysis indexes to determine the range that corresponds to each degree of hemolysis.

Results:

Visual assessment	Serum Hemolysis Index Range	Hemoglobin Concentration (g/dL)
Normal	<27	< 0.05
Mild Hemolysis	27 - 64	0.05 - 0.10
Hemolysis	65 - 170	0.11 - 0.35
Gross Hemolysis	>170	> 0.35

Conclusions: This study showed that there is a linear relationship between the serum hemoglobin index and the concentrations of hemoglobin. The serum hemolysis index ranges were determined and installed in our LIS to automatically attach an appropriate comment in a patient's result. Automated serum indices measurement minimizes technologist's intervention, save operator's time and provides consistent and reliable assessment of sample integrity.

A-55

Reduction of Specimen Rejection Rate using Clinical Practice Improvement Model

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Objective The objective of the project is to reduce the specimen rejection rate at Alexandra Hospital (AH). A surge in specimen rejection rate to >2% was observed across all departments during the transition period of taking over Alexandra Hospital by the new JurongHealth team. The benchmark for acceptable specimen rejection rate is targeted at <1%. We applied the Clinical Practice Improvement (CPI) Model, which is a proven methodology for improving clinical outcomes, efficiency and safety within the healthcare environment, to this project.

Methods There were 5 phases in the improvement process.

1. Project phase: A team with the relevant stakeholders representing all parts of the process was formed.

2. Diagnostic phase: Regular meetings and brainstorming sessions were conducted to

map the processes and evaluate root causes.

3. Intervention phase: The team identified the gaps where improvement is required and planned appropriate interventions.

4. Impact and Implementation phase: Interventions based on a “trial and learning” approach were carried out (see table below). A run chart is plotted to determine if the interventions have resulted in improvement.

Problem	Intervention	Start date
Hospital staff was not aware of the increasing specimen rejection rate.	Submission of Electronic Hospital Occurrence Report (eHOR) to create awareness.	09/2010
Incorrect sequence of draw and wrong tube type.	New hires are taught the correct blood taking techniques during orientation.	12/2010
Hospital staff not aware of specimen rejection criteria.	Rejected specimen is returned to requesting unit with form that highlights the reason for rejection.	01/2011
Mismatched specimen	Implement second tier check.	01/2011
Ward staff lack of phlebotomy skill	Extend phlebotomist service.	01/2011

5. Sustaining Improvement phase: The team review and keep track of the progress to ensure the interventions are executed consistently and systematically over time.

Results: We managed to achieve the specimen rejection rate below 1.0% for the first time in March 2011, after 6 months of interventions, and the result was sustained ever since.

Conclusions The use of CPI Model was successful in the reduction of specimen rejection rate at Alexandra Hospital.

A-56

High-dose hook effect on urine human chorionic gonadotropin sample from a patient with molar pregnancy.

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Background: One-step sandwich hCG (human chorionic gonadotropin) immunoassays are susceptible to a well-documented interference known as the high-dose hook effect. Current literature describes an association of the hCG hook effect with molar pregnancies and trophoblastic diseases, but rarely provide quantitative data illustrating this phenomenon. Understanding the occurrence and limitations of such assays in the clinical laboratory is important to elucidate patient diagnosis and perform appropriate management. Case: A 20-year-old woman (G3P1A1) presented to the University of Chicago Medical Center emergency department (ED) with chief complaints of vomiting and abdominal pains. She reported mild vaginal bleeding and bilateral abdominal pain in the past five to six days, and was concerned that these symptoms suggest pregnancy. Her last menstrual period occurred almost two and a half months ago. Given these preliminary findings, a differential diagnosis of threatened abortion versus pregnancy was ascertained. The patient had a qualitative urine hCG test done in the ED, and additional blood and urine samples drawn and sent for routine laboratory analyses.

Results: A qualitative urine β-hCG immunoassay (QuickVue+ One-Step hCG Combo, Quidel Corp., San Diego, CA) initially performed in the ED revealed a very faint positive result. Transvaginal limited ultrasound finding was negative for intrauterine pregnancy. However a quantitative serum β-hCG assay (Roche Elecsys®, Indianapolis, IN) showed an extremely elevated value of 1,142,700 mIU/ml (obtained on dilution). The discrepancy between the falsely low qualitative urine β-hCG reading and high serum β-hCG reading led to a suspicion of a high-dose hook effect on the urine sample, prompting a repeated laboratory analysis on a second urine sample. Serial dilutions of the second urine sample showed a progressively stronger positive signal on the Quickvue qualitative test with dilutions of 10-fold, 50-fold, and 100-fold, which weakened by the 10,000-fold dilution. This hook effect pattern was quantitatively reproduced on both the Elecsys β-hCG and ADVIA Centaur® XP total hCG + β (Siemens Diagnostic, Tarrytown, NY) assays, which showed a final hCG value of ~5,000,000 mIU/ml. Since results from all three β-hCG assays (QuickVue+, Elecsys, Centaur) confirmed extremely high urine hCG values on appropriately diluted urine samples, the patient was referred for a more thorough ultrasound study that now showed the presence of a hydatidiform mole. She was promptly taken to the operating room for a hysterectomy.

Conclusions: Clinical laboratory results revealed discordance between serum and urine hCG results. Additional studies showed that the initially trace positive urine hCG test was due to a high-dose hook effect caused by extremely high levels of hCG. Timely recognition and resolution of this artifact averted the misdiagnosis of the presence of a hydatidiform mole.

A-57

Reducing the rejection rate of NSE by detecting low hemoglobin in serum and cerebrospinal fluid using Nanodrop spectrophotometer

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Background: Neuron specific enolase (NSE) is a serum tumor marker for neuroendocrine neoplasms (e.g. small cell lung cancer and neuroblastoma), and more recently has also been used as a marker of hypoxic brain injury in patients with cardiac arrest. In cerebrospinal fluid (CSF), NSE is utilized as a biomarker of neuronal damage in neurodegenerative conditions like Creutzfeld-Jakob Disease. The accuracy of NSE measurements is greatly affected by even slight hemolysis, due to the abundance of NSE in red blood cells. We reject hemolyzed specimens containing >20 mg/dL of hemoglobin (Hb) when the NSE concentrations are above the reference range (serum =<15ng/mL, CSF <30ng/mL). Our NSE rejection rate due to hemolysis is 14% for serum and 1.5 % for CSF. In part, this might be due to our current method of measuring Hb with a HemoCue Plasma/Low Hb spectrophotometer, an instrument that is inaccurate at Hb levels <50mg/dL, overestimating lower Hb levels by up to 30%. In addition, this instrument requires expensive disposable microcuvettes.

Objective: Our aim was to validate the Nanodrop ND-1000 spectrophotometer as a more reliable and cheaper alternative to the HemoCue for measuring low free Hb concentrations in serum and cerebrospinal fluid.

Methods: We used de-identified residual hemolyzed patient samples to conduct these studies. Serum and CSF samples were centrifuged at 2000 rpm for 10 min. Free Hb concentrations were measured using the Nanodrop ND-1000 (2 uL of undiluted sample, 10 sec measurement) and compared to a validated Perkin Elmer Lambda 35 Spectrophotometer (100 uL of sample for dilution preparation, 3 min measurement). We measured sample absorbance at 415 nm (total hemoglobin), 450 nm (bilirubin) and 700 nm (sample turbidity) on both instruments and calculated the Hb concentrations as follows: Hemoglobin (mg/dL) = $154.7\ A_{415nm} - 130.7\ A_{450nm} - 123.9\ A_{700nm}$

Results: The Nanodrop’s intra-assay imprecision CVs were < 7% at 3, 60 and 125 mg/dL Hb in serum and < 7% at 3, 40 and 130 mg/dL Hb in CSF, respectively. The Hb measuring range extended from 3 to 200 mg/dL without dilution. No carryover was observed. The Nanodrop measurements agreed closely with those obtained on the Perkin Elmer Lambda 35 spectrophotometer: Serum (N = 42): y = 1.056x -0.349 and $R^2 = 0.995$; Cerebrospinal fluid (N = 22): y = 1.016x +0.255 and $R^2 = 1.000$. Hb recovery in samples spiked with human hemolysate ranged from 72 to 106% (mean 98%) in serum and from 91 to 111% (mean 103%) in CSF. Linearity ranged from 103 to 110% (mean 106%) in serum and from 87 to 105% (mean 99%) in CSF. Visible lipemia and bilirubin interfered with hemoglobin measurement.

Conclusions: The Nanodrop ND-1000 provides a fast, accurate and sensitive measurement of free Hb in serum and cerebrospinal fluid. Its more reliable measurements of low Hb concentrations reduce the NSE sample rejection rate in our practice.

A-58

Effect of stress on the blood Analytics “Somatostatin, Histamine and Serotonin” (neuropeptide and autoacoids) Indirect collective determination of the effect of stress

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Background: Comparisons have been performed for decades between analytics in the systemic and portal blood. The effect of the stress of major operation on the levels of portal blood analytics’ levels was not put in consideration . The effect of surgery (major stress factor) on Somatostatin , Histamine and Serotonin resulted in a change in their levels. The minute change of the levels due to the operative stress is the focus of this study. This is an indirect way of testing the effect of neuropeptides on levels of blood analytics.

Methods: 40 patients suffering of portal hypertension undergoing major operations were selected from Kasr El Aini Hospital, Cairo University. Three blood samples were taken from each patient for each of the tested parameters. Samples were taken from (a) systemic veins before the operation, (b) another sample from systemic veins after induction of anesthesia and start of the operation at a fixed timing, (c) the last sample from the portal veins taken by the help of the surgeon. Somatostatin was tested by RIA, Histamine and Serotonin by chemical flurometric methods. The sample (b) for each of the tested parameters is the indicator to the change in levels due to the stress. Two groups of patients were selected : bleeders and non bleeders from porto-systemic shunts.

Results: The mean Somatostatin level (pg/ml) in the bleeders was : sample (a) 26,6+/-6.2; sample (b) 31.6+/-6.2; sample (c) 47.2+/-13.4; where as its level in the non-bleeders was sample (a) 27.0+/-3.3; sample (b) 29.8+/-3.5; sample (c) 44.75+/-8.75. The mean Histamine level(µg/ml) in the bleeders was: sample (a) 154.3 +/-115; sample (b) 93,75+/-83.43; sample (c) 343.9+/-339.6. In the non-bleeders the mean was sample (a) 182.2+/-196,5; sample(b) 130+/- 179.5,sample (c) 292.5+/-266.7. The mean Serotonin level (ng/ml) was in the bleeders sample (a) 288,8 +/-149,5l; sample (b) 285.5+/-146.4; sample (c) 328+/-134.9. The mean level in the non-bleeders was: sample (a) 291+/-146; sample(b) 288+/-146.2; and sample (c) 272.1 +/-134.5. The stress factors affected the tested parameters as shown in the change in the levels in sample (b) in both groups.

Conclusions: The stress affects the level of secreted neuropeptides and autacoids. This is reflected on the level of hormones , neuropeptides and autocaoids of the gut as tested in the portal blood of portal hypertensive patients. The levels of analytics tested in the portal blood stream should be modulated according to the degree of change due to stress in order to obtain the real portal levels . This is an indirect way for collective detection of the effect of stress hormones and neuropeptides , that are released in stress situation.

A-59

Influence of HbE on measurement of HbA1C

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Introduction: The measurement of glycosolated hemoglobin (HbA_{1c}) provides an excellent measure of glycemic control for the vast majority of patients with diabetes; however, measuring this marker may be unreliable in some situations. The most common interference in HbA1c analysis is due to hemoglobin (Hb) variants. The four most common variants worldwide are HbS, HbE, HbC, and HbD. HbE is the most common variant in regions of Southeast Asia where the prevalence can be as high as 30% of the indigenous population. HbA_{1c} analysis is routinely performed by Variant II turbo 2.0 in our laboratory. The aim of this study was to investigate the influence of HbE trait on HbA_{1c} values measured by cation-exchange HPLC.

Methods: Hb variants were identified by Bio Rad Variant analyzer in 93 of 3522 samples sent to the laboratory in the month of Jan 2012. Hemoglobin electrophoresis was performed to identify the Hb variant. HbE trait was diagnosed in 81 samples. To study the influence of HbE trait on HbA_{1C} values by Variant II Tubo 2.0, boronate affinity HPLC method (Primus PDQ) was used as the comparison method. Boronate affinity method has been shown not to be affected by the presence of HbE variant. Bland Altman plot and Deming regression analysis were performed to analyze whether the presence of HbE trait produced a statistically significant difference in the results. The total allowable error by RCPA EQA is 5%. Hence clinically significant difference is being more than 5% at the medical decision level of 6 and 9%.

Results: Statistically and clinically significant higher results were observed in Variant II Turbo 2.0 due to the presence of HbE trait. A positive bias of ~ 10% was observed at the medical decision levels.

Conclusions: In managing diabetic patients, knowledge of Hb variants influencing HbA_{1C} determination methods is essential since this may result in mismanagement of diabetes owing to false HbA_{1C} results.

A-60

Influence of a combined collection method on hemolysis.

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Background: Objective. To measure the influence of different collection methods on hemolysis in a hospital emergency department. Relevance. Hemolysis is one of the main causes of error in the preanalytic phase. There are many causes and they have been widely studied. The repercussions are very important as they cause collections and tests to be repeated and a delay in diagnosis, which affects patient safety. To this must be added the economic impact which the repetition of tests has, and the number of hours that the patient must stay in the hospital emergency department. Hemolysis can oscillate between 8% - 18% of the total number of samples analyzed.

Methods: In order to measure the influence of a new blood collection system in the hospital emergency department, the nursing staff were trained for two hours. The new system was explained and basic aspects of venipuncture were tackled in the week before the change of system. Later, the usual vacuum collection systems were substituted by a system that combines the vacuum principle with that of aspiration, for one week, with that being the only system available for collection. After a week the original system was restored. Hemolysis was determined by measuring the serum rates (hemolysis) with the piece of equipment analyzer. All the samples with hemolysis serum rate higher than 1 were classified as hemolyzed. The percentage of hemolyzed samples and non-hemolyzed samples were measured, in the week when the new collection system was being used, the week immediately prior to the change, and the week immediately after the change. Also the percentage of hemolysis in the same week as the study but the year before was measured, to avoid bias. It is intended with this design to measure the influence of the new collection system, and of the training, on the hemolysis percentage. Validation. The total number of samples analyzed in the study was 1787. The statistical study was carried out by Chi-Square test, and the minimum number of samples for establishing comparisons was estimated at 420. The influence of the new collection system compared with the conventional one was measured, by means of the hemolysis percentage in the week of the trial and in the previous week; the influence of the training given was measured by means of the hemolysis percentage in the week of the trial and in the week afterwards. To avoid any bias or proximity effect, the hemolysis percentage was measured in the week of the trial and the same week the previous year.

Results: The hemolysis percentage with the newly introduced method was 6.9% compared to 17.45% reached the week before. In the week immediately afterwards a hemolysis percentage of 13.5% was obtained, and the hemolysis detected the year before amounted to 13.5%. The differences found in the usage of the new collection system were significant in all cases (p< 0.000).

Conclusions: The combined collection system significantly reduces hemolysis, thus contributing to an improvement in patient safety.

A-61

An Evaluation of Interference by Hemolysis, Lipemia and Icterus on the New AU5800® Clinical Chemistry System.

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Background: Hemolysis, lipemia and icterus in serum and plasma samples are common sources of potential interference in routine clinical chemistry assays. The aim of this study was to determine the levels of interference at which significant measurement bias was observed using AU® reagents on the Beckman Coulter AU5800® Clinical Chemistry System. The AU5800 is a series of ultra-high throughput clinical chemistry systems with up to four connected units completing up to 8000 photometric tests/hour plus electrolytes

Methods: 53 AU reagents were evaluated in this study. Pooled patient samples with values close to test levels recommended in the CLSI EP7-A2 guidelines were spiked with either hemolysate, Intralipid* or bilirubin solutions to simulate hemolysed, lipemic and icteric samples, respectively. For each interferent, samples were prepared with varying concentrations of interferent and the concentration at which interference occurred was determined by plotting the measured analyte value against the interference concentration. A recovery difference greater than 10% from the non-spiked sample was deemed to be a significant interference.

Results: Data in the Table below summarizes the results gathered using a representative panel of 12 out of the 53 reagents tested on the AU5800. The effect of interference on Glucose, GGT and α-Amylase was minimal with <5% bias demonstrated for each interferent. Creatinine, Urea and Calcium also show excellent interference performance. Lactate achieved an interference of <10% at 4mg/dL bilirubin, while CRP achieved interference of <10% at 28mg/dL bilirubin and of <10% at 600mg/dL Intralipid.

Conclusions: Of 53 reagents tested on the AU5800, interference performance data was demonstrated to achieve acceptable performance for clinical laboratory needs.

Reagent	Interference					
	Hemolysate		Intralipid		Bilirubin (Icterus)	
	Level	AU5800 Result	Level	AU5800 Result	Level	AU5800 Result
	mg/dL	Bias %	mg/dL	Bias %	mg/dL	Bias %
Glucose	500	<5%	700	<5%	40	<5%
Creatinine	500	<5%	700	<5%	20	<10%
Total Protein	500	<10%	1000	<10%	40	<10%
Albumin	450	<10%	800	<10%	40	<5%
Urea	250	<5%	500	<10%	20	<5%
Inorganic Phosphorous	350	<10%	900	<10%	40	<10%

Calcium Arsenazo	500	<5%	1000	<10%	40	<5%
GGT	350	<5%	1000	<5%	40	<5%
Cholesterol	500	<10%	1000	<5%	8	<10%
CRP	500	<10%	600	<10%	28	<10%
α-Amylase	350	<5%	1000	<5%	20	<5%
Lactate	500	<10%	1000	<10%	4	<10%

* All trademarks are property of their respective owners.

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Gender specific reference intervals for serum total bilirubin in healthy Korean adults

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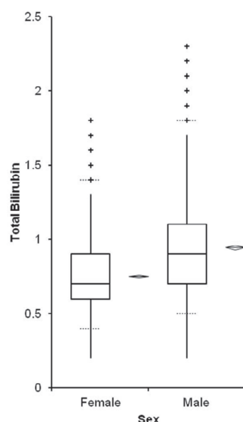
Background: Bilirubin is an endogenous product that is formed during the process of heme degradation. Generally, only one common reference interval for serum total bilirubin regardless of gender has been used in most clinical laboratories. Here, we evaluated between-gender difference of serum total bilirubin in a large Korean population.

Methods: Among apparently healthy adults aged 19 years or older who visited for a health check, 4,935 subjects (2,168 male and 2,767 female) were selected as reference individuals, excluding individuals with common latent disease and outliers. Since total bilirubin values were not normally distributed, the reference interval was determined using non-parametric method.

Results: Separate and combined reference intervals for total bilirubin were 0.5-1.8 mg/dl for men, 0.4-1.4 mg/dl for women, and 0.4-1.6 mg/dl for total. To test statistical significance of the gender-difference in total bilirubin we used three different methods, Mann-Whitney U test, the standard normal deviate test, introduced by Harris and Boyd and cited in CLSI guideline, and the criteria proposed by Lahti et al. Through all three different methods, we could consistently find that gender specific reference interval was recommended (Figure 1). The higher hemoglobin level in male might partially contribute to the higher bilirubin in men since bilirubin is the major metabolic product of hemoglobin. When we divided the study population to subgroups according to hemoglobin level (12-14, 14-16, and 16-18), we found significant mean and median difference of bilirubin between this subgroups. We also found significant correlation between bilirubin and hemoglobin ($R = 0.346$, $P < 0.001$). The marginal increase of total bilirubin in men has been problem for the clinician, often prompting a more extensive and expensive tests although without other clinical and laboratory evidence of disease.

Conclusions: Therefore the gender specific reference interval could result in the reduction of the marginal increase and unnecessary workup cases in male.



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Pseudohyperphosphatemia in a Multiple Myeloma Patient Caused by Paraprotein Interference

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Background: High blood levels of paraproteins in multiple myeloma patients have been reported to interfere with numerous chemistry and even some hematology tests. Specimens from these patients are a diagnostic challenge for the laboratory and often require special procedures for accurate measurements. Because paraprotein interference is not predictable as to analyte or degree of interference it is often undetected leading to unnecessary testing and/or diagnostic workups. The present case involves a 57 year old male inpatient with multiple myeloma on chemotherapy with an IgG-kappa paraprotein level of 5.2 g/dL. This patient was brought to the laboratory's attention because of an extremely elevated phosphorus result of 15 mg/dL (Ref. Interval 2.5 - 4.5) which was unexpected, clinically inconsistent, and, on subsequent investigation, found to be factitious.

Objective: To investigate whether the paraprotein was causing the dramatic elevation of phosphorus in this patient's plasma by utilizing several different methods to remove paraproteins or minimize their effect.

Methods: Serial dilutions are a practical first step to detect an interfering substance and in this case 1:2, 1:4, and 1:8 serial dilutions were performed with normal saline. Three other methods to remove paraproteins were utilized. (1). A protein-free supernatant was prepared by adding equal amounts of a 7% perchloric acid solution and serum, mixing on a vortex mixer for 1 minute, incubating for 10 minutes in an ice bath and centrifuging for 10 minutes at 3,500g. (2). A globulin-free supernatant was prepared by mixing equal volumes of polyethylene glycol 6000 and serum, mixing on a vortex mixer for 1 minute, incubating in an ice bath for 20 minutes and centrifuging at 3,500 x g for 20 minutes. (3). Preperation of a globulin-free ultrafiltrate with a centrifugal filter device, Amicon Ultra-4 10 K, which eliminates proteins of nominal molecular weight of 10,000 and above. 2 mL of plasma was added to the centrifugal filter device which was then centrifuged for 20 minutes at 3,500 x g and produced 0.5 mL of ultrafiltrate. Phosphorus measurements were performed on the Roche Modular P System.

Results: Serial dilution of this patient's plasma with normal saline demonstrated striking non-linearity and at a 1:8 dilution phosphorus was calculated to be 4.0 mg/dL in the original plasma sample. Since serial dilutions do not remove paraproteins, three other methods were used to remove paraproteins before measuring phosphorus. Phosphorus results were 4.1, 2.8 & 3.6 mg/dL after ultrafiltration, polyethylene glycol precipitation, and perchloric acid precipitation, respectively. All three methods demonstrated that removing the paraprotein produced phosphorus levels in the normal range for this patient.

Conclusions: We have demonstrated that the high levels of an IgG-kappa paraprotein in this patients plasma interfere with phosphorus measurements on the Roche Modular P System by an unknown mechanism, causing elevations of a normal phosphorus level to more than three times the upper limit of normal.

A-65

Evaluation of Biotin Interference in Beckman Coulter Immunoassays that Use Biotin-Streptavidin in their Assay Design

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Introduction: Biotin may interfere with immunoassays that rely on biotin-streptavidin interactions for analyte detection. Recently, our laboratory was involved in troubleshooting discrepant total T3 results between two immunoassays (Roche Elecsys® and Beckman Access®). In this case the total T3 concentration was >3000 ng/dL with the Beckman Coulter immunoassay and 320 ng/dL with the Roche immunoassay. It was determined that the total T3 levels with the Beckman Coulter immunoassay were falsely elevated due to the presence of high levels of biotin in the patient serum. At the time of this incident biotin was not recognized as a potential interference in the Beckman Coulter total T3 assay package insert. More recently, the package insert information has been updated to list biotin as a potential interference. However no information is provided regarding the concentration at which biotin might cause significant interference.

Objective: To determine the concentration at which biotin interferes in various Beckman Coulter (Brea, CA) immunoassays that use biotin-streptavidin in their assay design.

Methods: The following immunoassays were evaluated: free T3 (FT3), total T3 (TT3)

and thyroglobulin (Tg). Total T4 (TT4) was included as a control since biotinylated antibodies are not used in this assay. Three serum pools were generated for each test (FT3, TT3, TT4 and Tg). The respective concentrations were: low (1.5 pg/mL, 29 ng/dL, 5.0 mcg/dL, 1.90 ng/mL), intermediate (2.5 pg/mL, 85 ng/dL, 8.0 mcg/dL, 13 ng/mL) and high (20 pg/mL, 260 ng/dL, 12 mcg/dL, 540 ng/mL). A biotin (Sigma-Aldrich, St. Louis, MO) stock solution was prepared to a final concentration of 5000 ng/mL. The serum pools were spiked with biotin concentrations ranging from 6.3 to 500 ng/mL. The spiked serum pools were tested using a Beckman Coulter UniCel™ DxI 800 (Brea, CA). Clinically significant interference from Biotin was defined as a difference of > 20% from the control sample (non-spiked).

Results: The low pool for TT3 showed significant interference at all biotin concentrations tested. The intermediate pool for TT3 showed significant interference at all levels except the lowest biotin concentration tested (6.3 ng/mL). The high pool for TT3 showed significant interference at biotin concentrations = or > 25.0 ng/mL. All three pools of FT3 showed significant interference at biotin concentrations = or > 100 ng/mL. Tg showed significant interference at biotin concentrations = or > 50 ng/mL in all three pools. T4 serum pools were unaffected at all concentrations of biotin tested.

Conclusions: The TT3 assay was the most sensitive to biotin interference with lower concentrations of biotin having a significant effect on TT3 levels. The other assays (FT3 and Tg) were also affected but at much higher concentrations of biotin. This highlights just how important it is for the manufacturers of these assays to provide more specific information regarding the concentration at which biotin interferes with the assay and the expected magnitude of the change.

A-66

Effects of light exposure on serum folate, vitamin B12 and 25-hydroxyvitamin D measurements.

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Background: In many labs, serum samples for folate, vitamin B12 (B12) and total 25-hydroxyvitamin D (25OHD) measurements are drawn and kept in separate tubes under dark conditions. Although this may potentially reduce analyte degradation, it adds cost and inconvenience to the lab. To address this question, we compared serum levels of these 3 analytes in samples handled under light and dark conditions.

Methods: Ten men and 10 women ranging from 26 to 93 years participated in the study. In each patient, samples were drawn, centrifuged and kept under the same conditions in 2 primary tubes (Monovette Serum Gel Z, Sarstedt). One tube was wrapped with aluminum foil (Group 1), whereas the other was not handled under dark conditions (Group 2). Folate and B12 levels were measured in all samples by chemiluminescence in Vitros 5600 (Ortho Clinical Diagnostics), whereas total 25OHD was assayed by electrochemiluminescence in Modular Analytics E170 (Roche Diagnostics). Analytical measuring range and mean inter-assay variation were 0,6-20,0 ng/mL and 11,8% for folate; 159-1000 pg/mL and 7,2% for B12; 3-70 ng/mL and 4,3% for 25OHD, respectively. Results were expressed as mean ± SD and compared by paired t-tests (p at 0,05). They were also analyzed by EP Evaluator 9 software (Data Innovations) using Deming regression and an error index calculation based on the difference between values for each specimen. Results were considered equivalent (clinically identical) in both groups if they did not exceed allowable imprecision, which was set at 7,5% and 12,0% for folate and B12, respectively, according to biological variation database (Ricós C, 2010 edition). Since no such data is available for 25OHD, imprecision was defined as 15,3% for this analyte, based on the average CV of results reported by CAP survey participants.

Results: There were no significant differences between Groups 1 and 2 for serum folate (14.13 ± 4.27 vs 14.39 ± 4.44 ng/mL, $p = 0,24$) and 25OHD levels (28.3 ± 7.9 vs 28.6 ± 8.1 ng/mL, $p = 0,40$). B12 measurements were slightly lower in Group 1 than in 2 (397.7 ± 121.8 vs 378.0 ± 116.8 ng/mL, $p < 0,01$). There was a high correlation between groups for all analytes (R of 0.99, 0.96 and 0.99 for folate, B12 and 25OHD, respectively). All folate and 25OHD samples showed equivalent results in Groups 1 and 2 (both tests *passed*, according to EP Evaluator), with an average error index of 0.15 and 0.05, respectively. In contrast, only 17 B12 specimens were considered equivalent (test *failed*), with an average error index of -0.65.

Conclusions: Light exposure does not affect folate and 25OHD measurements, suggesting that sample handling under dark conditions is not required for these tests. However, light causes a small but significant reduction in B12 levels, which may not be clinically relevant.

A-67

Critical Appraisal of Biological Variation Data

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Biological variation data have many established applications ranging from the setting of analytical goals for imprecision and definition of quality specifications to, assessment of significance of change in serial results (reference change values (RCV)). Review of the literature on biological variation identifies a significant volume of work stretching back some 40 years. Much of this data has been incorporated into published reviews and web based databases that make them accessible to laboratory specialists. Given the importance of these applications, and the many others, there is an imperative that these fundamental data are fit for purpose. If the data are flawed in any way, or inapplicable to the population to which they are being applied, then the application must be considered to be erroneous. These data, like all data, will be subject to uncertainty that will impact on their usefulness. Uncertainty arises from design of the experiments from which the data are derived, the assay characteristics and integrity of the data analysis. Furthermore, extrapolation of published data to local populations of interest requires an understanding of the factors affecting commutability of those data and a clear understanding of the characteristics of the population originally studied. The complexity of these data cannot be underestimated and valid application of them in the fiield requires clear understanding of their defining characteristics and limitations. Biological variation data should in fact be considered reference data and schema should exist to enable their critical appraisal and to support their publication and application. A European Federation of Clinical Chemistry (EFCCC) Working Group on Biological Variation has been established and is undertaking work to deliver a proposal for a critical appraisal checklist applicable to biological variation data. An outline of a proposal is presented with examples of its application to published data. www.biologicalvariation.com

A-68

Discordant serum polyclonal IgA measurements revealed unusual presence of human anti-sheep antibodies in a patient serum sample

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Background: Interfering substances in immunological assays can produce serious analytical errors. Human anti-mammalian antibodies are common, but often unsuspected interfering substances found in patient samples. Such antibodies can have broad reactivity against mammalian immunoglobulins and can therefore react with a wide range of immunoassay reagents.

Total IgA and Hevylite (HLC) IgAK+IgAλ were assayed in a serum sample from a patient presenting with chronic intestinal inflammation. Polyclonal IgA measurements were 2.947 g/L for the total IgA assay and 4.441 g/L for summated HLC IgAK+IgAλ. The unanticipated poor agreement between the two polyclonal IgA measurements was assessed further.

Aim: To identify the cause of the disagreement between total IgA and Hevylite (HLC) IgAK+IgAλ measurements

Method and Results: The patient serum sample was analysed by serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE) to detect any serum abnormalities. SPE and IFE showed elevated serum polyclonal IgA and did not reveal any monoclonal protein. This sample was then further analysed by immunoelectrophoresis (IEP) to determine the nature of the analytes that react with the HLC reagents that were raised in sheep. IEP showed at least two arcs of precipitation which would indicate that patient sample contained antibodies to sheep proteins. Further analyses were carried out by Ouchterloney to confirm reactivity between the HLC reagents and the patient sample. Two arcs of identity were clearly identified. This prompted us to investigate presence of anti-sheep antibodies in the patient sample. Analysis of the sample against a non specific sheep serum alongside a panel of mammalian serum species was performed using the Ouchterloney technique. The patient sample showed strong reactivity against sheep serum and to a lesser extent other mammalian serum species included in the analysis. A latex blank assay performed with particles coated with non specific sheep IgG confirmed the IEP and Ouchterloney data indicating that the patient sample reacted specifically with sheep immunoglobulin.

Conclusions: Poor agreement between total IgA and summated HLC IgAK+IgAλ observed in an unusual patient sample is likely to be due to the presence of human anti-sheep immunoglobulin. We hypothesized that total IgA result, using reagents also raised in sheep, will also be affected but not to the same degree, as there will be cumulative effect for the two HLC assays. This interesting and unusual sample

provides an example of discordant measurements caused by human anti-mammalian antibody interference. Any analytical discrepancies should prompt laboratorians and clinicians to request further analysis to rule out or confirm the presence of circulating human anti-mammalian antibodies.

A-69

Are maternal serum screening biomarkers stable in serum *and* whole blood?

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Background: Non-invasive prenatal screening involves measurement of biomarkers in maternal serum during the first and/or second trimester of pregnancy. Because patient samples may be collected at distant sites, the time between sample collection and processing is an important source of pre-analytical variation. The objective of this study was to determine the stability of five maternal serum screening biomarkers in whole blood and serum, over a time period that reflected realistic delays in sample processing and storage.

Methods: Whole blood was collected by venipuncture from 22 pregnant women at 14-28 weeks of gestation. A baseline serum aliquot was frozen within 75 min of collection. Whole blood and serum were aliquoted and stored at room temperature (RT, 21-22°C) or refrigerated (3-5°C) for 2, 12, 24, 48 or 72 hours and then serum was recovered from the whole blood aliquots. All serum was frozen at -70°C until analysis. Alpha fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3), dimeric inhibin A (DIA), and pregnancy-associated plasma protein A (PAPP-A) were measured on a UniCel® DxI chemistry analyzer (Beckman Coulter Inc, Fulterton CA). Values were expressed as a percentage difference relative to baseline concentrations. Three-way within-subjects analysis of variance was used to determine the effect of sample type (serum or whole blood), storage temperature (RT or refrigerated) and time (five time-points), on each biomarker. Data analysis was performed using JMP 9.0.2 (SAS Institute Inc, Cary NC) and SPSS 11.0 (SPSS Inc, Chicago IL). The study was approved by the University of Utah Institutional Review Board.

Results: Storage temperature had a statistically significant effect on uE3 ($F(1,21)=9.21$, $p=0.006$). Concentrations were lower irrespective of whether serum was separated prior to storage (mean: -3.2% RT; -1.4% refrigerated, relative to baseline). Sample type had a significant effect on AFP and DIA concentrations although this was influenced by storage temperature (sample type x temperature interactions: $F(1,21)>15.5$, $p\leq 0.001$). At RT, mean AFP concentrations were 3.3% above baseline in whole blood, but -0.5% in serum; values were not different between sample types when refrigerated. Mean DIA was -0.05% of baseline when serum was refrigerated, but 2.4% higher in refrigerated whole blood; this difference was not apparent at RT. The effect of sample type on hCG and PAPP-A varied by temperature and time (sample type x temperature x time interactions: $F(4,84)>2.8$, $p\leq 0.031$). Follow-up testing identified significantly higher PAPP-A in whole blood stored at RT for 48 and 72 hours (average 6.6% above baseline), while hCG was lower in serum stored at RT for 72 hours (-4.2% of baseline). Despite statistically significant effects, differences in biomarker concentrations with time, storage temperature, or sample type may not have similar clinical significance. Ninety percent of biomarker concentrations were within 10% of baseline.

Conclusions: Concentrations of most biomarkers used for maternal serum screening were not significantly affected over a three-day storage time. Storage temperature had an effect on all biomarkers which often differed by sample type. The clinical impact of these findings however, is likely marginal given that most changes were within 10% of baseline concentrations.

A-70

Interference of carbamylated hemoglobin with HbA1c measurement in patients with chronic renal failure

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Carbamylated hemoglobin (carb Hb) is formed by non-enzymatic condensation of cyanate with the N-terminal valine of hemoglobin. In chronic renal failure carbamylated hemoglobin is increased due to elevated urea, which is dissociated *in vivo* to yield cyanate ions. Carbamylated hemoglobin has been shown to interfere with measurement of HbA1c in diabetic patients with chronic renal failure, especially when using assay methods that rely on separation of hemoglobins based on charge. We evaluated eight commercially available ion-exchange Methods. G7 and G8 (Tosoh Bioscience), Variant II NU, Variant II Turbo, Variant II Turbo 2.0, D-10 and D-10 Dual (Bio-Rad Laboratories), HA-8160 (A. Menarini Diagnostics), two immunoassay Methods: Integra 800 (Roche Diagnostics) and DCA 2000 (Siemens Healthcare Diagnostics), and one enzymatic method: Direct Enzymatic HbA1c (Diazyme Laboratories). Presumably, hemoglobin species modified by reactants other than glucose and not displaying a cis-1,1-diol group should not interfere with measurement of HbA1c by boronate affinity methods. Therefore, we used the boronate affinity ultra^2 HPLC (Trinity Biotech) as our comparative method. Approximately 120 EDTA whole blood samples drawn from normal subjects and subjects in various stages of renal failure were analyzed by all methods. Each patient's eGFR was used to estimate the level of carb Hb. A multiple regression model was used to determine if the relationship between HbA_{1c} results obtained from each test method and the ultra^2 method were significantly ($p<0.05$) affected by eGFR. The D-10, D-10 Dual, DCA 2000, G7 and Direct Enzymatic methods showed statistically significant effects. These methods were further evaluated for clinical significance by dividing the samples into quartiles based on eGFR results (eGFR ≤11, 11< eGFR ≤ 45, 45< eGFR ≤ 84, eGFR >84). Deming regression was then used to compare the relationships between each method and the ultra^2 for the highest and lowest quartiles; a difference between the quartiles of >7% at 6 or 9% HbA_{1c} was defined as being clinically significant. Only the Diazyme Direct HbA1c enzymatic assay was found to have a clinically significant (13% relative difference at 9%HbA1c) effect of eGFR, presumably due to interference from carb Hb. Healthcare providers need to be aware of potential interferences when interpreting HbA1c results in clinical settings.

A-71

Clinical Chemistry Sample Interferences Reporting Patterns in Ontario Laboratories

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Introduction: Endogenous and exogenous substances cause interferences in clinical chemistry analyses and compromise the accuracy of test results. Three major endogenous interferents, hemolysis, icterus and lipemia/turbidimetry, cause errors in results, and assessment and reporting them are important for achieving accurate interpretation of results. The Quality Management Program - Laboratory Services (QMP-LS), which provides a mandatory proficiency testing (PT) program in Ontario, conducted a survey to investigate interference reporting patterns of Ontario laboratories.

Methods: Survey questions regarding assessment and reporting patterns of the three common endogenous interferents, hemolysis, icterus and lipemia/turbidimetry, were distributed to licensed Ontario laboratories together with the regular wet chemistry schemes. Nineteen community and 164 hospital laboratories participated to the survey.

Results:

1. Types of interferents assessed: 98% of all laboratories were assessing at least one of the following interferents: hemolysis (96%), icterus (87%), and lipemia /turbidity (94%).
2. Instrument or visual interference assessment: For the interference assessment, 38% of the laboratories used chemistry analyzers, while 62% of them evaluated visually. Among laboratories performing visual assessment, only 34% of them were using reference cards. Of the laboratories using chemistry analyzers for interferent assessment, 29% report interferences quantitatively, 62%, semi-qualitatively and 9%, qualitatively.
3. Interference reporting style: 58% of all laboratories reported the presence of interferents only for the effected analytes; 40% of laboratories reported the presence of the interferent against all analytes for a given specimen.
4. Interference information reported: 89% of the laboratories reported the presence of interference, 64% indicated the level of interferent, 75 % included which analyte is affected, and only 40% report the direction of the impact.

5. Source of interference reference: 95% of the laboratories used the information provided by the manufacturer to determine the affected tests. A small number of laboratories (8.1%) performed in-house interference studies, while others (6.6%) use other means to source this information.
6. Specimen recollection for hemolysis Interference: 85% of the laboratories provided some feedback indicating the necessity of recollection on the patient report; however, 15% do not.
7. Types of lipid clearing procedures: 67% of the laboratories did not use any type of lipid clearing procedures for analysis of specimens for analytes affected by lipemia. 5% were using lipid clearing reagents, 19%, ultracentrifugation, and 8%, other methods that include referral, micro-centrifugation and dilution. Two laboratories did not respond.

Conclusion: Although, hemolysis, bilirubinemia, and lipemia can have significant effects on accurate interpretation of test results, not all laboratories report them. Only 38% of the laboratories were using clinical chemistry analyzers for assessment of interference and 66% of the labs performing visual assessment were not using reference cards. A preferred approach would be to use the analysers or reference cards to promote standardization in reporting interferences. Since 95% of the laboratories obtained interference data from the manufacturers and this information is not readily available to health-care providers, identification of the affected analytes and direction of impact when reporting interferences would provide greater clarity and clinical utility.

A-72

Additive Contamination and Its Effects on Laboratory Testing

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

Background: The majority of the errors in laboratory test results occur in the preanalytical phase; and most of the preanalytical phase errors are due to phlebotomy techniques with more than 60% of these errors are due to insufficient specimen quality and quantity. Carryover/cross contamination is one of the causes of erroneous results that are due to the transfer of additive from one tube to the next. It could happen when blood in an additive tube touches the needle during blood collection and blood remaining on the needle can be transferred to the next tube, or when the blood is transferred from one tube to another if the tubes is underfilled to correct the volume.

Methodology: we asked a phlebotomist mix the order of draw for one set (carryover of the additive); and in the next set of experiments we had the phlebotomist add blood, immediately after drawing, from different additive tube to a serum separator tubes and in the third set the blood from different additive were added to citrate tubes.

Results: Contamination of the serum/plasma tube with EDTA, citrate and oxalates cause change in the chemistry values, and contamination in the coagulation (citrate) tube with EDTA additive increases in PTT with no significant change in PT/INR, and increases PT/INR and PTT with heparin contamination.

	Citrate to SST	EDTA to SST	Oxalate to SST	EDTA to citrate	Heparin to citrate
Calcium	No significant change	Decrease (undetectable)	Decrease (undetectable)		
Potassium	No significant change	Increase	Increase		
Sodium	Increase	No significant change	Increase		
Total protein	Slight decrease	Slight increase	Increase		
Pt/INR				No significant change	Increase
PTT				Increase	Increase
Liver enzymes	No significant change	Decrease ALP	No significant change		

Conclusion: carryover/cross contamination is one of the errors in preanalytical phase that can be prevented by enhancing training of the phlebotomist, increasing error detection in the laboratory by educating laboratory technicians about the effect of this error on test results and keep the communication open between all sections of the laboratory to pass the information to the appropriate supervisor to provide and document the necessary retraining and in-servicing to the phlebotomist involved and use it as education to the rest of the team.

A-73

EC50 of reagents used in blocking heterophilic interference in immunoassays

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Objective: To demonstrate a quantitative model for evaluating the effectiveness of commercially available blocking reagents in blocking heterophilic interference in immunoassays. Relevance: Heterophile antibodies are well known interferences that affect many immunoassay formats. Among the most commonly encountered Heterophile antibodies are human anti mouse antibodies (HAMA) and rheumatoid factor (RF), a type of heterophilic antibody with non-specific affinity for the Fc fragment of IgG. To avoid false positive or falsely elevated results immunoassay manufacturers typically add commercially prepared blocking reagents to prevent interference from heterophilic antibodies. Unfortunately quantitative data on the performance of various blocking agents is scarce. We present quantitative performance data on reagents commercially used in blocking heterophilic antibody interference.

Methodology: Data are derived from a model utilizing a CE marked immunoassay kit. Serial dilutions of each blocking agent were tested against a range of human serum samples containing HAMA, RF, or both. At each concentration of interferent a dose response curve was created and the EC50 of the blocking agents was calculated. Validation: At a HAMA concentration of 97 ng/ml the EC50 for the three blocking agents varied from $3x 10^{-7}$ to $8 x 10^{-8}$ mg/ml. In addition we tested a panel of samples representing a clinical range of HAMA and RF against each blocking agent with variable results.

Conclusions: These data represent a novel and effective approach to evaluating the efficiency of commercially available blocking reagents used in immunoassays.

A-74

Delays in the availability and communication of critical versus non-critical values at three academic medical centers

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Objective: To compare critical versus non-critical value reporting times at three academic medical centers.

Background: Critical values are life-threatening results upon which immediate clinical action should be taken. The timeliness of reporting critical values is therefore vital to patient safety. Furthermore, laboratories are required by regulatory agencies to communicate all critical values to a licensed care provider with the intention that interventions will not be delayed.

Methodology: We surveyed three peer academic medical centers of similar size and patient complexity. The duration between the availability of the result on the instrument to the release of critical and non-critical values in the electronic medical record as well as when it was communicated to a healthcare provider was measured. Validation: Three tests in different sections of the clinical laboratory [chemistry (potassium), immunoassay (troponin), hematology (platelet count)] were used in the analysis. One week of patient results for each test was obtained from each institution's laboratory information system and divided into 2 categories, critical and non-critical values specific for each hospital. Significant differences ($P < .05$) were observed between critical and non-critical value turn-around-times (TATs) for all three tests within and across medical centers (Table 1).

Conclusions: Critical potassium, troponin, and platelet count values take longer to report in the medical record compared to non-critical values in the three surveyed medical centers. Those times were extended in all but one case when reporting critical values to providers. The reason for the overall significant delays observed in individual laboratories critical value reporting may be attributed to different protocols for releasing and/or ensuring proper documentation of verbal communication with providers. More studies are needed to determine the best practice for releasing critical values.

Table 1.	Potassium (TAT in minutes)		Troponin (TAT in minutes)		Platelet Count (TAT in minutes)	
	Non-Critical	Critical	Non-Critical	Critical	Non-Critical	Critical
Institution A	8	32*	3	31*	19	40*
Institution B	2	11 (34*)	2	6 (22*)	1	24 (39*)
Institution C	2	18 (24*)	No critical defined		2	25 (19*)

* Time (minutes) from result available on instrument to documented communication with a provider.

A-75

Validation of a Process Improvement for Streamlining Collection and Processing of Blood Collections for Lactate Analysis

D. A. Dalenberg, D. R. Block, N. A. Block. *Mayo Clinic and Foundation, Rochester, MN*

Background: Lactate is frequently measured in hospitalized patients. The use of blood collection tubes containing anti-glycolytics largely inhibits pre-analytical increases in lactate. The current practice at our institution is to collect samples for lactate analysis in gray top (sodium fluoride/potassium oxalate (F/Ox)) tubes, transport on-ice to the Central Clinical Laboratory (CCL) processing area via pneumatic tube, centrifuge, manually aliquot plasma, and deliver plasma aliquot to chemistry testing area. The goal of this study was to validate a process improvement where lactate samples may be transported ambient (eliminating need for ice) and reduce unnecessary steps in pre-analytic processing of plasma lactate samples (e.g. manual aliquoting). Objectives: (1) Determine stability of lactate in F/Ox whole blood kept on-ice versus ambient and (2) Determine stability of lactate in plasma kept on-ice and ambient.

Methods: Blood was collected in F/Ox tubes (2 tubes/volunteer, gray top, Becton-Dickinson, Franklin Lakes, NJ) from ten healthy volunteers on two separate occasions. Five of the volunteers performed forearm exercises before collection to increase lactate concentration. Each whole blood F/Ox sample was divided into five aliquots and kept on-ice or ambient. At each time point (0, 1, 4, 8, and 24 hours) one aliquot from each set (on-ice or ambient) was centrifuged and lactate was measured in plasma (Vitros 350, Ortho Clinical Diagnostics, Rochester, NY). In the second collection, one set of samples was incubated on-ice and one set was kept ambient. Both sets of samples were centrifuged 30 minutes after collection (5 min, 3500*g*) and kept upright (on-ice or ambient). Plasma was sampled for lactate measurement at 0, 1, 2, 8, and 24 hours. Lactate concentrations at each time point were compared to the 0 hr time point.

Results: The mean(±SD) lactate concentration in healthy volunteers with and without forearm exercise was 4.4±2.1 and 1.0±0.2 mmol/L, respectively. In whole blood samples kept ambient for 1,4,8 and 24 hrs before centrifuging, the mean absolute difference (range) in lactate concentrations compared to the 0 time point was 0.0(0.0-0.1), 0.0(0.0-0.1), 0.1(0.1-0.2) and 0.1(0.1-0.2) mmol/L, respectively. For samples kept on ice, the mean absolute differences were comparable with a maximum of 0.1(0.0-0.1) mmol/L at 24 hours. The mean absolute difference from 0 hr (range) for ambient plasma samples (centrifuged and kept upright) were 0.0(0.0-0.1), 0.1(0.0-0.1), 0.2(0.1-0.2), and 0.2(0.1-0.2) mmol/L at 1,2,8 and 24 hours, respectively. Lactate concentrations in samples kept on ice were comparable with the maximum mean absolute difference of 0.1(0.0-0.2) mmol/L at 24 hours. Least squared linear regression analyses of the on-ice versus ambient samples centrifuged after 30 minutes and at various times points was y=1.05x-0.2 (R^2=0.99) and y=1.01x+0.1 (R^2=1.00), respectively.

Conclusions: Lactate concentrations in the ambient samples increased at a faster rate than the on-ice samples in both experiments, however the increase was never greater than 0.2 mmol/L. It is not necessary to transport lactate samples on ice or to manually aliquot plasma prior to testing leading to increased efficiency in processing of samples.

Tuesday AM, July 17, 2012

Poster Session: 10:00 AM - 12:30 PM

Lipids/Lipoproteins

A-78

The levels of lipids and apolipoproteins in patients with Vascular Dementia

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Background. The apolipoprotein A (apoA) is a main protein component of HDL holesterol and it accounts for approximately 65 % of the toltal protein HDL. The apolipoprotein B (apo B), is a main protein component of LDL holesterol and it accounts for approximately 95 % of the toltal protein LDL. If concentration of apo B is normal decrease apo A level may be a risk factor for atherosclerosis processes. Elevated levels of apo B is frequently found in patients with atherosclerotic vascular changes is a risk factor for atherosclerosis. The study was to determined concentrations of total cholesterol, HDL cholesterol, apo B, apoA and Lpa at patients' serum with vascular dementia.

Methods. The study included 400 subjects, 200 patients diagnosed with vascular dementia developed as a consequence of ischemic brain stroke, i.e. of many small ischemic focus of various age and 200 healthy subjects. Lipids were determined using DIMENSION LxR automatic analyser of DADE BEHRING. The cholesterol method is based on used of cholesterol esterase and HDL cholesterol is a homogeneous method for directly measurements. The apolipoproteins were determined using SIEMENS automatic analyser. The apo A, apoB and Lpa method is quantitative imunonefelometric measurement. Collected data were statistically analyzed using programs SPSS version 11.0 and Microsoft Office Excel 2003.

Results. Our results show that the concentration of HDL cholesterol and apo A were significantly lower in the group with and vascular dementia than in the control group. Average concentrations of cholesterol, apo B and Lpa were significantly higher in the group with vascular dementia than in the control group. In the group with vascular dementia, the concentration of apo B was higher than 1.33 g / L in about 60% (mean concentration 1,35 g/L) of patients then in control group whereas the concentration of Lpa was higher than the upper reference value in about 20 % of patients (mean concentration 0,37 g/L). The mean concentration of cholesterol 5.59 mmol/L was significantly higher in the group with vascular dementia then in control group 4.96 mmol/L. Cholesterol concentration is significantly different between the group vascular dementia, and the control group for $p<0.05$ (mean difference 0.628 $p = 0.006$). The HDL mean concentration in group with vascular dementia was 1.08 mmol/L and control group was 1.39 mmol/L. The HDL cholesterol concentration is significantly different between the group vascular dementia, and the control group for $p<0.05$ (mean difference 0.322 $p = 0.000$).

Conclusions. Increasing concentrations of apo B and Lpa in the serum of patients with vascular dementia affects the further development of atherosclerosis and the development of new stroke. The apo A is associated with higher cardiovascular risk in humans and it has cardioprotective role. The low concentration of HDL holesterol and apo A are connected with future cardiovalcular illneses and possible development of vascular dementia. Therefore measurement of lipids and apolipoproteins is important at patient with vacular disease.

A-82

Lipid Profile in oropharyngeal cancers in southern India

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Background: Oropharyngeal cancers are the most common cancers in India especially in rural population in North Coastal Andhra Pradesh where people practice reverse smoking and Tobacco chewing. These cancers are the mostly affects in terms of quality of life suffering from dysphagia and breathlesness. The present study was designed to evaluate the pattern of serum lipid profile in patients suffering from oropharyngeal carcinomas.

Materials and Methods: 59 patients with oropharyngeal carcinoma starting from oral cavity upto the level of esophagus have been selected from an age group ranging from 25 to 70 years with a mean age of 45 years from the department of Radiotherapy ,King George Hospital, Visakhapatnam from March 2011 to Aug 2011.72 healthy controls with an age group from 25 to 70 years with a mean age of 45 years were also selected. Out of the 59 cases of oropharyngeal cancers all were malignant histologically. Serum Lipid profile had been done to all patients after overnight fasting . Cholesterol, HDL- cholesterol and Triglycerides were measured by authentic methods and LDL cholesterol was measured by Friedwal's formula.

Results: Serum cholesterol and HDL cholesterol has been significantly elevated in patients with oropharyngeal cancers when compared with controls (p=0.007). whereas total cholesterol and LDL cholesterol was significantly lowered in patients when compared with controls (0.017 and 0.0062 respectively). Serum triglycerides and VLDL cholesterol were also lowered in cases but not significant.

Conclusions: The present study is showing that there is association between lipid changes and oropharyngeal tumors . Serum lipid profile is showing a beneficial effect in these cases needs further evaluation in larger scale.

Table

	TC	TG	HDL	VLDL	LDL
CTRL Mean	169.43	142.26	41.50	28.47	99.45
CTRL SD	37.31	76.06	07.33	15.19	36.24
Cases Mean	153.81	131.04	44.40	25.87	82.82
Cases SD	36.67	67.60	04.76	13.80	31.06
p-value	0.0177	0.3790	0.007	0.3118	0.0062

A-83

Homogenous Assay Compared to Ultracentrifugation of Small, Dense Low Density Lipoproteins in Healthy Individuals

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Objective Atherosclerosis is a chronic disease driven by several risk factors and one of the leading causes of morbidity and mortality in the developed world. Results from epidemiological studies have shown that sdLDL is an atherogenic lipoprotein fraction. In 2001 small, dense LDL particles (sdLDL) have been declared as an emerging cardiovascular risk factor and in 2008 it was suggested to measure concentrations of atherogenic LDL in patients at high risk for cardiometabolic disorders.

Methods We assessed the cholesterol content of small, dense LDL (sdLDL) measured by an automated homogenous assay (Denka Seiken, Japan) in 5280 probands, 1702 males aged 34-66 years and 3578 females aged 34-63 years, without any signs of metabolic or cardiovascular disorder. In 1071 probands we compared these measurements to the cholesterol content of LDL-5 and LDL-6, also denoted as sdLDL, separated by ultracentrifugation, the reference method. Measurements have been performed on Roche cobas® 6000 analyser system (cobas c 501).

Results Linear regression function for all probands is $y=0.993x+14.194$ with a 2-sided Pearson Correlation of 0.675 ($p<0.001$). However, there seem to be some outliers possibly due to elevated triglycerides (TG). If only probands with TG <400 mg/dl (n=1012) the regression function changed to $y=0.9687x+13.111$ with a correlation of 0.724 ($p<0.001$). We further assessed the association of the homogenous sdLDL assay with sex and age. As expected, concentrations of sdLDL are higher in males than in females and rise continuously with age. For patients with TG<400 mg/dL where data from both, the homogenous assay and ultracentrifugation were available the 80th percentile of homogenous sdLDL cholesterol for females was 45.7 mg/dl and 61.3 mg/dL for males, respectively. The corresponding values for apoB in sdLDL assessed by ultracentrifugation were 22.1 and 36.0 mg/dL, respectively. This corresponds well to the previously suggested threshold of 25 mg/dL apoB in sdLDL defining an atherogenic sdLDL profile in patients with type 2 diabetes.

Conclusions: The homogenous assay for sdLDL correlates well to sdLDL-fractions separated by ultracentrifugation. However, readings of both methods may not be identical because they address different properties of sdLDL. In general the investigated assay may provide a promising tool of measuring sdLDL suitable for large scale applications in research studies and in laboratory routine. However, the clinical usefulness of this new assay needs further evaluation.

A-84

Metabolic Syndrome in Menopausal Transition

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Background: This study was carried out in order to search for prevalence of metabolic syndrome during menopausal transition.

Methods: The material of our study were 532 women aged 45,4 ± 6,9 years, of which 60.2% had a positive family history of cardiovascular diseases, 46.6% were smokers and 9.1% had frequent alcohol use. All women were initially submitted to anthropometric examination (measurement of waist circumference, body weight and height), systolic and diastolic blood pressure while calculating the average pressure. Participants were categorized into three groups of pre-menopause, menopause and post-menopause. Leisure time physical activity and global dietary index were included as life style factors. The association of metabolic syndrome and its components with menopausal transition considering other factors such as age and life style was analyzed.

Results: There were 102, 70 and 360 women in premenopausal, early menopausal and postmenopausal groups respectively. Metabolic syndrome was found in 49 (48%) premenopausal participants and significantly increased to 41 (58.57%) and 270 (75%) in early menopausal and postmenopausal participants respectively. Except for hypertension and hypertriglyceridemia, there was no significant difference between three groups of menopausal transition when metabolic syndrome's components were considered. On the relationship of total physical activity with all laboratory measurements and blood pressure found no significant correlation.

Conclusions: In contrary to the claims regarding the role of waist circumference and blood glucose in increasing of metabolic syndrome during the menopausal transition, this study showed this phenomenon could be independence of them.

A-85

New enzymatic assay method for measuring pancreatic lipase activity in serum

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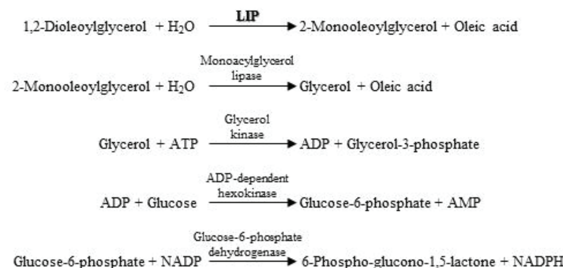
Background: The clinical importance of serum pancreatic lipase (LIP) concentration is well established for the diagnosis and monitoring of acute pancreatitis. We developed a novel LIP assay method, because there is a need for a reference assay to provide an accurate base to which routine methods can trace.

Methods: LIP activity is measured in a coupled enzymatic reaction from the increase in absorbance at 340 nm with production of NADPH at 37 °C, in which 1,2-dioleoylglycerol is the substrate (Figure).

Results: Sample blank measurements are essential, because nonspecific absorbance increase was occasionally observed. The method requires no special hardware and the constituent chemicals are all commercially available. The reactions were stoichiometric and approximately zero order. The K_m value of serum pancreatic lipase was calculated to be about 0.2 mM from the Substrate-Velocity curve. When pooled serum samples (containing about 54.1 and 164 U/L, respectively) were assayed using the manual procedure, the coefficients of variation (CV) of within-day reproducibility were 8.45 % and 1.56 %, the between-day reproducibility were 10.9 % and 2.3 %, respectively. LIP activity was linear up to 440 U/L (8-times expected upper limit of physiological concentration). The differences between the measured values and the theoretical values were -4, -15 or -46 U/L when the measured values were 369, 440 or 492 U/L, respectively. The reagents appear to be stable for at least 7 days at 4-10 °C, as they retained their initial sensitivity (99.2 %-103 %). The between laboratory variation for six samples surveyed at five laboratories was 3.80-26.4 % (CV) for samples containing about 20-290 U/L LIP activity. Interferences by > 5 mM glycerol and low specificity with post-heparin samples were noted.

Conclusions: Although further studies are required, the method may reduce methodological discrepancies of LIP activity.



A-87

Validation of Ultracentrifugation of Lipemic Serum Samples for Chemistry and Immunoassay Testing on the Roche Modular Platform

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Background: Lipemia is a common interference in serum samples. Ultracentrifugation is one method many labs utilize to clear lipemic samples prior to analysis. We extensively validated a new ultracentrifuge to confirm the process does not affect the accuracy of analyte measurements. We also investigated LipoClear treatment as an alternative to ultracentrifugation.

Objective: Validate a method to clear lipemic samples prior to analysis.

Methods: All analytes were measured on Roche Modular Chemistry (D or P modular) and Immunoassay (e601, Indianapolis, IN) instruments. Lipemia was estimated by measuring the L-index (measure of sample turbidity). LipoClear (Iris Sample Processing, Westwood Ma.) was used according to the manufacturer's package insert. Ultracentrifugation was performed using a Sorvall MX-120 micro-ultracentrifuge with capped microtubes (volume=1.5 mL, 55000 rpm, 7 minutes). Residual serum samples with varying extents of lipemia near the current L-index thresholds for electrolyte measurement (L-index = 80-250) were obtained. L-indices were measured before and after ultracentrifugation or treatment of sample with LipoClear. Residual serum samples with L-indices below the interference threshold for each assay were analyzed pre- and post-ultracentrifugation or before and after sample treatment with LipoClear. Bias between pre- and post-ultracentrifugation results and pre- and post-Lipoclear treatment results was calculated.

Results: Ultracentrifugation or Lipoclear treatment of samples (L-index range 45-250) reduced the L-index by 87±8% (mean±SD) or 95±3%, respectively. In all cases, lipemia post-treatment was reduced to an L-index of <18. For each test (n=5 samples or n=3 for immunoassays), maximum bias (absolute or percent) between results from pre- and post-ultracentrifuged samples was as follows (analyte, mean bias (range)): Sodium, 1.4 mmol/L (0-2 mmol/L); Potassium, <0.1 mmol/L (0.0-0.1 mmol/L); Chloride, 1 mmol/L (1-2 mmol/L); Bicarbonate, <1 mmol/L (0-1 mmol/L); Creatinine, 0.1 mg/dL (0.0-0.1 mg/dL); BUN, <1 mg/dL (0-1 mg/dL); Calcium, -0.1 mg/dL (-0.2-0.2 mg/dL); AST, 3.7% (0-8%); Alkaline phosphatase, 1.3% (-0.8-2.1%); Creatine kinase, 2.3% (-1.8-6.9%); ALT, -2.5% (-11.8-5.3%); GGT, -1.1% (-3.0-0.0 %); Phosphorus, <0.1 mg/dL (0.0-0.2 mg/dL); Albumin, 0.2 mg/dL (0.1-0.3 mg/dL); Glucose, 1.3% (-1.6-4.2%); Total protein, 0.2 mg/dL (0.1-0.3 mg/dL); Lactate dehydrogenase, -0.7% (-4.9-4.3%); C-reactive protein, 0.7% (-0.6-4.3%); Total bilirubin, -0.2 mg/dL (-1.1-0.1 mg/dL); Direct bilirubin, -0.2 mg/dL (-0.6-0.0 mg/dL); Magnesium, <0.1 mg/dL (0.0-0.1 mg/dL); Amylase, 2.3% (0.0-4.4%); Lipase, -2.8% (-7.3-1.3%); Uric acid, <0.1 mg/dL (-0.3-0.2 mg/dL); NT-ProBNP, 0.3% (-1.2-1.1%); TroponinT, -0.03 ng/mL (-0.10-0.01 ng/mL); TSH, 2.4% (2.1-3.0 %); PSA, 0.4 ng/mL (0.2-0.6 ng/mL); HCG, 0.1% (-13.4-8.5%); Estradiol, 3.1% (-0.8-5.5 %); PTH, -0.4 ng/mL (-1.0-0.4 ng/mL); C-peptide, 4.4% (2.8-7.1 %); Insulin, 2.4% (-0.4-4.5%). For Lipoclear: TSH, -44.0% (-47.6 to -38.9%); Calcium,-0.4 mg/dL (-0.5 to -0.2 mg/dL); Phosphorus, 0.4mg/dL (0.4-0.6 mg/dL); Total bilirubin, -0.4 mg/dL (-1.8-0.0 mg/dL); and Direct bilirubin, -0.1 mg/dL (-0.8-0.1 mg/dL).

Conclusions: Ultracentrifugation and LipoClear treatment were effective at removing lipemia from serum samples. Bias between pre- and post-ultracentrifugation results was acceptable for all chemistry and immunoassay analytes measured. LipoClear treatment produced bias that was not acceptable for several analytes suggesting laboratories performing chemistry testing should internally validate methods used to reduce lipemic interference.

A-89

Inter-laboratory comparison of cholesterol reference measurement procedures with NIST GC-MS primary reference method using fresh frozen and lyophilized serum

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Background: The LSP and the CRMLN forms a sophisticated but functional network to standardize lipid measurements worldwide. This system enables a seamless transfer of accuracy of cholesterol measurements between the well-established national reference system (NRS) and routine clinical lipid profile characterization. Such an effective process of transfering measurment accuracy between laboratory measurements and the NRS for cholesterol depends on a solid and consistent performance of the reference measurement procedures (RMP) over time and space. Both the CDC reference laboratory and CRMLN laboratories operate the modified Abell-Kendall (ABLK) RMP which is the 'gold standard' for cholesterol measurements. Most comparisons of the ABLK RMP and IDMS for cholesterol have utilized pooled frozen or lyophilized reference materials. In previous comparison of the RMPs for cholesterol, CRMLN laboratories were not involved and the performance of the CDC RMP versus the primary RMP served as the basis to indirectly assess the RMPs used in the CRMLN relative to the primary RMP. We conducted an inter-laboratory comparison of the cholesterol reference measurement procedures and compare its performance with to the NIST primary RMP using fresh frozen pools, individual patient samples, and lyophilized sera.

Method: Frozen serum pools which were prepared at Solomon Park Research Laboratories following CLSI standardized protocol C-37A and 10 native serum samples were shipped on dry ice to each participating laboratory. Samples were stored at-70^{0}C upon receipt until time of the study. NIST provided one set (2 levels) of Standard Reference Material (SRM) 1951b. Two sets of lyophilized serum (2 levels) used in two cycles of the IFCC ring trial were obtained from IFCC. The protocol called for two analytical runs which were performed on independent days. The CDC reference materials and aliquots of the IFCC ring trial lyophilized samples were analyzed in both runs. SRM 1951b was analyzed in a single run.

Results: The ALBK cholesterol values ranged from 111.9 mg/dL to 267.76 mg/dL and the NIST's ID/GC-MS values range from 110 mg/dL to 268.4 mg/dL. The difference between the CDC ALBK measurements and the IDMS measurements for fresh frozen serum samples ranged from -0.83 mg/dL (244.4 mg/dL) to 2.72 (258.44 mg/dL). For the IFCC RELA lyophilized serum samples the difference ranged from 3.6 mg/dL (156.5 mg/dL) to 6.7 mg/dL (237.8 mg/dL). Average biases for fresh frozen serum samples (pools and individual specimen) from all laboratories and methods ranged from -1.4% to 1.7% versus CDC and -1.3% to 1.7% versus NIST. The average biases for all of the participating laboratories for the RELA lyophilized serum samples ranged from -1.2% to 1.2% versus CDC ABLK and from 1.0 to 3.9% versus NIST's ID/GC-MS.

Conclusion: CDC reference laboratory and laboratories in the CRMLN have demonstrated continuous agreement for cholesterol measurement and have shown good agreement with NIST GC-MS measurement for fresh frozen pools and individual patient samples. In general CRMLN laboratories and CDC's reference laboratory performs with a consistent positive bias versus NIST for IFCC RELA lyophilized serum samples.

A-91

Quantitative measurement of serum phosphatidylcholine and lysophosphatidylcholine using matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry

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Background: Phosphatidylcholine (PC) is the most abundant phospholipid (PL) in the human body. PC hydrolyzes to lysophosphatidylcholine (LPC), which is reported to be the major lipid constituent of oxidized low density lipoprotein, and is also reported to be pro-atherogenic and may also be pro-inflammatory. A method for quantitative measurement of serum PC and LPC concentrations is highly desirable.

The present report describes the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to quantitatively measure serum PC and LPC levels.

Materials and Methods: Serum samples were collected from 20 healthy volunteers. Subjects gave informed consent, and the study was approved by the local ethics review board. Dimyristoyl phosphatidylcholine and monomyristoyl phosphatidylcholine were used as internal standards (ISs). Serum lipids were prepared according to the procedure described Folch et al. PC and LPC preparations from commercial PC from egg yolk or serum lipids were mixed with ISs and 2,5-DHB (matrix agent), and the mixtures then subjected to MALDI-TOF MS analysis.

Results: MALDI-TOF mass spectrograms were examined to identify mass-charge ranges and peaks. The mixture containing serum phospholipids plus ISs resulted in peaks ranging from m/z 400-850. The sample containing ISs alone resulted in peaks at m/z 468.5, 490.5, 678.3 and 700.8, which corresponded to LPC16:0$[+H]^+$, LPC16:0$[+Na]^+$, PC16:0/16:0$[+H]^+$ and PC16:0/16:0$[+Na]^+$, respectively. In the serum lipid samples, peaks at m/z 496.5, 518.5, 524.5 and 546.7 represented LPCs, and peaks at m/z 761.0, 781.0, 783.0, 809.0 and 811.0 represented PCs. The m/z 725.9 peak corresponded to sphingomyelin. Assays using various PC:IS or LPC:IS ratios showed that there was a linear relationship between both PC and LPC level and peak intensity. The reproducibility of within-run assay (mean ±SD) of PC and LPC were 126.4 ± 8.4 mg/dL (CV 6.6%) and 14.9±1.2 mg/dL (CV 7.7%) respectively (n=10). The ratio of LPC-C16:0 to LPC-C18:0 was constant between 12.5 - 100 mg/dL LPC. Serum PC and LPC levels were 100±25 mg/dL and 15±4 mg/dL (mean ± SD), respectively, according to enzymatic assay. Regression analyses using the mass spectrometry and enzyme assay data showed there was a linear relationship for PC where y=0.92x-30.00 (correlation coefficient (r) = 0.8838), and also for LPC, where y=1.28x-6.40(r=0.8889).

Conclusions: MALDI-TOF MS analysis can be used to quantitatively measure serum PC and LPC concentrations. Such measurement is possible even when PLs contain a variety of fatty acid side chains.

A-94

Development of multiple measurements of HDL-C subfractions by a new homogeneous assay

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Background: 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. 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. Herein, we developed a new assay which can simultaneously measure apoE-containing HDL-C, apoE -deficient HDL-C and total HDL-C by a single test on general chemistry analyzers.

Methods: We identified suitable surfactants by screening for those selective for the HDL-C subfractions and for the dissociation of other lipoproteins. We designed the new assay to consist of the following 3 steps: the 1st step to decompose and eliminate non-HDL-C, the 2nd step to quantify apoE-deficient HDL and the 3rd step to quantify apoE-containing HDL. We used 13% PEG precipitation method for total HDL-C, sulfate/phosphotungstate/ Mg++ precipitation method for apoE-deficient HDL-C as comparison methods. ApoE-containing HDL-C was calculated from apoE-deficient HDL-C and total HDL-C for comparison with the new assay

Results: We found a certain type of surfactant (polyoxyethylene benzylphenyl ether derivative) can selectively react with only HDL particles among various lipoproteins. We also found that apoE-deficient HDL is preferably reacted with the low level surfactant and all HDL particles are reacted with the high level surfactant. The new assay showed excellent correlations with the comparison methods for apoE-containing HDL-C, apoE-deficient HDL-C and total HDL-C, respectively ($r > 0.9$).

Conclusions: The new homogeneous assay allows reproducible measurement of HDL subclasses within 10 min and appears promising in further investigations of the clinical significance of HDL subclasses.

A-95

Development of a new homogeneous assay for LDL-Triglyceride

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Background and aim: Low density lipoprotein (LDL) has been regarded as one of the important risk factors for coronary heart disease (CHD). Conventionally LDL has been assessed by its cholesterol content. However, recent studies suggested measurement of triglycerides in LDL particles (LDL-TG) could give more clinically useful information and could be more closely related to CHD risk than LDL-cholesterol (LDL-C). Some laboratories measure LDL-TG by ultracentrifugation or electrophoresis based methods but such test methods are laborious and technically demanding, and not suitable for routine testing. We report development of a fully automated homogeneous assay for LDL-TG quantification which can be run on general chemistry analyzers.

Methods: We followed the same strategy as employed to a homogenous assay for LDL-C that we previously developed. In the 1st step reaction, lipoproteins other than LDL are decomposed and in the 2nd step reaction, triglycerides in the remaining LDL particles are quantified by enzymatic reactions. For the decomposition of non-LDL lipoproteins in the 1st step reaction, some specific surfactants and cholesterol esterase are applied.

Results: We found polyoxyethylene benzylphenyl ether derivatives and cholesterol esterase can decompose non-LDL lipoproteins. We designed the 1st step reaction to degrade triglycerides from such non-LDL lipoproteins to water and oxygen by lipoprotein lipase, glycerol kinase, L-α-glycerophosphate oxidase and catalase. We then designed the 2nd step to quantify triglycerides from LDL as color development under the presence of another surfactant, lipoprotein lipase, glycerol kinase, L-α-glycerophosphate oxidase and peroxidase. Catalase is inhibited in the 2nd step reaction by sodium azide contained in the 2nd reagent. The new assay exhibited reactivity against Chylomicron, VLDL and HDL less than 10%. CVs from within-run precision study were below 5% at all the LDL-TG levels tested. Dilution linearity study was conducted and excellent linearity was observed up to 80 mg/dL. The new assay also showed good correlation with ultracentrifugation method ($r > 0.9$).

Conclusions: Our new homogeneous assay gives a LDL-TG result in 10 min in a fully automated manner and thus allows analysis of large number of samples in routine Laboratories.

A-96

Comparison of small dense LDL cholesterol obtained between polyacrylamide tube gel electrophoresis and homogenous enzymatic methods

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Background: Small-dense low density lipoprotein (sdLDL), as a result of abnormal lipoprotein metabolism, has been linked to the progression of coronary artery disease. There are various techniques for determining of sdLDL. The aim of this study was to compare the polyacrylamide tube gel electrophoresis (PTGE) and homogenous enzymatic method (sdLDL-EX) methods for determining sdLDL particles.

Methods: A total of 225 individual patient sera were analyzed for lipid profile and sdLDL-C using the PTGE (Quantimetrix Lipoprint™, CA), and the sdLDL-EX assay (Denka Seiken, Japan). The PTGE method electrophoretically separate plasma lipoproteins into maximum twelve bands ranked by size, from largest to smallest: very low density (VLDL), midbands (primarily intermediate low density, IDL) (MID-C, MID-B and MID-A), larger-buoyant LDL (LDL1 and LDL2), small-dense LDL (LDL3 to LDL7) and high density lipoproteins (HDL). By densitometry, the relative area for each lipoprotein band is determined and multiplied by total cholesterol concentration, yielding the amount of cholesterol for each band. The estimation of sdLDL-C is calculated as the sum of the cholesterol concentrations of LDL3 to LDL7. The sdLDL-EX assay is based on interactions of specific surfactants with various phospholipid components on the surface of lipoproteins.

Results: Linear regression revealed a good relationship between the sdLDL-EX (y) and the PTGE (x) as $y_{mg/dL} = 0.755x + 25.95$, $r = 0.720$. The mean difference (sdLDL-EX minus PTGE) and the SD of the difference were 23.4 and 10 mg/dL, respectively. The bias was significantly correlated with MID-C, MID-B, MID-A, LDL1 and LDL2 ($P < 0.01$) but was not with VLDL and HDL ($P > 0.50$) cholesterol concentrations. Correlation analyses of each sdLDL-C assay with other lipoproteins show that PTGE correlated positively with VLDL, MID-C, MID-B and LDL2, and negatively with MID-A and LDL1 while sdLDL-EX strongly correlated with VLDL, MID-C, MID-B and LDL2 but did not with MID-A and LDL1.

Conclusions: The sdLDL-C concentrations obtained from sdLDL-EX were higher than those from PTGE. The significant relationship between sdLDL-EX and MID-C, MID-B and LDL2 indicates that this assay may determine cholesterol concentrations not only in sdLDL particles but also in the other lipoprotein particles found in the IDL and larger LDL zones. These lipoproteins have a chemical composition similar to sdLDL particles (resulting remodeling of TG enriched lipoprotein particles) and play an important role in atherogenesis. Because the sdLDL-EX method detects more fractions of atherogenic particles and is easy to perform, it may be a better predictor of cardiovascular risk and marker of therapy.

A-97

Heterogeneous performances of intermediate and low density lipoprotein subpopulations

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Background: Plasma low-density lipoprotein (LDL) and intermediate-density lipoprotein (IDL) are believed to contribute directly to plaque formation and progression of cardiovascular disease. Because these lipoproteins consist of heterogeneous particles, they may demonstrate distinct atherogenic characteristics. Our objective was to examine whether these subpopulations measured by polyacrylamide gel electrophoresis (Quantimetrix Lipoprint™, CA) differ in the relationship with other lipids and lipoproteins.

Methods: The Lipoprint assay separates plasma lipoproteins by their specific electrophoretic mobility according to particle size. In this assay, IDL (diameter 27-35 nm) separates into three midbands (MID-C, MID-B and MID-A) and LDL (diameter 21.8-27.5 nm) has potentially divided into seven subfractions (LDL-1 to LDL-7). The LDL-1 and LDL-2 bands correspond to large buoyant LDL, whereas bands LDL-3 to LDL-7 comprise small dense LDL (sdLDL) particles. A total of 225 individual patient sera were analyzed.

Results: The associations of MID-C, MID-B, MID-A, LDL-1, LDL-2 and sdLDL with VLDL and HDL fractions were performed. MID-C, MID-B, LDL-2 and sdLDL positively correlate with VLDL (independent variable) while MID-A and LDL-1 inversely correlate with VLDL ($p < 0.04$). In contrast, the MID-A and LDL-1 fractions strongly correlate with HDL, in a positive fashion ($p < 0.001$). Correlation coefficients with other biomarkers are displayed in Table below.

Conclusions: Each subfraction of IDL and LDL may vary in their ability to predict risk for coronary artery disease. The variation may explain why a high LDL-cholesterol concentration, as typically measured in conventional laboratory methods, is not a perfect predictor of coronary artery disease. Further studies of their atherogenicity may be required to provide suitable clinical value.

Correlation coefficients of each IDL and LDL subfraction with biochemical markers						
Biochemical markers	MID-C	MID-B	MID-A	LDL-1	LDL-2	sdLDL
Triglycerides (mg/dL)	0.406a	0.222a	-0.341a	-0.442a	0.326a	0.601a
Total cholesterol (mg/dL)	0.356a	0.617a	0.521a	0.591a	0.680a	0.487a
Apolipoprotein A-I (mg/dL)	-0.137b	0.055	0.151b	0.213a	-0.001	-0.065
Apolipoprotein B (mg/dL)	0.418a	0.630a	0.355a	0.332a	0.727a	0.641a
HDL-C (mg/dL)	-0.172a	-0.018	0.297a	0.399a	-0.102	-0.195a
LDL-C (mg/dL)	0.352a	0.613a	0.567a	0.617a	0.687a	0.436a
VLDL-C (mg/dL)	0.169b	0.286a	-0.142b	-0.138b	0.547a	0.663a
Non HDL-C (mg/dL)	0.320a	0.630a	0.455a	0.500a	0.727a	0.541a
Glucose (mg/dL)	0.027	0.031	-0.135b	-0.193a	0.101	0.161b
HbA1C (%)	0.086	0.092	-0.089	-0.181a	0.107	0.178a
Creatinine (mg/dL)	0.136b	0.105	-0.093	-0.248a	-0.028	0.084
Cystatin C (mg/dL)	0.232a	0.213a	0.000	-0.136b	0.011	0.046
Mean LDL particle size (nm)	-0.116	-0.165b	0.432a	0.506a	-0.496a	-0.908a
a Correlation is significant at the 0.01 level b Correlation is significant at the 0.05 level						

A-98

HDL function are Impaired in diabetic nephropathy

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Background: HDL cholesterol help decrease the risk of diabetic nephropathy. However, new research suggests that it may not be the amount of HDL cholesterol that you have, but whether or not you have the kind that functions more effectively. The objective of this study was to evaluate whether lipoprotein cholesterol (HDL) function is impaired in diabetic nephropathy.

Methods: In this study, the urinary albumin to creatinine ratio (ACR) was done in 146 diabetic patients. Serum total cholesterol, triglyceride were measured by ROCHE P800 and the HDL inflammatory index were measured using a cell-free assay. The HDL inflammatory index was used to evaluate HDL function. An ACR of ≥300mg/g was defined as macro-albuminuria, ≥30mg/g as micro-albuminuria and <30 mg/g as normal albuminuria.

Results: Among 146 patients, 56 were normal albuminuria, 60 were micro-albuminuria and 30 were macro-albuminuria. The levels of total cholesterol and triglyceride were significantly higher in albuminuric group than normal buminuric group (all P<0.05). No significant differences of total cholesterol and triglyceride were obeserved between micro-albuminuria group and macro- albuminuria group. The HDL inflammatory index was 1.95 ± 1.07 in normal albuminuria group, 2.34 ±1.04 in micro-albuminuric group, and 2.83 ± 1.14 in macro- albuminuria group (all $P < 0.05$). In a multiple linear regression model, HDL inflammatory index was associated with ACR (r=0.756, p<0.05).

Conclusions: There was significant correlation between HDL inflammatory index and urinary albumin to creatinine ratio. Our findings suggest that the HDL function is impaired in diabetic nephropathy.

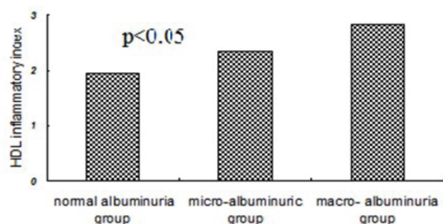

Fig. The HDL inflammatory index in normal albuminuria group, micro-albuminuric group and macro- albuminuria group. P value<0.05 was considered statistically significant.

A-100

Small dense low-density lipoprotein cholesterol can predict incident coronary arterydisease in an urban Japanese cohort: The Suita study

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Background: Several lines of evidence indicate that small dense LDL (sd LDL) particles are more atherogenic than large buoyant LDL particles. Few studies have addressed the association between sd LDL and cardiovascular disease, because there was no easy assay to measure the amount of sd LDL. Therefore, we examined the association between sd LDL cholesterol (sd LDL -C) and coronary artery disease (CAD) using a new assay kit for sd LDL -C in an urban Japanese cohort.

Methods: We performed an 11.7-year prospective study in general population aged 30-79 without history of cardiovascular disease. Homogeneous LDL-C and sd LDL -C were measured in samples from 2034 participants (968 men and 1066 women). During follow-up period, there were 63 incident cases of CAD. We calculated the multivariable-adjusted hazard ratios of sd LDL -C and sd LDL -C/LDL-C ratio for CAD using a proportional hazards regression model after adjusting for age, sex, hypertension, diabetes, use of lipid lowering drugs, body mass index, and current smoking and alcohol drinking.

Results: Increasing quartiles of sd LDL -C were associated with increased risk of CVD. The multivariable-adjusted hazard ratios for the subjects in the highest quartile were 1.60 (p=0.014) for CVD and 3.26 (p=0.003) for CHD. Sex-specific analysis also showed a significant positive association between sd LDL -C and CVD in men (multivariable-adjusted hazard ratio was 1.85, p=0.026), but not in women. We also found that sd LDL -C/LDL-C ratio was a significant risk for CVD in both men and women (multivariable-adjusted hazard ratio was 2.51 in all subjects, p=0.003).

Conclusions: We demonstrated that sd LDL -C and sd LDL -C/LDL-C are significantly associated with the development of CAD in Japanese, providing evidence of sd LDL -C as an important biomarker to predict CAD.

A-101

Effect of treatment with CPAP on oxidized LDL

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Background The obstructive sleep apnea (OSA) has been associated as a condition that promotes oxidative stress and lipid peroxidation. Recent studies have shown an increase of concentration of oxidized LDL in patients with OSA especially when associated with comorbidites. The increased lipid peroxidation may explain the high incidence of deaths from cardiovascular diseases in individuals with OSA. We hypothesized that measures the concentration of oxidized LDL may be decreased with effective treatment of OSA with 6 months to a year with continuous positive pressure (CPAP). This study we tested this hypothesis.

Methods: We evaluated the concentration of oxidized LDL in 30 patients (22 men) with moderate and severe OSA diagnosed at the Sleep Disorders Institute, with average BMI = 30.20 ± 9.12 kg/m^2 without other diseases were randomized and treated effectively 6 months and one year with CPAP, use average of 5 hours per night. Oxidized LDL concentrations of these patients were performed by ELISA (Mercordia- USA) after 12 hours of fast to baseline, and after 6 and 12 months of treatment. Statistics: ANOVA calculations were performed for comparison over time. It was used *cutoff* suggested by the manufacturer of 100 U/l, for normal subjects. Test comparisons were carried out in pairs.

Results: The averages of concentrations of oxidized LDL in patients with moderate and severe OSA and who were treated with CPAP and sham CPAP showed averages of the baseline, 6 months and 12 months after treatment (140.84 ± 51.95 U/l 143.52 ± 49.64 U/l and 141.26 ± 47.99 U/l respectively. The comparisons between the baseline measurements of untreated and 6 months of CPAP was showed a p = 0.20, while that between the baseline and after 12 months of CPAP was set at p = 0.12 and between 6 months and 12 months p = 0.23. No significant differences in concentration of oxidized LDL.

Conclusion: The treatment of OSA with CPAP showed no difference in lipid peroxidation measured concentrations of oxidized LDL in our study. However we know that the OSA is a risk factor for increased oxidized LDL especially when associated with dyslipidemia and hypertension demonstrated in previous study by our group.

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Antioxidant ability of apolipoprotein E-containing high-density lipoprotein

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Background: Some cholesteryl ester transfer protein (CETP) inhibitors are currently undergoing clinical evaluation. Although robust effects of those inhibitors on plasma high-density lipoprotein (HDL) levels were reported, there is almost no report about the character and functionality of increased large-size HDL particles. HDL has the diverse anti-atherogenic functions, and one of those is antioxidant ability. Oxidized low-density lipoprotein (oxLDL), which is promising risk factor for the development of cardiovascular diseases, contains multiple oxidant products, including lipid hydroperoxides (LOOH). Those products induce oxidative and inflammatory injuries. HDL not only protects LDL from oxidation by free radicals but also removes oxidized

lipids from oxLDL. However, it is reported that HDL lacking antioxidant ability is atherogenic. In this study, we estimated the composition and anti-oxidant ability of apolipoprotein (apo) E-containing HDL, which is a HDL fraction known to be increased by CETP inhibitor.

Methods: HDL (1.063<d<1.210 g/mL), isolated from pooled serum obtained from healthy volunteers by ultracentrifugation, was separated into apoE-containing and apoE-deficient HDL by Heparin-Sepharose choromatography. The compositions of HDL were determined by Lowry's Method (protein) and enzymatic test kit (cholesterol and phospholipids). The antioxidant ability of HDL was estimated by the effect on LDL oxidation induced by $CuSO_4$. Namely, on the LDL oxidation curve obtained by monitoring absorbance at 234nm (induction of diene), the time period of relatively inhibited oxidation, termed "lag time" just before the extreme increase of absorbance, was defined as an index of antioxidant ability. LOOH was measured by the previously reported method. Briefly, HDL was mixed with the reagent solution (4.5 mmol/L $FeSO_4$•$7H_2O$ in 0.2 mol/L HCl was mixed with the same volume of 3% methanolic solution of KSCN) followed by the incubation for 30 min. The absorbance was measured at 500 nm and LOOH concentration was calculated using molecular absorption coefficient of $Fe(SCN)_2$.

Results: The lipid contents of apoE-containing and apoE-deficient HDL were 0.58 and 0.24 mg/mg protein for cholesterol, respectively, and 0.53 and 0.41 mg/mg protein for phospholipids, respectively. ApoE-containing HDL was represented as cholesterol enriched particles in comparison with apoE-deficient HDL. The lag time of LDL oxidation in the presence of apoE-containing HDL (105±17 min) was statistically shorter than that in the presence of whole HDL (160±16 min) or apoE-deficient HDL(135±12 min), indicating that antioxidant ability of apoE-containing HDL was lower than that of apoE-deficient HDL. LOOH concentration of apoE-containing HDL was 48.5 nmol/mg protein, extremely high compared with 1.4 nmol/mg protein of apoE-deficient HDL. Although only 8% of total HDL protein existed in apoE-containing HDL fraction, 74% of LOOH conjugated with total HDL existed in the same fraction, strongly suggesting that LOOH was unevenly distributed to apoE-containing HDL.

Conclusions: ApoE-containing HDL has lower antioxidant ability and higher level of LOOH than apoE-deficient HDL. These suggest that apoE-containing HDL would protect LDL from oxidation due to its higher susceptibility to oxidation or its higher ability to accept LOOH from oxLDL. Consequently, apoE-containing HDL possibly failed to indicate the similar antioxidant ability (protection of diene formation) to apoE-deficient HDL *in vitro*.

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Identification of the most susceptible lysine residue on *N*-homocysteinylation of apolipoprotein AI

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Background: Homocysteine (Hcy), a sulfur containing amino acid, is generated from methionine (Met) as a metabolite. Hcy is normally removed by remethylation or transsulfuration, however; a part of Hcy is transformed to Hcy-thiolactone (HcyT) by methionyl-tRNA synthetase in order to prevent to incorporate into the protein biosynthesis in place of Met. HcyT indicates a high reactivity with ε-amino group of lysine residue and induces *N*-homocysteinylated (*N*-Hcy)-protein. Some *N*-Hcy-proteins lose those original structures and functions, and are associated with various disorders. Apolipoprotein AI (apoAI), a main protein of high-density lipoprotein (HDL), is believed to play the leading part for anti-atherosclerotic action of HDL. We have developed the new method to identify and semi-quantify *N*-Hcy-apoAI in human serum using isoelectric focusing (IEF) with cysteamine treatment. In the present study, we tried to determine lysine residue(s) in apoAI which is the most susceptible to *N*-homocysteinylation by HcyT. It will be support to consider the change of function in connection with the structure of *N*-Hcy-apoAI.

Methods: To obtain a relatively large amount of *N*-homocysteinylated fraction, purified apoAI was incubated with the final concentration of 1 mmol/L HcyT at 37°C. The mixture was treated with cysteamine to separate *N*-Hcy-apoAI from intact apoAI by two-dimensional gel electrophoresis (2D-GE), isoelectric focusing followed by SDS-PAGE. It is based on the increase of isoelectric point of *N*-Hcy-apoAI after the reaction between -SH group of conjugated Hcy and cysteamine. In contrast, the isoelectric point of intact apoAI is not changed by cysteamine treatment because of the absence of cysteine residue in its molecule. After 2D-GE, the protein spots were stained by CBB R-250. The spot including *N*-homocysteinylated apoAI at one lysine residue was cut out for the in-gel digestion using trypsin or endoproteinase Glu-C. Finally, the fragmented peptides were analyzed by MALDI-TOF-MS to identify *N*-homocysteinylated peptides by peptide mass fingerprinting.

Results: Four lysine residues, K59, K77, K107 and K140, were identified as the main sites of *N*-homocysteinylation by MS/MS. The frequency of the detection for the peptides including *N*-homocysteinylated K59, K77, K107, and K140, calculated from 15 times MS results, were 0.53, 0.20, 0.20, and 0.20, respectively. These results suggest that K59 is one of the most susceptible sites for *N*-homocysteinylation.

Conclusions: It was considered not that the specified one lysine residue has been induced *N*-homocysteinylation by HcyT in *N*-Hcy-apoAI conjugated with one molecular of Hcy. The plural number of lysine residues, at least 4, was susceptible, however; K59 is a candidate for the main target of *N*-homocysteinylation in apoAI. The previous our result that *N*-homocysteinylated apoAI was reduced its antioxidant ability could attribute to the modification of these lysine residues. The next our interest is to investigate the relation between modification site and function of apoAI, such as cholesterol efflux and anti-inflammatory ability.

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The difference of high-density lipoprotein subfractions evaluated by non-denaturing polyacrylamide gel electrophoresis method between non-alcoholic fatty liver disease and alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) defined as fat accumulation in the liver exceeding 5-10% by weight and is considered the hepatic manifestation of the metabolic syndrome. Low HDL-cholesterol (HDL-C) is one of the features of metabolic syndrome. However, there are no data on the quality of HDL (HDL particle size) in NAFLD. In the present study, we examined the HDL subfractions by electrophoretic methods in the general population. All participants were recruited through an annual health check-up in Mima city, Tokushima prefecture, Japan. A total of 185 Japanese community-dwelling men (59±14 years) were enrolled in this study. We included subjects who were apparently healthy without any known medical history of chronic diseases. The study protocol was approved by the Ethics Committee of the National Hospital Organization, Kyoto Medical Center. All of the subjects signed an informed consent form after being fully informed about all aspects of the study, before they were enrolled in the study. After an overnight fast, venous blood samples were then drawn for blood tests. Medical histories and lifestyle including drinking were confirmed by a public health questionnaire or interview. Increased liver fat content was defined as liver fat >5.6% (based on the Dallas Heart Study) and corresponded to AST concentrations of 33 U/I and to ALT concentrations of 43 U/I. Subjects with increased fasting serum ALT or/and fasting serum AST concentrations cosuming ≤20g (about 1 "gou") of ethanol per day was considered to have NAFLD, while those consuming >20g of ethanol per day was considered to have alcoholic fatty liver disease (AFLD). Serum HDL subfractions (large, intermediate, and small-sized HDL) was measured by electrophoretic separation of lipoproteins employing the Lipoprint™ system. Briefly, 25μL of serum sample and 300μL of loading gel were applied to an 8.0% polyacrylamide gel tube and mixed well. The sample was photopolymerized at room temperature for 30 minutes and then electrophoresed for 50 minutes (3mA/gel tube). The sample was then left standing for 30 minutes to prevent dehydration of the gel and fading of the bands. All of the HDL subfractions were calculated based on a flotation rate (Rf) between the very low-density lipoprotein (VLDL) fraction and low-density lipoprotein (LDL) fraction of Rf = 0.0, and the albumin fraction of =1.0. Subfractions HDL1-3 was defined as large-sized HDL, and HDL8-10 were defined as small-sized HDL.

There were no differences in age, body weight, body mass index, blood pressure, AST, ALT, plasma glucose concentration, insulin, total cholesterol, low-density lipoprotein cholesterol, and triglycerides between the two groups. The HDL-C levels was marginally higher in the subjects with AFLD compared with that in the subjects with NAFLD (1.42±0.44 vs. 1.24±0.52 mmol/l; p=0.056). However, in the subjects with AFLD, the proportion of the small-sized HDL was significantly greater compared with that in the subjects with NAFLD (6.7±5.6 vs. 3.8±4.9 %; p=0.022). Our findings indicate that the HDL subfraction distribution taken from non-denaturing polyacrylamide gel electrophoresis method is different pattern between NAFLD and AFLD.

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Validity of Calculated LDL Cholesterol by the Friedewald Formula Compared to LDL Cholesterol Measured by Ultracentrifugation in Patients with Very Low LDL

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Background: Reduction of LDL cholesterol (LDLc) is the cornerstone of prevention of cardiovascular disease (CVD). Based on CVD outcome trials national targets for LDLc keep decreasing and new drugs are being developed to achieve treatment goals. As LDLc with many of these new drugs fall below 50 mg/dL it is important to assess the accuracy of LDLc measurements in this range. Prior well conducted studies of detergent based 'direct' online LDLc measurements have been shown to vary by manufacturer and have significant biases against the accepted reference procedure by ultracentrifugation (BQuant). Calculated LDLc (Calc LDLc) by Friedewald formula remains the accepted next best procedure for both clinical and research purposes. We evaluate Calc LDLc compared to BQuant in patients with low and very low LDLc .

Methods: From 23,650 BQuant samples analyzed over 6 years and with triglycerides (TG) ≤400 mg/dL we compared Calc LDLc and BQuant LDLc at various cut points; 7879 LDLc ≤100 mg/dL; 1193 LDLc ≤50 mg/dL and 361 LDLc ≤25 mg/dL. We also assessed relationships at each level based on various TG. Cholesterol measurements were performed in CDC Part III standardized laboratories. Linearity of cholesterol measurement was 2 mg/dL.

Results: A proportional bias at LDLc ≤100 mg/dL was observed; with the % difference increasing at lower LDLc. % difference for LDLc ≤100 mg/dL was -5.29; % difference for LDLc ≤50 mg/dL was -18.8; % difference for LDLc ≤25 mg/dL was -27.6.

Conclusions: Calc LDLc and BQuant compare favorably between 51 and 100 mg/dL but deteriorate significantly as LDLc falls to ≤50 mg/dL and even more so ≤25 mg/dL where Calc LDLc may underestimate the true LDLc by BQuant by nearly 50%. At lower LDLc levels TG play an increasing role.

LDLc range mg/dL	n	mean TG	mean LDLc Calc mg/dL	mean BQuant LDLc mg/dL	% diff	p value	r value
<= 25	361	147.3	17.04	23.55	-27.6	<0.0001	0.36
<= 50	1193	174.2	33.90	41.75	-18.8	<0.0001	0.83
<= 100	7879	182.4	72.06	76.73	-5.29	<0.0001	0.92

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Lipoprotein associated Phospholipase A2 activity is increased in newly diagnosed patients of Type-2 Diabetes and correlates with fasting glucose levels

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Background: Lipoprotein associated Phospholipase A2 (Lp PLA2) is an independent predictor of cardiovascular diseases (CVD). Though patients of T2DM have high incidence of CVD, not much literature is available on Lp-PLA2 activity in these patients specially those who are recently diagnosed and are not under any kind of treatment. Accordingly, the objectives of this study were: i) to quantify Lp-PLA2 activity and oxLDL in plasma/serum ii) to determine the association of Lp-PLA2 with oxLDL, plasma glucose levels, serum insulin levels and lipid profile and compare with age and sex matched healthy individuals.

Methods: The study was conducted on patients of type 2 diabetes (n=45) diagnosed on the basis of FBG and 2 hours PPBG and HbA1c for the first time in our hospital and age and sex matched healthy controls (n=45). Individuals with abnormal hepatic/renal function tests, present or past history of CAD and endocrine disorders were excluded from the study. Anthropometric measurements were recorded and serum lipid profile, ox LDL, Lp-PLA2 activity, apoA1, apoB and serum insulin levels were measured in both groups before any treatment was started for patients. While comparing the two groups, students' 't' test was used. Value of p<0.05 was considered significant. Correlation between different parameters was carried out by Pearson correlation analysis in both groups.

Results and observations: Lp-PLA2 activity in patients (24.48±4.909 nmol/mt/ml) was significantly higher (p<0.001) than in controls (18.63±5.285nmol/mt/ml). Ox-LDL levels were also significantly higher (p< 0.01) in patients (52.46±40.19μmol/L) than in healthy controls (33.26±12.53μmol/L). Of all the lipid profile parameters only HDL-C was observed to be significantly (p<0.05) lower in patients (37.73±8.06 mg/dl) as compared with controls (42.03±7.81mg/dl). Though HDL/LDL ratio was not significantly different in the two groups, apoA1/apoB ratio was significantly low in patients (p<0.01). Lp-PLA2 activity showed positive correlation with the ox-LDL in both controls (r=0.421,p<0.01), as well in patients (r=0.414,p<0.01). A positive correlation between Lp-PLA2 activity and fasting plasma glucose levels was observed only in patients (r=0.351,p<0.02). A positive correlation of Lp-PLA2 activity with LDL-C (r=0.320,p<0.05), and negative correlation with HDL-C(r=-0.341,p<0.05), HDL/LDL ratio(r=-0.308, p<0.05) and apoA1/apoB (r=-0.470, p<0.01)in controls was not observed in patients.

Conclusions: This study implicates that oxLDL and LpPLA2 activity both are increased in type 2 DM. A positive correlation between ox LDL and LpPLA2 activity indicates that ox LDL being a substrate for this enzyme induces LpPLA2. Increase in the activity of LpPLA2 may explain a higher incidence of CVD in diabetic patients. Since there is a positive correlation between enzyme activity and fasting plasma glucose, it is apparent that maintenance of blood glucose levels may reduce incidence of CVD in diabetic patients. Further in this study we propose that loss of positive correlation with LDL-C and negative correlation with HDL-C, HDL/LDL as well as apoA1/apoB in freshly diagnosed diabetic individuals may probably be due to redistribution of the enzyme in early stages of diabetes.

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Pseudohypertriglyceridemia secondary to hyperglycerolemia

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Background: Hyperglycerolemia is a very rare genetic disorder caused by glycerol kinase deficiency. Although usually is presented unexpectedly in routine checks, there are severe forms, especially in children. In general, glycerol and glycerol kinase activity analyses are not include in routine laboratory determination. Glycerol presents positive interferences with some biochemical analytic techniques, e.g. in the determination of serum triglycerides and plasma ethylene glycol levels. Here, we present a Spanish patient with a pseudohypertriglyceridemia, a falsely elevated triglycerides concentration that not corrected with lipid-lowering therapy for 3 years.

Case report: A 21 years old man with high triglycerides serum levels for 3-years refractory to treatment with fibrates (Gemfibrozil 600mg/12h). He was asymptomatic, sportsman, normal BMI and blood pressure, no previous history of cardiovascular disease neither diabetes. The patient reported that do not smoke, drink alcohol or made fat diet. Parents showed normal serum triglycerides levels and no previous cardiovascular events. Familial history of cardiovascular disease was reported. The patient was remitted since Primary Care to Cardiovascular Risk Laboratory for lipid study. Initial laboratory investigations revealed high levels of triglycerides, 536 mg/dl (reference interval, <150 mg/dl) and total bilirubin, 2.7 mg/dl (reference interval, 0-1.0 mg/dl). Serum glucose, creatinine, alanine aminotransferase, aspartate aminotransferase, and thyroid hormone values were within reference limits. Cardiovascular Risk profile, including the emerging risk factors (fibrinogen and Lp(a)) and apolipoproteins (ApoA-I and ApoB-100), was normal. The analytical indices (lipemia and hemolysis) were within acceptable limits. Separation of serum lipoprotein in density gradient and electrophoresis revealed qualitative and quantitative normal lipoproteins (very low density lipoprotein, VLDL, particles triglycerides-rich and partially delipidated, normal and low density LDL and normal HDL).

Discussion: Glycerol is a halfway product in triglycerides determination. Most enzymatic methods used in routine laboratories do not involve a glycerol blank and determine both glycerol and triglycerides. We performed serum glycerol determination and we obtained 4.17 mmol/L of serum glycerol (reference interval, 0.03-0.19 mmol/L). These levels of serum glycerol are equivalent about 360 mg/dl of triglycerides. In our case, the lack of symptoms leads us to suspect in isolated GK deficiency. It may be important to consider glycerol kinase deficiency in the differential diagnosis of hypertriglyceridemia resistant to treatment. Although clinical symptoms in adults are extremely rare, asymptomatic patients should be advised of possible extreme catabolic situations.

A-110

Clinical Significance of apoAI-AII heterodimer in HDL induced by the oxidization with myeloperoxidase

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Background: Myeloperoxidase (MPO) is one of the biomarkers for acute coronary syndromes. In the advanced atherosclerotic lesions, MPO, secreted from macrophages and neutrophils, is known to induce the oxidized apoAI, such as 2-chloro- or 2-nitro-tyrosyl apoAI and apoAI-AII heterodimer bound with tyrosine-tyrosine bond. These products would be expected to give us the different informations from MPO activities in plasma, likely more specific to cardiac disease. In the present study, we tried to evaluate the effects of oxidation by MPO on antioxidant ability of HDL and apoAI. Additionally, a quantitative assay method for apoAI-AII heterodimer was developed to compare with the plasma levels of MPO in the patients with myocardial infarction.

Methods: 1) Oxidation of HDL by MPO; HDL (1.063<d<1.210 g/mL) was incubated with phosphate buffer (pH 7.4) containing hydrogen peroxide, diethylenetriamine pentaacid, L-tyrosine, and MPO for 24 hrs at 37[[Unsupported Character - ]]C. After the oxidation by MPO, a part of mixture was delipidated by ethanol and diethyl ether (3:2). Then, apoAI, apoAII, and apoAI-AII heterodimer rich fraction were partially purified with gelfiltration followed by affinity chromatography. 2) Evaluation of antioxidant ability; LDL was incubated with $CuSO_4$ at room temperature in the absence or the presence of HDL or its apolipoproteins. The kinetics of LDL oxidation (induction of diene) was analyzed by monitoring the absorbance at 234 nm. Antioxidant ability defined as the prolonged lag time was compared between before and after the oxidation of HDL by MPO. 3) Determination of MPO and apoAI-AII heterodimer; Plasma MPO levels were measured by the sandwich ELISA using one polyclonal anti-MPO antibody with and without biotinylation. ApoAI-AII heterodimer was also measured by the sandwich ELISA using anti-apoAII antibody as a capture antibody and a biotinylated anti-apoAI antibody as a detection antibody.

Results: ApoAI-AII heterodimer in HDL was apparently increased by the incubation with MPO, which was confirmed by SDS-PAGE followed by CBB R250 staining and immunoblotting, and ELISA assay. LDL oxidation was clearly delayed by HDL regardless of its pre-incubation with or without MPO. Untreated apoAI also indicated higher antioxidant ability, however; apoAI purified from MPO treated HDL was attenuated its antioxidant ability by 50 %. ApoAII and apoAI-AII heterodimer rich fraction did not indicate a significant effect on the diene formation in LDL by $CuSO_4$. The plasma levels of apoAI-apoAII heterodimer of the patients urgently hospitalized for the treatment of acute myocardial infarction (AMI) were significantly higher than those of the healthy subjects. MPO mass in plasma, measured for the samples (n=16) obtained from the healthy subjects and the patients with AMI, indicated the high correlation with apoAI-apoAII heterodimer levels (r=0.831).

Conclusions: MPO could not largely affect the antioxidant ability of HDL in in vivo. The measurement of apoAI-apoAII heterodimer is possibly useful because of the high correlation with MPO in plasma. It would be more specific to evaluate the progression of atherosclerotic lesion, since MPO levels in plasma are decided by macrophages and neutrophils activated in any infectious or inflammatory disease.

A-111

Monitoring Apolipoprotein A-I and Apolipoprotein B Measurements in the CDC-Lipid Standardization Program - A Two Year Report

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Background: Apolipoprotein A-I (apo A-I) and apolipoprotein B (apo B) are important biomarkers for assessing risk for cardiovascular diseases (CVD). To improve the diagnosis, treatment and prevention of CVD, the measurements of these biomarkers need standardization.

The Centers for Disease Control and Prevention (CDC) established the Lipid Standardization Program (LSP) that provides services to clinical and research laboratories to improve the accuracy and comparability of lipid and lipoprotein measurements. LSP provides serum materials with reference values determined by the CDC reference method for total cholesterol, HDL-cholesterol, and triglycerides. In 2010, CDC began offering apolipoprotein surveys in the LSP in addition to the established lipid surveys. The Northwest Lipid Metabolism and Diabetes Research Laboratory (NWLMDRL) collaborates with CDC by providing target values to the LSP materials using its designated comparison method (DCM). Long-term performance data on apo A-I and apo B measurements conducted in research and routine laboratories are not available. This study provides information on apo A-I and apo B measurement performance using data from the LSP.

Methods: Participants were instructed to measure, along with regular patient samples, one LSP sample in duplicate in one analytical run per week for a total of 12 weeks. CDC provided blind-coded samples that consisted of 9 different levels per year. Participant results were compared against the target values. The data reported from LSP participants were gathered from 2010-2011.

Results: 45 laboratories were enrolled in the LSP program during the last 2 years for apo A-I and apo B measurements using nephelometric and immunoturbidometric assay technologies from 8 different manufacturers. The median from all measurements (apo A-I, n=966; apo B, n=972) differed from the DCM target values by 2.65% (5th-95th: −7.57% - 10.00%) and −6.62% (5th-95th: −14.04% - 1.68%) for apo A-I and apo B, respectively. No trends in measurement bias over time were observed across all participants. The variability in measurement bias was slightly higher for immunoturbidometric methods (5th-95th for apo B: −15.11% - 3.78%, n=576 and apo A-I: 5th-95th: −9.85% - 10.54%, n=558) than than nephelometric methods (5th-95th for apo B: −10.81% - 0.16%, n=396 and apo A-I: 5th-95th: −2.30% - 8.97%, n=408), while the median biases remained similar between the methods. The median bias between individual assay manufacturers and the DCM method ranged between −5.74% - 4.41% for apo A-I and −12.08% - 6.59% for apo B. The bias does not seem to change with increasing analyte concentration.

Conclusions: By receiving commutable serum materials with target values assigned by a DCM, LSP participants were able to improve measurement accuracy and assure consistency of measurements over time. Thus, the LSP can improve the reliability of apo A-I and apo B measurements in clinical and research laboratories. CDC is working with its partners to improve apo A-I and apo B measurements, and continues to help establish traceability of the assay calibrators to the WHO/IFCC reference materials.

A-113

Performance Evaluation of a New Lipoprotein(a) Assay on the High-Throughput ADVIA Chemistry Systems

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Background: Lipoprotein(a) [Lp(a)] consists of two components, a low-density lipoprotein-like particle and apolipoprotein(a), which are linked by a disulfide bridge. The concentration of Lp(a) in the serum depends on genetic factors; the range of variation in a population is relatively large.[1] Elevated Lp(a) is a possible risk factor for coronary heart disease (CHD). Determination of Lp(a) may be useful to guide management of individuals with a family history of CHD or with existing disease.[2] A new assay* for Lp(a) on the automated ADVIA® Clinical Chemistry Systems is under development. The objective of this study was to evaluate the performance of this new Lp(a) assay on the ADVIA Chemistry Systems.

Methods: In the ADVIA Chemistry LPA assay, sample is diluted and reacted with latex particles coated with Lp(a)-specific antibodies. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured at 694 nm. The Lp(a) concentration in a sample is determined from a six-level standard curve using assay-specific serum-based calibrators. The performance evaluation in this study included precision, interference, linearity, and correlation with a commercially available Lp(a) assay run on the Hitachi 911 system. Data were collected for all ADVIA Chemistry Systems (ADVIA 1200, ADVIA 1650, ADVIA 1800, and ADVIA 2400), which use the same ADVIA Chemistry LPA reagent packs, calibrators, and commercial controls.

Results: The imprecision (%CV) of the new ADVIA Chemistry LPA assay with three-level commercial controls and three serum pools ranging from ~9 to ~85 mg/dL (n = 80) on all ADVIA Chemistry Systems (1200/1650/1800/2400) was ≤1.7% (within-run) and ≤2.6% (total). The analytical range of the new assay was from 1.40 mg/dL to the Lp(a) level in the highest calibrator (110 mg/dL). The assay correlated well with the commercially available Lp(a) assay run on the Hitachi 911 system: ADVIA = 0.97 [Commercial Lp(a)] + 0.01 (r = 0.99; n = 76; sample range: 3-82 mg/dL). The new assay also showed no interference at an Lp(a) level of ~18 mg/dL with unconjugated or conjugated bilirubin (up to 60 mg/dL), hemoglobin (up to 1000 mg/dL), and lipids (INTRALIPID, Fresenius Kabi AB; up to 1000 mg/dL). Minimum on-system stability and calibration frequency were both 60 days.

Conclusions: The data demonstrate good performance of the LPA assay on the high-throughput ADVIA Chemistry Systems from Siemens Healthcare Diagnostics.

* Under development. Not available for sale.

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A-114

Performance evaluation of the measurement of LDL particle number on the Vantera® Clinical Analyzer.

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Background: Atherosclerotic risk resulting from exposure of the artery wall to low density lipoprotein (LDL) particles has historically been assessed by measuring LDL cholesterol (LDL-C). In recent years, large clinical studies have demonstrated that measurement of LDL particle concentration (LDL-P) using nuclear magnetic resonance (NMR) spectroscopy is a better predictor of cardiovascular events than LDL-C. This is due in part because LDL-P is not affected by the variable cholesterol content inside LDL particles, which frequently over- or underestimates a patient's LDL level. LDL-P measurement by NMR is currently provided as a laboratory-developed test performed at a central laboratory, LipoScience, using the NMR Profiler® system. The Vantera Clinical Analyzer has been developed to enable routine, fully-automated measurement of LDL-P and other lipoproteins in any clinical laboratory. This study was designed to evaluate the performance of the Vantera LDL-P assay and compare it to the NMR Profiler.

Methods: Measurement of LDL-P using the Vantera Clinical Analyzer was evaluated in a manner consistent with CLSI Guidelines for precision, correlation with reference device (NMR Profiler), limits of detection (LoD) and quantitation (LoQ), linearity, and effects of interfering substances.

Results: LoD and LoQ were 41 and 132 nmol/L, respectively. Linearity was established for a range of 225-4320 nmol/L. Within-run imprecision (n=20) gave coefficients of variation (CVs) of 2.7-5.8% for three test levels. Total imprecision (within-lab) showed CVs of 3.9-5.3% over 20 days of testing, with repeatability of 2.6-4.4% CV. Linear regression for method comparison with the NMR Profiler gave the regression line, y=1.03*x-36.6 (r =0.978, n=1483). Bias at medical decision limits was ≤ 1% and reference ranges were equivalent for the two systems. No clinically significant interference was observed for 28 substances tested. The Vantera LDL-P test was compatible with Greiner Bio-One and plain serum, as well as EDTA and Heparin plasma collection tubes. Samples were stable for 6 days at 2-8ºC and 6 hours at room temperature. Sample-to-sample carryover was not detectable.

Conclusions: Results indicate the Vantera LDL-P performance characteristics are equivalent to that of the NMR Profiler. This study demonstrates the successful development of a fully automated platform directed toward the measurement of LDL-P based on NMR technology to allow the decentralization of testing in a clinical laboratory.

A-115

Performance characteristics for HDL particle measurement using the Vantera® Clinical Analyzer

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Background: High density lipoprotein (HDL) particles are believed to be anti-atherogenic based on the observed inverse associations of HDL cholesterol (HDL-C), apolipoprotein A-1 (apoA-1), and HDL particle number (HDL-P) with cardiovascular disease (CVD) risk. These 3 clinical measures of HDL are not equivalent because the per-particle cholesterol and apoA-1 contents of HDL vary between individuals and are altered differentially by HDL-raising treatments. Results from recent CVD outcome studies indicate HDL-P levels are more independent of metabolic and obesity-related markers and more predictive of future coronary events than HDL-C and apoA-1. HDL-P measurement by nuclear magnetic resonance (NMR) spectroscopy is currently provided as a laboratory-developed test performed at LipoScience using the NMR Profiler® system. The Vantera Clinical Analyzer has been developed to enable fully-automated, NMR-based measurement of HDL-P and other lipoproteins in any clinical laboratory. This study was designed to evaluate the performance of the Vantera HDL-P assay and compare its HDL-P values to those produced by the NMR Profiler system.

Methods: Measurement of HDL-P using the Vantera Clinical Analyzer was evaluated in a manner consistent with CLSI Guidelines for precision, correlation with reference device (NMR Profiler), limits of detection (LoD) and quantitation (LoQ), linearity, and effects of interfering substances. Specimen stability, collection tube comparability, and sample-to-sample carryover were also investigated.

Results: For the Vantera Clinical Analyzer, HDL-P LoD and LoQ were determined to be 4.6 µmol/L. Linear range was determined to be 5.0 - 95.0 µmol/L. Total imprecision tested over 20 days was determined to be 3.0%, 2.4%, and 2.0% from low (27.1 µmol/L), medium (36.6 µmol/L), and high (45.8 µmol/L) HDL-P materials, respectively. Linear regression between Vantera HDL-P and the reference method was determined to be y=1.04x-0.59 (r=0.978, n=1520). Ibuprofen concentration ≥ 140 µg/mL was found to cause interference with HDL-P measurement. No other sources of interference were identified among 28 compounds tested. The Vantera HDL-P test was compatible with Greiner Bio-One and plain serum, as well as EDTA and Heparin plasma collection tubes. Specimen stability was determined to be 6 days refrigerated (2-8ºC) and 6 hours at room temperature. Sample-to sample carryover of HDL-P was not detected from the Vantera Clinical Analyzer.

Conclusions: Results indicate the Vantera HDL-P performance characteristics are equivalent to that of the NMR Profiler. This study demonstrates the successful development of a fully automated, NMR-based platform to allow decentralized HDL-P testing in the clinical laboratory.

A-116

Quantitative determination of Lipoprotein-X by agarose gel electrophoresis and enzymatic staining for total and free cholesterol.

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Background: Lipoprotein X (LP-X) is an abnormal lipoprotein found in lethicin cholesterol actyltransferase (LCAT) deficiency and frequently associated with severe hypercholesterolaemia in cholestatic liver disease, normal lipoproteins being reduced or absent. LP-X is composed predominantly of free cholesterol (66%) and phospholipid (23%) Normal lipoproteins contain much less free cholesterol. LP-X displays a unique cathodic mobility on agarose gel electrophoresis however it overlaps significantly with low density lipoprotein. In view of the diagnostic significance it is important to distinguish LP-X from LDL-C, however currently there are no commercial methods for detection and quantification of LP-X. We describe a novel adaptation of an established commercial electrophoretic method for simultaneous estimation of the total and free cholesterol enabling quantification of LP-X in dyslipidaemic samples.

Methods: Electrophoresis of duplicate plasma samples in the HDL plus agarose gel with a tris buffer (Helena Laboratories, Beaumont, Texas) produced separation of lipoprotein in serum samples. Simultaneous enzymatic staining for cholesterol was performed by cholesterol dehydrogenase (Vis cholesterol kit, Helena Laboratories), with and without the presence of cholesterol esterase in duplicate plasma samples. Using densiometry (570nm) the area under the curve for the total and free cholesterol was quantified in non-HDL-C fractions. This allowed a ratio between total and free cholesterol to be calculated and LP-X in the non-HDL-C fraction to be quantitated. To establish a normal range, samples from 54 patients were analysed after exclusion of secondary causes of dyslipidaemia and any values outlying the three standard deviation range from the mean. The ratio of total:free cholesterol in combination with the electrophoretic mobility was used to detect the presence of LP-X in samples from a patient S with bilary obstruction pre and post treatment and the quantities of total and free cholesterol, cholesterol ester and LP-X were calculated.

Validation: Between gel precision was calculated using 18 repeated samples giving a coefficient of variation (CV) of the total:free cholesterol ratio of 14%. Within gel precision of six samples gave a ratio CV of 7%. Linearity for the method has been demonstrated up to 6.5mmol/L of non-HDL-C.

Results: The mean total cholesterol to free cholesterol ratio in non-HDL lipoproteins in control samples was 3.9 (range 2.6-5.3). Values below this range indicate the presence of LP-X, confirmed by the presence of lipoprotein with cathodal electrophoretic mobility. At the time of referral a patient, S, with dyslipidaemia due to primary bilary cirrhosis had a total cholesterol (TC) of 28.8mmol/L, HDL-C 0.5mmol/L, non HDL-C 27.6mmol/L, apolipoprotein B 1.8 g/L and apolipoprotein B:TC ratio reduced at 0.06 (NR 0.16-0.24). LP-X was estimated at 19.6 mmol/L. With colsevelam treatment the TC and LP-X values decreased to 18.5mmol/L and 8.3 mmol/L respectively. Following addition of low dose simvastatin TC fell to 10 mmol/L and LP-X to 3.0 mmol/L, with partial normalisation of the lipoprotein electrophoresis pattern.

Conclusions: Agarose electrophoresis with simultaneous enzymatic staining for total and free cholesterol permits accurate identification and quantification of LP-X. Application of this method may prove useful in diagnosis and monitoring of patients with cholestatic liver disease.

A-117

Quantitation of Erythrocyte Omega-3 Fatty Acids Using On-Line Extraction and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).

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Introduction: Eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) are polyunsaturated omega-3 (n-3) fatty acids utilized as a contemporary treatment modality in patients with severe hypertriglyceridemia (>500 mg/dL) and in those individuals with documented coronary heart disease due to a demonstrated reduction in cardiovascular mortality in secondary prevention trials. There are currently no biomarkers which are adequate in assessing long-term fatty acid intake and use of serum or plasma n-3 fatty acid concentrations is difficult due to the various free and complexed (phospholipid and lipoprotein) forms. Serum n-3 fatty acids also vary considerably with dietary intake, thus membrane-bound erythrocyte n-3 fatty acids are believed to be a more accurate and representative indicator of long-term intake. We developed a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay which does not require derivitization or manual extraction to quantitate n-3 fatty acids (EPA and DHA) in erythrocytes.

Methods: Erythrocytes were separated from plasma and white blood cells, washed with saline, and frozen at -80º C until hydrolysis. Dueterated-labeled internal standards were added to the erythrocyte specimens, controls, and standards, which underwent subsequent hydrolysis in an acidic ACN solution for 1 hour at 100º C. Hydrolyzed samples were then cooled and filtered through a 0.2 micron PTFE filter prior to on-line extraction and purification with high-throughput liquid chromatography (Turbo Flow Cyclone MAX Column, Thermo Fisher) followed by conventional liquid chromatography on a C-18 XBridge analytical column (Waters Corp). EPA, DHA, d_5-EPA and d_5-DHA were analyzed using ESI and tandem mass spectrometry (AB Sciex API 5000 MS/MS) using the following transitions: *m/z* 301.2/301.2, 301.2/257.20 (EPA); *m/z* 327.28/327.28, 327.28/283.22 (DHA); *m/z* 306.4/306.4, 306.4/262.20 (d_5-EPA) and *m/z* 332.4/332.4, 332.4/288.25 (d_5-DHA). Total analysis time was 11 minutes 10 seconds per sample.

Results: Intra-assay precision (CV) ranged from 0.6 - 12% (mean 3.5%, 7.8 ug/mL) for EPA and between 0.3 - 8.0% (mean 3.4%, 63.3 ug/mL) for DHA in erythrocytes pooled specimens. Inter-assay precision was 12.2% at an EPA mean concentration of 9.1 ug/mL and 7.7% at a DHA mean concentration of 74.8 ug/mL. Method linearity was assessed through mixing studies conducted with red blood cell matrix and demonstrated the method was linear for both EPA ($y = 0.8759x + 1.192$, $r^2 = 0.9984$) and DHA ($y = 0.9947x + 0.2296$, $r^2 = 0.9996$). Standard curve variability and accuracy showed minimal variance and bias over several days (EPA: $y = 0.9427x + 0.0005$, $r^2 = 0.9993$; DHA: $y = 0.8711x + 0.0011$, $r^2 = 0.9989$). Red blood cells obtained from adult donors with normal hematological parameters yielded EPA concentrations between 2.16 to 22.4 ug/ml and DHA concentrations between 50.3 to 134 ug/ml.

Conclusions: We have developed a sensitive and precise LC-MS/MS assay to quantitate erythrocyte concentrations of the n-3 fatty acids EPA and DHA. Our method improves upon previously published methods, as there is no requirement for time consuming liquid/liquid extractions or derivitization and may be multiplexed to enhance throughput. Additional clinical validation studies are warranted to derive an appropriate interpretive and/or therapeutic range for these n-3 fatty acids.

A-119

The association between fasting plasma glucose and serum oxidized high-density lipoprotein levels in patients with dyslipidemia

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Background: High-density lipoprotein (HDL) protects against atherosclerosis. Apolipoprotein A-I (apoA-I) is the major protein component of HDL and plays a critical role in the reverse cholesterol transport and anti-oxidant properties of HDL. When HDL particles become dysfunctional, their lipids and proteins, apoA-I in particular, may be oxidatively modified, during conditions such as dyslipidemia and glucose intolerance. However, there are very few studies on the relationship of the biochemical measurements of oxidized HDL (oxHDL) with glucose metabolism in association with dyslipidemia. The aim of this study was to investigate a new assay we developed for oxidized apoA-I (oxHDL) for its association with fasting plasma glucose (PG) in dyslipidemc patients.

Methods: A total of 134 asymptomatic, non-medicated and cardiovascular disease-free women (mean age of 57.9 years) with dyslipidemia were consecutively studied. All data, such as body mass index (BMI), blood pressure (BP), serum lipids and PG, were obtained from subjects in an overnight fasted state. The serum oxHDL levels were quantified using a sandwich ELISA system, which utilizes monoclonal antibodies prepared by immunization with the H_2O_2-oxidized human apoA-I.

Results: The mean/median levels of the measured variables were as follows: BMI, 23.9 kg/m^2; mean BP, 96 mmHg; total cholesterol, 6.21 mmol/L; low-density lipoprotein cholesterol, 4.01 mmol/L; triglycerides, 1.39 mmol/L; HDL-cholesterol, 1.82 mmol/L; PG, 5.50 mmol/L and oxHDL, 140.3 U/mL. A simple linear regression analysis showed oxHDL levels to be significantly and positively correlated with those of HDL-cholesterol ($r = 0.42$, $p < 0.01$) and PG ($r = 0.25$, $p < 0.01$). A multiple linear regression analysis, adjusted for BP, lipid panels and PG, revealed that oxHDL levels remained to be independently, significantly and positively correlated with those of HDL-cholesterol ($\beta = 0.43$, $p < 0.01$) and PG ($\beta = 0.25$, $p < 0.01$). In contrast, a similar simple linear regression analysis showed that HDL-cholesterol levels were not significantly correlated with PG levels ($r = 0.07$, $p = 0.42$). A multiple linear regression analysis revealed that HDL-cholesterol levels remained to be independently, significantly and positively correlated with those of oxHDL ($\beta = 0.35$, $p < 0.01$) but not PG ($\beta = 0.13$, $p = 0.10$).

Conclusions: The present findings of the significant positive association between fasting PG and serum oxHDL, but not HDL-cholesterol, among dyslipidemic women suggest that high glucose concentrations may oxidatively modify HDL particles, irrespective of HDL-cholesterol levels. The oxHDL test that we developed may, therefore, be useful to assess the pathophysiology of glucose and lipoprotein metabolism in subjects with dyslipidemia.

Tuesday AM, July 17, 2012

Poster Session: 10:00 AM - 12:30 PM

Molecular Pathology/Probes

A-120

A novel blood collection device minimizes cellular DNA release in blood during sample storage and shipping

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Background: Cell-free DNA (cfDNA) naturally occurs in blood and is currently being used for non-invasive prenatal diagnostics and for detection, monitoring, and molecular analysis of cancer biomarkers (1). Due to the rarity of cfDNA targets, it is important to minimize release of genomic DNA (gDNA) following blood draw in order to accurately measure cfDNA concentrations (2). Pre-analytical conditions can affect the release of background gDNA into plasma, decreasing the proportion of specific cfDNA targets and masking their detection in downstream applications. We have evaluated the ability of a novel blood collection device and traditional K_3EDTA tubes to minimize cellular gDNA release into plasma when subjected to conditions that can occur during sample storage, shipping and processing.

Methods: Blood samples were drawn from donors into K_3EDTA and Cell-Free DNA™ BCT tubes. To demonstrate the effect of elevated background gDNA on accurate cfDNA detection, a known concentration of plasmid containing a Y-chromosome specific SRY sequence was spiked into non-pregnant female blood that had been drawn each tube type; tubes were then stored at 22 °C for 14 days. The β-actin assay was used to determine total plasma DNA (pDNA) concentration over time and the SRY assay was used to measure the simulated cfDNA target. To evaluate the result of agitation during the transportation of samples, blood draws were either shaken or unshaken at 22 °C for 24 hours. To assess temperature variations, samples were stored at 6 °C, 22 °C and 37 °C for 14 days. For each experiment, plasma was harvested at various time points by centrifugation. Total plasma DNA (pDNA) was extracted and assayed by quantitative real-time PCR (qPCR).

Results: The plasmid spiking experiment illustrated the dramatic effect of elevated background gDNA in plasma on detection of rare cfDNA sequences; 50- and 90-fold increases in β-actin levels were seen in K_3EDTA over 7 and 14 days, while detection of SRY levels decreased 3- and 95-fold. In comparison, Cell-Free DNA BCT showed no change in β-actin levels at day 7 and an 8-fold increase at day 14, while detectable SRY levels remained steady throughout the time course. Shaking of blood drawn into K_3EDTA tubes showed a significant increase in pDNA over 24 h, whereas no change was seen in Cell-Free DNA BCT tubes. Blood incubated at 6 °C, 22 °C and 37 °C showed significant increases in pDNA isolated from K_3EDTA tubes, while pDNA levels from Cell-Free DNA BCT remained stable over 14 days.

Conclusions: Cell-Free DNA BCT tubes prevent increases in background gDNA levels caused by temperature fluctuations or agitation that can occur during blood sample storage, shipping and processing. This novel blood collection tube provides a method for obtaining high quality stabilized samples for rare DNA target detection and determining accurate cfDNA concentrations.

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A-121

A cell stabilizing reagent in Cell-Free DNA™ BCT is formaldehyde free and has no adverse effects on DNA integrity

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Background: Cell-free DNA (cfDNA) found in blood plasma has potential uses in clinical diagnosis (1). Release of cellular genomic DNA (gDNA) into plasma during blood processing, storage and shipping can hinder the clinical utility of cfDNA. Our previous investigations have shown that Cell-Free DNA™ BCT, a collection device that contains a cell stabilizing reagent, minimizes gDNA release into plasma as measured by real-time quantitative PCR (2). Traditional chemicals used in cell stabilization, such as formaldehyde and glutaraldehyde, are known to damage the double helical structure of DNA (3). In this study we wished to investigate whether the cell stabilizing reagent present in Cell-Free DNA BCT contained formaldehyde and affected the integrity of DNA as compared to traditional cell stabilizers formaldehyde and glutaraldehyde.

Methods: Quantitative detection of formaldehyde in Cell-Free DNA BCT reagent was performed by ^{13}C-NMR spectroscopy, assisted by a relaxation agent and using 500 MHz NMR spectrometer (Bruker Biospin Corp., Billerica, MA). Damage to the double helical conformation of DNA was determined by Circular-Dichroism (CD) spectroscopy (between 200-320 nm wavelength using J-185, Jasco CD spectrophotometer), fluorescence spectroscopy (using SYBR Green dye and iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, CA), agarose gel electrophoresis and PCR reaction.

Results: ^{13}C-NMR analyses, carried out using an internal reference, showed that there is no detectable amounts of free formaldehyde in the cell stabilizing reagent present in Cell-Free DNA BCT tubes. CD and fluorescence spectra of human genomic DNA incubated with formaldehyde and glutaraldehyde at room temperature for 3, 7 and 14 days, showed damage to the double helical structure of DNA as compared to a control DNA sample incubated without either formaldehyde or glutaraldehyde. Conversely, CD and fluorescence spectra of the control DNA samples and the DNA samples incubated with Cell-Free DNA BCT reagent at room temperature for 3, 7 and 14 days were similar, indicating this stabilizing reagent has no adverse effects on the DNA structure. Agarose gel electrophoresis showed there is no damage to DNA integrity in the Cell-Free DNA BCT reagent treated DNA, compared to DNA samples treated with formaldehyde and glutaraldehyde.

Conclusions: The cell stabilizing reagent present in Cell-Free DNA BCT is free of formaldehyde and does not damage DNA like traditional cell stabilizing agents such as formaldehyde and glutaraldehyde.

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A-122

The role of TGF-β in the interaction between colon cancer cells and mesenchymal cells

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Transforming growth factor (TGF)-β is a low-molecular-weight dimeric peptide and belongs to a family of growth factors. TGF-β functions have dual role, as a tumor suppressor in premalignant cells and as a tumor promoter in late stages of cancer. TGF-β also has important roles in host-tumor interactions between stromal fibroblasts and tumor cells. The stroma including fibroblast derived mesenchymal cells, collectively create an environment that promotes tumor progression by facilitate proliferation, angiogenesis, invasion, and metastasis. Because of these roles, TGF-β is an attractive therapeutic target. Understanding the mechanisms underlying the dual role of the TGF-β signaling pathway is critical for the development of specific and efficient TGF-β- targeted therapy.

The objective of this study to investigate the role of TGF-β in the interaction between colon cancer cells and mesenchymal stem cells (MSCs).

Methodology. We evaluated the recruitment of bone marrow-mesenchymal stem cells (BM-MSC) into the srtoma of adenocarcinoma colon cases and correlated the expression of alpha-SMA, as myofibroblasts markers, to incidence of lymph node metastasis in 57 adenocarcinoma colon cases. To know causes of differentiation BM-MSC into carcinoma associated fibroblasts (CAF), We compared SW480 (colon cancer cell line) and UE6E7T-12 (BM-MSC) in two conditions. The first condition is direct co-culture between SW480 and UE6E7T-12 cells by comparison 1:1 until almost 100% confluent using D-MEM containing 10% fetal bovine serum (FBS). After 24-48 hours, separating cells were performed using anti-CD326 (EpCAM) microbeads and AutoMacs separator. The second condition is indirect co-culture which performed using cell culture insert with 8.0 μm pore size. Than, we evaluated the expression of TGF-β isoform (TGF-β1, TGF-β2, TGF-β3) and their receptors (TGFBR1, TGFBR2, TGFBR3), and the impact in differentiation and metastasis

using RT-PCR and immunostaining. To evaluated the role of TGF-β1, we treated UE6E7T-12 with TGF-β1 and inhibitor SB431542 or siTGF-β.

Results. We find the recruitment of BM-MSC into tumor's stroma, and the expression of alpha-SMA was significantly increased in LNM cases. The alpha-SMA expression was clearly up-regulated in direct co-culture compared indirect co-culture and original of BM-MSC, and time dependent. In BM-MSC, direct co-culture induced the up-regulation of TGF-β2 and TGF-β3, whereas in SW480 cells, indirect and direct co-culture induced the up-regulation of TGF-β1 and TGF-β3. After TGF-β1 treatment, the expression of alpha-SMA was increased by dose and time dependent. And SB431542 and siTGF-β1 were successfully suppressed differentiation BM-MSC into CAF with dose and time dependent. In other hand, After direct co-culture, SW480 cells showed the increasing of MMP2 and MMP9 levels and the number of migrating cells compared original. Suppressing of TGF-β1 using SB431542 and siTGF-β1 decreased MMP9 expression in SW480.

Conclusions. TGF-βs originated from epithelial cancer cell nor mesenchymal cell influence differentiation cancer cell to more invasive, and mesenchymal cell to CAF. Treatment with target TGF-β may be effective to prevent metastasis tumor.

A-123

Mineral-bone metabolism markers in ederly patients with chronic kidney disease

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Background: Chronic kidney disease (CKD) is associated with accelerated atherosclerosis, and it has been observed that more of deaths in dialysis patients could be attributed to atherosclerotic complications. In this study we determined the most important markers of mineral-bone metabolism in elderly CKD patients in order to detect any metabolism changes that could lead to the vascular calcification frequently observed in these patients.

Methods: The analysis was realized in 94 CKD patients (52 males and 42 females) on haemodialysis for >12 months and in 72 controls (32 males and 32 females), with an age range of 55-70 years in both groups. No participants were receiving treatments related to mineral metabolism. Fasting blood samples were obtained in all subjects. In patients, blood was drawn immediately before initiation of hemodialysis. Serum was separated from cells by centrifugation, and stored at -80º C until analysis. Calcium and phosphorus were determined using the analyzers Roche/ Hitachi Modular CAN 679. Intact PTH (iPTH) was measured by using the N-tact PTH, (IRMA, Diasorin), with intra-and inter-assay coefficients of variance (CVs) of <8%. 1,25$(OH)_2$ D_3 was measured after extraction by using the 1,25-Dihydroxyvitamin D ^{125}I RIA Kit (Diasorin), with an intra- and inter-assay CVs of 6.8% and 13.5%, respectively. 25$(OH)D_3$ was measured by using LIAISON 25OH Vitamin D TOTAL (Diasorin), with intra- and inter-assay CVs of <3% and <9%, respectively. Osteoprotegerin (OPG) was measured by a specific enzyme immunoassay (Biomedica Medizinprodukte) with intra-and inter-assay CVs of 4% and 8%, respectively. Total soluble receptor activator of nuclear factor-κB ligand (sRANKL) was measured with an enzyme immunoassay (Biomedica Medizinprodukte) with intra- and inter-assay CVs of <3% and <9%, respectively. Osteocalcin (OC) was measured with an ELISA kit (Nordic Bioscience Diagnostics), with intra-and inter-assay CVs of <5%. Bone-specific alkaline phosphatase (BAP) was measured by using the Ostase BAP *immunoenzymometric* assay ((IDS) with intra- and inter-assay CVs of 3.7% and 7.35%, respectively. Tartrate-resistant acid phosphatase (TRACP-5b) was measured using a bone TRAP *immunoenzymometric* assay (IDS), with intra-and inter-assay CVs of 4.7% and 9%, respectively. *Statistical analysis* Means and standard errors were calculated for each group. After Kolmogorov-Smirnov goodness-of-fit tests, Student's *t* test was used for comparison among groups. Significance level was set at $p<0.05$.

Results: No differences in sRANKL (1,1±0.2 vs 0,9±0,2 pm/l) TRACP-5b (2,1±03 vs 1,8±0,2 U/l) and Calcium (9,1±0,1 vs 8,9±0,1 mg/dl) were found between CKD patients and controls. CKD patients had significantly higher levels of Phosphorous (5,0±0,07 vs 2,1±0,01 mg/dl), OPG (11,8± 0,2 vs 2,8 ±0,1 pm/l), BAP (20,1±0,3 vs 9,5±0,1 ug/dl), iPTH (380±1,2 vs 64±0,2 pg/ml) and OC (86 ±0,5 vs 16 ±0,2 ng/ml) and significantly lower levels of sRANKL/OPG ratio (0,07±0,02 vs 0,32 ±0,01), 25$(OH)D_3$ (14,1±0,8 vs 29±0,9 ng/ml) and 1,25$(OH)_2D_3$ (10,1±0.6,2 vs 41,6±0.8 pg/ml) compared with controls.

Conclusions: Our results indicate that bone formation and resorption parameters are alterered in CKD patients. The elevated OPG levels and the low levels of vitamin D, may lead to vascular calcifications and cardiovascular complications, in these patients.

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Newborn gene screening for high risk deafness-associated mutations with a new tetra-primer ARMS PCR kit

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Background: The study was to develop a simple kit using tetra-primer amplification refractory mutation system PCR (tetra-primer ARMS PCR) for high risk deafness-associated mutations screening in Chinese. In China, more than 0.8million children less than 7 years old with hearing impairment, and this number continues to increase more than 30,000 children annually. Previous epidemiological studies indicate that among the hearing impaired Chinese children, GJB2, SLC26A4 or mtDNA 12S rRNA mutations were responsible for hearing disable (about 36%). A cost-effective method for screening deafness-associated mutations at early age is needed.

Methods: In 2010, total 1,181 newborns in China were enrolled. All blood samples were collected by Chinese PLA General Hospital. A tetra-primer ARMS PCR deafness mutations screening kit was designed for detecting high risk deafness-associated mutations (GJB2 235delC, SLC26A4 919-2A>G, 12S rRNA mt.1555A>G and mt.1494C>T). The kit was able to amplify both wild-type and mutant alleles with a control fragment. It included Blood Specimen Collection Card, Genomic DNA extracting, and PCR amplification. DNA was extracted from 10μm venous blood spots in Specimen Collection Card. The quantity and quality of the extracted DNA were tested using UV at 260 nm and agarose gel electrophoresis, respectively. The proposed method was conducted to genotype four deafness gene mutations of GJB2 235delC, SLC26A4 919-2A>G, 12S rRNA mt.1555A>G, and mt.1494C>T. in four PCR reactions. Each mutation was genotyped by a set of four primers, two allele-specific primers, and two common primers. A mismatch at the penultimate or third nucleotide of the 3' terminus was introduced in order to maximize specificity. The 16 primers were used for the amplification of genomic DNA as template. The reaction was separated by electrophoresis. All samples with mutations were further validated with Sanger sequencing.

Results: Among 1,181 newborns, 29 individuals had one or two mutant alleles, with the carrier rate 2.46% (29/1,181). For GJB2 235delC mutation, one case was homozygote, 12 cases were heterozygote carriers; For SLC26A4 919-2A>G mutation, 12 cases were heterozygotes carriers, no homozygote; for 12S rRNA mt.1555A>G mutation, one case was identified; three cases of 12S rRNA mt.1494C>T mutation were detected. All mutations were detected with high specificity. Mutation samples were confirmed via Sanger sequencing. No false positive was found.

Conclusions: The user-friendly tetra-primer ARMS PCR deafness mutations screening kit was successfully developed. It provided rapid, reproducible, and cost-effective detection of deafness gene mutation without special equipment. The kit allowed the detection of four high risk deafness-associated mutations of GJB2 235delC, SLC26A4 919-2A>G, 12S rRNA mt.1555A>G and mt.1494C>T with only 4 single tube PCR reactions. In the future, the kit could be applied to large population-based epidemiologic studies for newborn hearing defects screening.

A-125

Amplification refractory mutation system qPCR for genotyping clinical relevant genomic variations: A concept modification resulting in objective data evaluation and exemption from endogenous controls

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Background: The amplification refractory mutation system (ARMS) is a general technique for genotyping point mutations or small deletions in predefined DNA regions. It is based on the fact that mismatches at the 3'-OH end of oligonucleotides primers results in loss of efficiency of the PCR reaction, delaying the DNA amplification process. Since its description, the ARMS has been adapted in various ways, including its migration to qPCR, which combines the advantages of both techniques, homogeneous and of high-throughput (qPCR), but also convenient and fast (ARMS). However, all descriptions in the literature still maintain the original ARMS concept of stopping the reaction at the point in time when the specific (matched) PCR products are generated, while the delayed (mismatch) ones are not. In this sense, we developed a concept modification, allowing the qPCR reaction to continue until amplification of the delayed PCR products. By allowing both reactions to occur, specific and delayed,

the exact moment in time when each amplification product reach the exponential phase is determined. Then, the genotype assignment will be based on the ΔCq, or the difference between the specific and delayed quantification cycles (Cq).

Methods: DNA was isolated from leukocytes by the Chelex-100® method (Bio-Rad). The amplification reaction was performed with Maxima SYBR® Green/ROX qPCR Master Mix 2X (Fermentas), 100ηg of each primer, 5µl of Chelex DNA, in a total volume of 20µl. The thermal cycling was carried out in a StepOne™ Real-Time PCR System (AppliedBiosystems). The amplification specificity was verified by melting analysis. Both products, specific and delayed, must yield the same result during melting analysis. After that, allele A Cq is subtracted from the allele B Cq, and the patient genotype is assigned following the ΔCq intervals reference described below.

Results: This ARMS assay concept modification has proved to be robust, and has shown perfect correlation with standard genotyping techniques. The following ΔCq intervals rules shown to be useful for all of our genotyping tests: homozygote for allele A (ΔCq>5); Heterozygote AB (-2<ΔCq<2); Homozygote for allele B (ΔCq<-5). In over two years, 5,000 genotyping reactions of clinically relevant genomic variations have been performed using this procedure (Factor V Leiden, APOE E2/E3/E4 genotyping, G20201A in F2 gene, C-13910T in lactase promoter, C282Y/H63D in hemochromatosis gene and others). Besides of its speed, low cost, and homogeneity, this assay always allows the amplification of the same genomic region in both reactions, only at different times depending on the genotype. Therefore, one reaction serves as amplification/endogenous control to the other. Moreover, the use of a numerical interval reference during genotype assignment incorporates objective criteria in the results interpretation, eliminating the visual evaluation of the amplification curves (subjective), and allowing the release of automatic test results.

Conclusion: The adaptation of ARMS-qPCR described here allows for rapid and robust genotyping, exempting the use of endogenous controls, while combining the advantages of qPCR and ARMS.

A-126

Purification of genomic DNA from FFPE tissue using a Qiagen EZ1 tissue kit with minor modifications

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Background: Isolation of DNA from formalin-fixed paraffin embedded (FFPE) tissue remains a laborious task for clinical laboratories and researchers screening several samples for genetic analysis. The removal of the paraffin wax encasing the thin layer of tissue and isolation of sufficient intact DNA are major obstacles to working with these precious samples. The objective of this study was to bring about an efficient method with less hands-on time to obtain DNA of optimum concentration and purity for use in downstream applications

Methods: Three different Qiagen DNA purification methods were employed in the study: Gentra Puregene Tissue isolation method, EZ1 DNA Tissue Kit and QIAamp MinElute column technique. Samples consisted of FFPE tissue rolls (2x10um) and unstained slides (4x5um) obtained from paraffin blocks of head/neck and lung tumor resections. The concentration and purity of isolated DNA was analyzed using the Nanodrop 1000 spectrophotometer. Further, EZ1 isolated DNA sample concentrations were normalized and quantitative real-time PCR was performed for a housekeeping gene, *GAPDH,* using the Applied Biosystems 7500 Fast real-time PCR system

Results: Purification of DNA was a two-day procedure for all the three protocols. The hands-on-time required was less for the EZ1 protocol (as little as 10 minutes) compared to the QIAamp MinElute column (45 minutes) and Gentra Puregene (3 hours) extraction methods. Concentration of DNA obtained from all three methods was between 50 and 100ng/ul, and the QIAamp MinElute column method produced relatively lower yield. However, purity was comparable for all the samples isolated using the three protocols. The purity and yield of DNA obtained from rolls and slides specimens were nearly similar between each of the methods used

Conclusions: Different methods are currently available for purifying genomic DNA from FFPE tissues. In comparison with the methods investigated for isolation of paraffin embedded tissues, the Qiagen EZ1 FFPE isolation method outperformed other methods especially with reduced hands-on-time in order to purify the DNA. This is a significant factor while considering the fact that the technique has to be routinely employed in isolating FFPE samples in clinical laboratories for various genetic analyses. The EZ1 DNA Tissue Kit utilizes the magnetic-particle technology which in turn provides high-quality DNA that is suitable for direct use in downstream applications such as amplification or other enzymatic reactions.

A-127

Evaluating the Thermostability of Commercial Fast SYBR Green Real-Time PCR Master Mixes

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Background: The lack of clinical diagnostic tests at low-infrastructure sites highly contributes to the devastating effects of many diseases in the developing world. The absence of routinely performed molecular diagnostic tests, mainly real-time PCR-based assays, is due to several crucial factors including unreliable electricity and the consequent lack of cold storage required for PCR reagent stability. In an attempt to circumvent this limitation, we sought to evaluate the thermostability of cost-effective, intercalating dye-based, commercial PCR master mixes. Following varied time incubations at elevated temperatures, we tested the performance of several, commercial SYBR® Green I master mixes using a fast-ramping real-time PCR instrument (the Cepheid® Smartcycler® II).

Methods: Five SYBR® Green I master mixes were evaluated: Quanta® PerfeCta® SYBR Green Fastmix®, Qiagen QuantiFast® SYBR® Green, Qiagen QuantiTect® SYBR® Green, Bio-rad SsoFast™ EvaGreen® Supermix, and ABI Fast SYBR® Green Master Mix. Aliquots of each of the master mixes were incubated at 20°C, 40°C, or 45°C for varied periods of time. At each temperature, the ability of each master mix to amplify a 295bp region of human genomic DNA (extracted from whole-blood sample using a Qiagen EZ1 robot) was assessed using the Smartcycler after 1, 2, 7, 14, 21, 30, and 44 days of incubation. PCR specificity was confirmed by including a final melt-curve analysis in the PCR protocol and by subsequent gel electrophoresis. Each condition (defined by master mix, temperature, incubation time) was run in duplicate and mean endpoint fluorescence was calculated. Performance was assessed by normalizing to the mean endpoint fluorescence of the corresponding un-incubated master mix in each run.

Results: Surprisingly, incubating most master mixes for up to 6 weeks at 20°C did not affect their performance as assayed by comparing Ct values and mean endpoint fluorescence relative to control un-incubated master mixes. This was also the case when most master mixes were incubated for up to 4 weeks at 40°C. However, with the exception of the Qiagen QuantiFast® SYBR® Green master mix which showed robust performance when incubated at 45°C for up to 4 weeks, other master mixes exhibited a decline in their performance starting at 2 weeks of a 45°C incubation.

Conclusions: In addition to their cost-effective advantage over other probe-based chemistries, these findings suggest that commercial SYBR® Green I master mixes are also highly thermostable and can reliably withstand elevated temperatures (20°C to 45°C) for prolonged periods of time (2 to 6 weeks). Hence, the SYBR® Green I master mixes tested here will be very useful in providing inexpensive, robust qualitative real-time PCR assays in resource-limited settings with unreliable cold storage.

A-128

Genetic polymorphisms of IL-6, IL-10, COX-2, VDR and periodontal disease.

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Background: Periodontal disease is a complex and multifactorial disorder, in which genetic and environmental factors strongly interplay. Although several genetic polymorphisms have been variably associated with the pathogenesis of this pathology, no definitive data are available on the influence of Interelukin (IL)-6 [-174G/C], IL-10 [-1082G>A], IL-10 [-819C>T], IL-10 [-592C>A], cyclooxygenase-2 (COX-2) [-765G/C] and vitamin D receptor (VDR) [Taql] in Caucasian populations.

Methods: The study population consisted in 42 Caucasian patients (27 females and 12 males, mean age of 60±9 years) with chronic periodontal disease established according to the criteria of the International Workshop for a Classification of Periodontal Diseases and Conditions, and a control group of 39 Caucasian subjects matched for age and gender (30 females and 9 males, mean age 56±15 years) not affected by periodontal disease. Genomic testing was carried out after collecting material with a sterile foam tipped applicator. The DNA was extracted with a commercial kit purchased from Qiagen (QIAGEN GmbH, Hilden, Germany). Allelic analysis was performed using SNP Genotyping Assays (Applied Biosystems, Forster City, CA, USA), which are based on a predesigned mix of unlabeled polymerase chain reaction (PCR) primers and the TaqMan® minor groove binding group (MGB) probe

(FAM™ and VIC® dye-labeled), on the instrument StepOne (Applied Biosystems). The potential interactions among the different polymorphisms has been assessed by multivariate analysis of variance (MANOVA), with statistical significance established at $p<0.05$.

Results: The analysis of cases and controls according to MANOVA revealed that the genotype distribution of IL-6 and COX-2 was mostly overlapping between cases and controls (p=0.182 and p=0.682, respectively), whereas that of IL-10 and VDR achieved a statistically significant difference (p=0.012 and $p<0.01$, respectively). The statistically different genotype distribution of both IL-10 (p=0.030) and VDR ($p<0.01$) was unaffected after removing IL-6 and COX-2 from the model. In particular, the IL-10 ATA/ACC and VDR tt genotypes appeared to confer protection towards periodontal disease, since their frequency was significantly lower in patients with periodontal disease than in controls. It is also noteworthy that although IL-6 and COX-2 gene polymorphisms did not show significant association with periodontal disease when taken singularly, their combination yielded instead a marginal statistical significance (p=0.016).

Conclusions: The results of this study show for the first time in a Caucasian population that IL-10 and VDR gene polymorphisms, as well as the combination of IL-6 and COX-2 genotypes, might influence the pathogenesis of periodontal disease, so that their assessment might be helpful for genotype-based risk assessment, early prevention of predisposed individuals and more aggressive treatment of patients at increased risk.

A-129

Investigation of IL28β Genotypes and Virological Response in an Inner City Population Infected with Hepatitis C Virus

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Background: A Single nucleotide polymorphism *rs12979860,* in the promoter region of the IL28β gene has been recently linked with spontaneous clearance of the Hepatitis C virus (HCV) and sustained virological response (SVR) to combination Pegylated interferon and Ribavarin therapy. The prevalence of this polymorphism is known to vary across ethnic groups and partially explains the differing responses to therapy in these groups. Since the patient population at University Hospital in Newark is diverse, this study is aimed at investigating the IL28β genotype distribution in our patient population and its association with ethnicity, HCV genotype and viral clearance.

Methods: Quantification of Hepatitis C viral RNA followed by HCV genotyping was performed on 100 plasma specimens at the University Hospital. These specimens were further genotyped for IL28β polymorphism *(rs12979860C/T)* using a laboratory developed real time PCR assay and melt curve analysis. Three possible IL28b variants were apparent - TT; C/T; and CC. Demographic data was obtained by chart review. The patient population included in this preliminary study included an equivalent distribution of African-Americans (30%), Hispanic (28%) and Caucasian populations (28%) and others (13%) accounting for the rest.

Results: 69.8% of patients genotyped were HCV genotype 1; 5.7% were genotype 2; 9.4% were genotype 3 and 3.8% were genotype 4. Two patients included in this study harbored multiple HCV genotypes. 78% patients infected with HCV genotype 1 had the C/T or TT SNP of IL28β. This trend was reversed with patients with HCV genotypes 2 (66% CC) and HCV genotype 3 (60% CC). Monitoring the viral load (VL) for at least 12 months following HCV quantification revealed a significant decrease in VL ($\geq$ 2.0 log) in patients with the CC SNP of IL28β.

Conclusions: This preliminary investigation including all ethnicities has attempted to evaluate the significance of IL28β CC variant in clearance of HCV in our patient population. Preliminary data suggests that patients with the IL28β CC genotype are able to achieve SVR when infected with HCV genotypes 2 or 3. Similarly patients infected with HCV genotype 1 who had the IL28β CC variant also showed decreasing trends in VL in response to combination therapy compared to the patients with the IL28β TT or T/C variants. Our data further shows that this correlation between the I IL28β genotype and SVR is observed across all ethnicities.

A-130

Improving PGx Testing by Determining the Cis/Trans Orientation of Multiple Gene Variants

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Objective. To determine the cis/trans orientation of two heterozygous variations in any gene by allele specific amplification

Relevance. The interpretation of genetic information in clinical samples is often incomplete because the relationship between two or more heterozygous variations in the DNA sequence cannot be established. It is often clinically relevant to determine if alterations in the sequence are inherited on the same copy of a gene (inherited in cis), or inherited on different copies of the gene (inherited in trans). Inferred phenotype may differ dependent upon inheritance in cis or in trans.

Methodology. An allele-specific PCR method has been developed that determines if two or more heterozygous genetic variations are located on the same or different copies of the gene. Two allele-specific primers (one wild type and one mutant) are made and paired with a primer that amplifies either the 3'-end or 5'-end of a DNA segment in question. The allele-specific reactions are performed in different vessels and are tested separately. Each of the amplified reactions is analyzed by DNA sequencing to identify the exact sequence in each of the amplicons which establishes the cis/trans orientation of the variations in question.

Results. We have validated an assay to determine the cis/trans orientation between the *17 (c.-806C>T) promoter variation and the *2 (c.681G>A) or *4 (c.1A>G) null alleles in the cytochrome P450 2C19 (*CYP2C19*) gene. This assay was validated using DNA from 26 previously run samples which had known genotypes (fifteen compound heterozygous *17 and *2, one compound heterozygous *17 and *4, five homozygous *17/*17, and five *1/*1). Three of these samples were challenged with inter-assay (three samples on three different runs) and intra-assay (three samples repeated in triplicate on the same run) reproducibility. The assay was verified using a range of DNA concentration from 3-300 ng/uL. This method has also been applied to additional genes including; *UGT1A1* to verifying the cis/trans orientation of the heterozygous *6 (c.211G>A) variation and the TA promoter repeat region (specifically heterozygous *28 [TA7] and *37 [TA8]), *CYP2D6* where it was used to determine the complete haplotype of the *11 (c.883G>C) allele from the promoter region through the 3' UTR of the gene. This method has also been used with additional variations within the *CYP2C19* gene to assess novel functional and rare variations when found as compound heterozygous with other variations. One example of this is a novel function variation we identified (c.486G>T) in exon 3 which causes a premature stop codon. A patient was compound heterozygous for this novel variation, *2 and *17. Using this technique we were able to ascertain the orientation of all three of the identified variations.

Conclusion. We have developed a novel method to accurately establish the cis/trans relationship between compound heterozygous variations, and applied it to multiple genes. Thus, enabling a more accurate and comprehensive prediction of phenotype in clinical samples.

A-136

Real-time nucleic acid sequence based amplification (NASBA) for detection of influenza A virus subtypes

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Background: Influenza A virus of the *Orthomyxoviridae* family is a contagious respiratory pathogen that continues to evolve and burden in the human public health. It is able to spread efficiently from human to human and has the potential to cause pandemics with significant morbidity and mortality. It has been estimated that every year about 500 million people are infected with this virus, causing approximately 0.5 million people deaths worldwide. Influenza A viruses are classified into different subtypes by antigenicity base on their hemagglutinin (HA) and neuraminidase (NA) proteins. The sudden emergence of influenza A virus subtypes and access for epidemiological analysis of this subtypes demanded a rapid development of specific diagnostic tools. Also, rapid identification of the subtypes can help to determine the antiviral treatment, because the different subtypes have a different antiviral drug resistance patterns.The aim of this study was to detection of influenza A virus subtypes by using real-time NASBA which has high sensitivity and specificity through molecular beacon. Real-time NASBA is the method that able to shorten the time

compare to other molecular diagnostic tools and is performed by isothermal condition.

Methods: We selected major pandemic influenza A virus subtypes H3N2 and H5N1. Then, the three RNA gene fragments of influenza A virus were targeted, heamgglutinin (HA) gene, neuraminidase (NA) gene and Matrix protein (M) gene, respectively. M gene is distinguished influenza A virus from other influenza virus. Specific primers and molecular beacons for each targeted RNA segments were designed prior to testing in NASBA assay. We performed RT-PCR with each of subtype reference genes specific primer to confirm specificity.

Results: In case of RT-PCR, M gene, H3N2 and H5N1 genes primer showed good specificity; however, H3N2 NA2 gene primer showed relatively low specificity. PCR product which has amplified by H5N1 gene primer has shown lower sensitivity than others. To confirm the optimal condition of real-time NASBA, we tested with positive and negative controls. It was confirmed that optimal cut-off signal value was 2, and then the amplification signals of each gene have rise up since 21 to 41 minutes. We performed each of subtype HA/NA gene specific molecular beacon to confirm the specificity. As a result, molecular beacons detecting all subtypes of HA/NA genes had higher specificity than RT-PCR.

Conclusions: The real-time NASBA was able to detect and distinguish H3N2/H5N1 subtypes from other subtypes of influenza A virus. Positive results can be achieved with this system within 41 minutes. Also, the specificity and the sensitivity of the method using real-time NASBA were better than those of RT-PCR. This study suggests that detection of neo-appearance pandemic influenza A virus using real-time NASBA has the potential to determine the subtypes of respiratory pathogen.

A-140

Validation of Alpha 1-antitrypsin genotyping to identify Pi*S and Pi*Z alleles using Real-time PCR

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Background: Alpha 1-antitrypsin (AAT) glycoprotein is a member of serine protease inhibitor family synthesized mainly in the liver. The protein plays a critical role in maintaining protease-antiprotease balance by inhibiting neutrophil elastase, a powerful protease capable of destroying lung parenchyma. AAT deficiency is a genetic disorder caused by defective production of the protein, leading to decreased AAT activity in the lungs, and deposition of excessive abnormal AAT protein in liver cells, resulting in chronic obstructive pulmonary disease (COPD), various liver diseases and rarely panniculitis. The purpose of this study was to validate a genotyping method to identify specific alleles associated with AAT deficiency.

Methods: Fourty eight samples referred for AAT phenotype analysis were used for validation of the genotyping assay. Genomic DNA was isolated from EDTA-anticoagulated whole blood samples using the Qiagen EZ1 blood extraction kit. In addition, DNA from twenty samples blinded for AAT genotypes obtained from another institution (Mayo Clinic, Rochester, MN), was used for the assay. Genotyping was carried out using an allelic discrimination assay on the Applied Biosystems 7500 Fast Real-time PCR instrument with Taqman probes. All the samples were genotyped for the most common Pi*S (rs17580) and Pi*Z (rs28929474) deficient allelic variants. TaqMan® MGB probe and primer sets for the alleles were designed using Primer Express Software, Version 3.0 (Applied Biosystems). Positive controls were gBlocks™ gene fragments (Integrated DNA Technologies) of wild type and mutant genotypes for Pi*S and Pi*Z.

Results: The optimized Real-time PCR assay was evaluated using the samples obtained from individuals who were clinically suspected to have AAT deficiency. Our genotyping results were in 100% concordance with the phenotyping or genotyping data of the samples analyzed at the referral lab and Mayo clinic respectively. The genotypes of fourty eight samples were MM (31), MS (11), MZ (5) and SZ (1). The twenty Mayo samples represented a mix of different genotypes: MM (5), MS (5), MZ (4), SZ (3), and ZZ (3). The real-time PCR assay was validated for specificity, sensitivity and precision.

Conclusions: According to the National Human Genome Institute report (January 2012), AAT deficiency occurs in approximately 1 in 2,500 individuals. Mutations in the *serine proteinase inhibitor A1* gene cause AAT deficiency. The M allele is considered to have normal levels of AAT protein production with corresponding serum concentrations of 100-250 mg/dL. The S and Z alleles account for the majority of the harmful variants found in patients with AAT deficiency and are caused by single base substitutions that substantially reduce AAT levels. The homozygous ZZ genotype often results in development of severe COPD and hepatic cirrhosis. The SZ and SS genotypes are associated with pulmonary problems, particularly in smokers. The validated AAT assay would facilitate genotyping of AAT samples routinely for the hospital where currently about 800 samples are tested annually. Validation of AAT serum quantification and protein phenotyping are warranted in the future to generate an algorithm in order to test individuals who are clinically suspected to have AAT deficiency.

A-141

The human melanocortin four receptor gene: A new candidate gene for second generation antipsychotic-related weight gain?

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Background: The human melanocortin four receptor gene (MC4R) is one of the most important causative genes regarding monogenic obesity. The alpha-melanocyte stimulating hormone suppresses food intake and activates energy expenditure via MC4R. In a large scale genome wide association study of obesity[1] the common polymorphism rs17782313 showed the strongest association signal. Its position downstream suggests an influence on MC4R expression. It might, therefore, be a new and interesting candidate gene for second generation antipsychotic (SGA) related weight gain, a frequent adverse effect and major problem of treatment with many antipsychotic drugs.

Objective: Detection of the rs17782313 polymorphism by using the LightCycler™ (Roche, Mannheim, Germany) and investigation of its influence on SGA-related weight gain.

Methods: We developed a new rapid-cycle polymerase chain reaction on a LightCycler 1.5 which was cross-validated with allele-specific-PCR on the GenAmp PCR system 9700 (Applied Biosystems, Foster City, USA). The best results were achieved with the following setup: forward-primer: 5´TTGTGTGCCAGAGGAAACAG3´, reverse-primer: 5´ACCTCAATCCCAGATGCTAAA3´, sensor-probe: 5´GAGATTGTATCCCGATGGAAATGACAAGAA-fluorescein3´, anchor-probe: 5´LC640-GCTTCAGGGGGAAGGTGACATTTAAGTTGG-phosphate3´, 35x (95°C-10sec, 58°C-20sec, 72°C-30sec), 625nM MC4R primers, 75nM hybridization probes, 100ng DNA, 2.75mM $MgCl_2$, 1µl DMSO and 2µl master hybridization mixture (Roche Diagnostics, Mannheim, Germany), total volume 20µl.

Three hundred forty-five Caucasian inpatients who received an SGA (clozapine, olanzapine, risperidone, paliperidone, quetiapine or amisulpride) as part of their treatment for at least 4 weeks were included in our retrospective investigation. Body weight was measured on admission to hospital and on day 28. All recruited patients were inpatients on standard hospital diet. Weight measurement was taken in light nightwear before breakfast. Weight gain and BMI increase were compared by analysis of variance. Two-tailed p-values <0.05 were considered to be of statistical significance.

Results: All 345 patients were genotyped successfully on the LightCycler and 54 samples were cross-checked with allele-specific PCR. The results were in complete concordance. The entire LightCycler run takes 50 minutes.

After 4 weeks of treatment, patients homozygous for the rs17782313 C-allele had a significantly higher risk of weight gain and BMI increase with a dose effect of the C-allele: Carriers of the CC-genotype (n=21) gained 1.5 kg more weight and also showed a 0.5 kg/m² higher BMI increase than the TT-genotype (n=198, ANOVA, p=0.041 and p=0.047 for n=345). In a subpopulation without additional weight gain inducing co-medication, the 106 TT-allele carriers gained on average 0.75 kg (1.09% of their baseline weight) within the 4 weeks of treatment whereas the 57 CT-allele carriers and the 9 CC-allele carriers gained 2.0 kg (3.28%) and 3.9 kg (5.47%, p=0.003, for weight and percent weight gain).

Conclusions: We developed a fast and reliable method for detecting rs17782313. The investigated polymorphism significantly affected weight gain, a common, undesirable and therapy limiting side effect of SGA treatment. Our findings indicate for the first time that the rs17782313 polymorphism could increase the amount of SGA-related weight gain and may influence MC4R expression. Further studies are needed to confirm the role of MC4R before pre-therapeutic testing may be used to improve the allocation of optimal antipsychotic drug therapy.

1. Loos RJ *et al*. *Nat Genet* 2008;40:768-775

A-142

MiRNA Profile Associated with Chronic Calcineurin Inhibitor Nephrotoxicity in Renal Transplant Biopsies

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Background: Chronic calcineurin inhibitor (CNI) nephrotoxicity is associated with progressive renal damage and is a major cause of late kidney allograft failure. It is traditionally diagnosed by histological findings in kidney biopsies. The molecular mechanism associated with this condition is not well elucidated.

Methods: We used a genome-wide microRNA (miRNAs) microfluidic biochip array to compare the expression levels of 1212 human miRNAs in 11 of 25 biopsies (4 with chronic CNI toxicity *vs* 7 with no significant histological abnormalities (discovery set)). We then used qRT-PCR to validate our findings in the remaining 14 biopsies (8 with toxicity and 6 without toxicity (validation set)). We finally explored whether miRNA expression level changes let us detect CNI toxicity before histological changes are detected.

Results: We identified 8 miRNAs differentially expressed with P <0.01 in the discovery set. A total of 21 miRNAs were differentially expressed with P<0.05. Among the top 8 miRNAs, hsa-miR-10b, hsa-miR-30c, hsa-let-7e and hsa-let-7d were validated to be down-regulated in the validation set (P<0.05). Areas-under-the-curve (AUCs) of 0.7-0.9, sensitivities of 82-100% and specificities of 46-92% were achieved when these miRNAs were tested as diagnostic markers for chronic CNI toxicity. MiRNA profile consistent with toxicity was detected in 3 biopsies with no histological features of toxicity, and taken from patients later developed chronic CNI nephrotoxicity.

Conclusions: We identified a unique miRNA expression profile that may complement, or even predate histological findings to diagnose and predict chronic toxicity. These miRNAs may also play important roles in the pathogenesis of toxicity and late graft failure.

A-143

MiRNA Profile Associated with Kidney Graft Reperfusion Injury

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Background: About 4-10% of transplanted kidneys from living donors do not function immediately after transplantation. The frequency of delayed graft function is even higher after cadaver donor transplantation. This is thought to be due to graft injury secondary to reperfusion. The diagnosis of reperfusion injury is made by histology examination of the graft biopsies. No molecular biomarkers for reperfusion injury are established. The molecular mechanism associated with this condition is not well elucidated.

Methods: To explore possible miRNA markers associated with reperfusion injury, 10 biopsies were subjected to next-generation RNA sequencing using the Illumina Genome Analyzer. Of these biopsies, 5 were diagnosed with reperfusion injury and 5 were from kidneys with immediate graft function.

Results: Three thousand seven hundred and nineteen unique miRNAs were detected from the sequencing data. Out of these, 1,325 were mapped to human specific miRNAs in miRbase; 72 reads were known mammalian miRNAs, but novel to human; the rest of the 2322 reads were predicted mammalian miRNAs. The length of reads ranged from 15-30 nucleotides, with the largest fraction (37%) being 22 nucleotides. Fourteen miRNAs were found to be differentially expressed with P <0.05 between biopsies with vs. without reperfusion injury. Out of these, hsa-let-7d*, hsa-miR-708, hsa-miR-1180, hsa-miR-106a are previously described human miRNAs. The rest of the 10 miRNAs were novel miRNAs discovered by this sequencing project.

Conclusions: A miRNA expression profile containing novel miRNAs was discovered by next-generation RNA sequencing for kidney graft reperfusion injury. This profile is being validated using rt-PCR in an independent sample set of 20 biopsies. Validation of this profile in post-transplant serum or urine samples is also in progress. Our data serves as a pilot study for further investigations of reperfusion injury molecular biomarkers.

A-146

Development of a Sequencing Assay for New Pheochromocytoma Susceptibility TMEM127 gene

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Background: Recently, the transmembrane-encoding gene TMEM127 has been identified as a new pheochromocytoma susceptibility gene. Germline *TMEM127* mutations has been found both in familial and sporadic-appearing pheochromocytomas. TMEM127, a candidate for tumor suppressor, controls cell proliferation and acts as a negative regulator of the mTOR signaling pathway.

Objective: To develop and validate a method for *TMEM127* mutation detection that could be incorporated into our current clinical panel for pheochromocytoma and paraganglioma genetic testing.

Methods: Point mutations and small deletions were detected by DNA sequencing of the entire coding region and flanking introns of all three coding exons of *TMEM127* gene and a 5'UTR region. Four primer pairs were used to amplify *TMEM127*, 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. Reproducibility was assessed by running three samples in triplicate on one run and by running three samples in three different runs. DNA from four individuals previously identified as TMEM127 mutation positive by another laboratory was used to asses assay accuracy.

Results: Results showed 100% concordance within and between runs. The average sequencing quality score (represent signal to noise ratio where quality score of 20 represents 99% accuracy) for bidirectional sequencing of *TMEM127* was 39 (range: 18-46) with average intra- and interassay CVs for quality scores of 9.0% (range: 4.5-12) and 15% (range: 9-24%), respectively. Fifteen normal control samples were tested and shown to be free of any disease-causing mutations or small deletions. All mutations in the previously genotyped samples were detected.

Conclusions: We have developed and validated a full gene sequencing assay for detection of mutations in *TMEM127* gene. Genetic testing of TMEM127 should be considered in patients with sporadic pheochromocytoma, who have a single adrenal tumor and who lack mutations or deletions in VHL or SDH genes. Together with *VHL* and *SDHB*, *SDHC*, *SDHD*, SDHAF2 genotyping, which we recently developed and implemented as clinical assays, addition of TMEM127 gene mutation testing will improve diagnostic work-up of pheochromocytoma and paraganglioma.

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Are the single nucleotide polymorphisms in the von Hippel-Lindau (VHL) tumor suppressor gene associated with renal cell carcinoma (RCC) occurrence?

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Inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene has been implicated with the tumorigenic pathway of renal cell carcinoma (RCC), a most common malignant tumor arising from the kidney. RCC occurred 3.62 and 1.95 cases for men and women respectively per 100 thousand people yearly in Taiwan, and 80% of the cases were classified as clear-cell RCC. Our previous studies have noted that two allelic polymorphisms, both A or G, at position 19 and 1149 of the nucleotide sequence in the VHL gene may be informative and implicated with the occurrence of RCC worldwide. Therefore, we hypothesized that these genetic polymorphisms may be risk factors for RCC. To test this hypothesis we examined these allelic frequencies in nineteen RCC patients including normal and tumor tissues and compared the results of healthy subjects from Taiwan. The samples were screened for allele polymorphisms by using restriction fragment length polymorphism (RFLP) of BsaJ I and Acc I digesting. Our results indicated that the 1149 single nucleotide polymorphism was a significant risk factor implicated with sporadic RCC. Also, consistent with the Knudson's two-hit theory, 85.7% of the RCC patients with AG heterozygote at 19 SNP were identified as loss of heterozygosity (LOH) and all the RCC patients with AG heterozygote at 1149 SNP were also confirmed of LOH. Even though larger sample size has been considered to determine the exact power of correlation between these two genetic polymorphisms and RCC, our results in the current study have clearly demonstrated that at least 1149 SNP is strongly implicated with sporadic RCC.

Genotype and allele frequency at 19 and 1149 SNPs in the VHL tumor suppressor gene						
	Number of samples	Genotype frequency (counts)			Allele frequency	
		AA	AG	GG	A	G
19 SNP:						
Healthy subjects	616	0.763 (470)	0.214 (132)	0.023 (14)	0.870	0.130
RCC patients	19	0.632 (12)	0.368 (7)	0 (0)	0.816	0.184
1149 SNP:						
Healthy subjects	616	0.758 (467)	0.218 (134)	0.024 (15)	0.867	0.133
RCC patients	19	0.579 (11)	0.421 (8)*	0 (0)	0.790	0.210
*, p < 0.05 compared with healthy controls.						

A-150

TGFβ1 -509 C>T and XRCC1 399 Arg>Gln (28152G>A) correlate with increased side effects induced by radiotherapy in prostate cancer patients

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Background: Current literature is not rich in information regarding the association of genetic polymorphisms and the risk of developing adverse reactions in patients with prostate cancer (PCa) post radiotherapy. Radiation can lead to various side effects, such as urinary incontinence or difficulties, rectal bleeding and erectile dysfunctions in PCa patients. The risk of developing such adverse reactions depends on a multitude of factors of which the genetic background is a part. The objective of this study is to identify potential correlations between certain DNA single nucleotide polymorphisms (SNPs) within genes involved in DNA repair, regulation of cell growth, cellular antioxidant defense, and telomere maintenance including TGFβ1 −509C>T, XRCC1 399 Arg>Gln (28152G>A), SOD 16Val>Ala, hTERT -245C>T, XRCC3 241 Thr>Met and ATM 5557G>A and the risk of developing side effects post radiotherapy in PCa patients.

Methods: Eighty seven PCa patients treated with radiotherapy was observed for one year following the treatment. Urinary tract symptoms were scored using the American Urology Association (AUA) Symptom Score. The Quality of Life (QoL) due to urinary symptoms was assessed by grading urinary conditions using the scoring system recommended by the AUA. Erectile function was assessed using the Mount Sinai Erectile Functioning scoring system and International Index of Erectile Function. Rectal toxicity was graded according to the Radiation Therapy Oncology Group morbidity criteria.DNA isolated from peripheral blood was used for genotyping using the allele specific TaqMan SNP assay. The incidences of adverse reactions in wide-type and variant (heterozygous and homozygous variants) groups were compared using two-sided Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results: In the wild-type group of TGFβ1 −509C>T, 16 of 41 patients experienced a significant increase in AUA symptom scores post-radiotherapy, while in the variant group, 7 of 46 experienced such an increase. The incidences of these increases were 0.39 and 0.15 in the wild-type and variant groups, respectively, and the difference between these two groups were statistically significant ($p=0.0153$). In the wild-type group of XRCC1 399 Arg>Gln (28152G>A), 2 of 48 experienced significant deterioration in QoL due to urinary symptoms induced by radiotherapy, while in the variant group, 9 of 39 had deterioration in QoL. The incidences of deterioration in QoL were 0.04 and 0.23 in the wild-type and variant groups, respectively, and the difference was statistically significant ($p=0.0105$). The above two SNPs are not associated with other radiation induced adverse reactions in PCa patients. In terms of other SNPs including SOD 16Val>Ala, hTERT -245C>T; XRCC3 241 Thr>Met and ATM 5557G>A), the incidences of all adverse reactions are not statistically different between wild-type and variant groups.

Conclusions: The CC genotype of the TGFβ1 −509C>T is associated with an increased risk for developing urinary side effects in PCa patients after radiotherapy. Additionally, the variant genotype of XRCC1 399 Arg>Gln (28152G>A) is associated with a higher incidence of deterioration in QoL due to urinary symptoms induced by radiotherapy in PCa patients. Further studies are needed to identify other SNPs involved in the risk of radiotherapy-induced adverse reactions in these patients.

A-153

Genotype frequencies of interleukin-28B in patients with hepatitis C virus infection.

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The single nucleotide polymorphism (SNP) rs12979860 (C>T) in the IL28B locus is strongly associated with sustained virological reponse (SVR) to the treatment with interferon-alpha (IFN-) and rivabirin (RBV) in patients with chronic hepatitis C virus (HCV) infection and patients co-infected with HCV and human immunodeficiency virus (HIV). The rs12979860 genotype C/C Alis associated with a higher probability of achieving SVR in patients monoinfected with HCV and co-infected HCV/HIV treated with IFN-/RVB.

Objectives: 1. Implement a method to detect the SNP rs12979860 in the region of the IL28B gene. 2. To study the frequency of the rs12979860 genotypes in patients analyzed.

Methods: 200 Spanish individuals infected with HCV from our hospital were included in this study. Moreover 67 patients are coinfected with HCV/HIV. Genotyping of the rs12979860 was performed using: DNA extraction with QIAamp DNA Blood Mini Kit in QIACube® (Qiagen). Real-time PCR system LightCycler® 2.0 (Roche Diagnostics). Hybridization probes and primers LightMix® Kit IL28B (Roche Diagnostics) and Imegen® IL28B (Imegen). rs12979860 genotype of 54 patients was analyzed with both methods whilst the remaining 146 samples were genotyped with one of the two methods indistinctly.

Results: Genotypes of the rs12979860 were unequivocally assigned in all the cases. The temperature of melting value (Tm) varied ±1 ºC among the different experiments respecting to Tm established by the manufacturer. There is a 100% concordance between both methods.

Conclusions: I. The protocols of IL28B Imegen and IL28B LightMix Kit for LightCycler 2.0 unequivocally detected rs12979860 in IL28B. II. Our data showed 33.0% of patients with favorable genotype C/C, 51.5% of genotype C/T and also 15.5% of genotype T/T. C/C genotype frequency observed was similar to previously reported studies for European population. III. 71% of the patients where infected with G1. The C/C genotype was underrepresented among G1 (31.0 %) on comparing with non-G1 (43.3%).

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Development of a BCR-ABL RT-PCR assay using the ICEPlex Platform

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Introduction: *BCR-ABL* fusion gene transcripts result from a translocation involving chromosomes 9 and 22 and are present in most cases of chronic myelogenous leukemia (CML) and some cases of acute lymphoblastic leukemia (ALL). *BCR-ABL* testing is performed by a variety of cytogenetic and molecular methods for diagnostic, prognostic and therapeutic monitoring purposes. Cytogenetic methods are able to detect (but not discriminate between) the various fusions but are not as analytically sensitive as molecular methods which normally detect only the most common transcripts. Therefore, clinical laboratories offer a variety of BCR-ABL tests and algorithms to accommodate all clinical scenarios requiring *BCR-ABL* analysis. The ICEPlex platform is capable of performing quantitative RT-PCR with high multiplexing capabilities and is used in this study to develop a multiplexed *BCR-ABL* assay that could eliminate the need for multiple clinical *BCR-ABL* tests. As an initial step in the development of such an assay, we evaluate the qualitative performance of a multiplexed *BCR-ABL* assay on the ICEPlex platform.

Methods: A multiplexed RT-PCR assay was designed to detect and discriminate nine *BCR*-ABL fusion transcripts (e13a2, e13a3, e19a2, e6a2, e8a2, e14a2, e14a3, e1a2, and e1a3) along with an *ABL* transcript. The primers for each transcript were designed to yield amplicons of different lengths to allow for the identification of transcripts by capillary electrophoresis. RNA samples were extracted from 18 whole blood samples and one bone marrow sample that were submitted for clinical testing. The 19 RNA samples were tested using the multiplexed *BCR-ABL* ICEPlex assay and the results were compared qualitatively to the GeneXpert assay (using a cut-off of 0.01% *BCR-ABL:ABL*).

Results: Fourteen samples were negative for BCR-ABL transcript by both assays and three samples were BCR-ABL-positive by both assays. Of the two remaining samples, one was positive by the GeneXpert assay only and one was positive by the ICEPlex assay only. The discrepant sample positive by the ICEPlex assay contained

a transcript (e1a2) that the GeneXpert assay is not designed to detect. Additionally, plasmid templates representing all transcripts were amplified and produced amplicons of the predicted sizes.

Conclusions: We developed and evaluated a novel *BCR-ABL* assay capable of detecting the four most common fusion transcripts and five rare transcripts. In an initial qualitative evaluation of this assay using clinical specimens, its increased multiplexing allowed for detection of a *BCR-ABL* transcript that otherwise could have been missed. The ICEPlex assay failed to detect a low-level *BCR-ABL* transcript in one sample but it is unclear whether this discrepant result can be attributed to a delay of 1-2 days in processing the specimens for the ICEPlex assay. Additional studies will evaluate the quantitative performance of this assay.

A-155

Impact of cholesterol-lowering therapies on gene expression of adhesion molecules in mononuclear cells from hypercholesterolemic patients

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Backgroud: L-selectin, P-selectin glicoprotein ligand 1 (PSGL-1), α4β1 integrin (VLA-4), αLβ2 integrin (LFA-1) and αMβ2 integrin (Mac-1) are important adhesion molecules present in the surface of monocytes with a key role in the inflammation process by modulating the interaction of monocytes with endothelial cells (ECs). Whereas L-selectin interacts with PSGL-1 also present in ECs, monocyte PSGL-1 interacts with the selectins on the endothelium mediating the initial monocyte-EC interaction or rolling. Rolling causes further expression of monocyte integrins VLA-4, LFA-1 and Mac-1 resulting in stable adhesion to ECs through binding to intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). The objective of the present work was to investigate the effect of cholesterol-lowering drugs - inhibitors of cholesterol synthesis and absorption- on gene expression of monocyte adhesion molecules in peripheral blood mononuclear cells (PBMC) from hypercholesterolemic (HC) patients.

Methods: Thirty HC individuals were selected from the University Hospital, Sao Paulo, Brazil. After a 4-week washout period, HC patients were treated with ezetimibe (10mg/day/4-weeks), followed by simvastatin (10mg/day/8-weeks) and simvastatin plus ezetimibe (10mg each/day/4-weeks). Total and HDL cholesterol, as well as triglycerides, apoAI and apoB were determinated by common laboratory techniques, whereas LDL and VLDL cholestol were calculated by Friedewald's formula. RNA was extracted from PBMC at baseline (after washout period) and after each treatment. Relative mRNA expression of genes that codified for L-selectin (*SELL*), PSGL-1 (*SELPLG*), VLA-4 (*ITGA4*), LFA-1 (*ITGAL*) and Mac-1 (*ITGAM*) were measured by Taqman® assays, using ubiquitin c (*UBC*) as endogenous reference that was the most stable among various endogenous reference genes tested.

Results: Simvastatin causes a greater reduction of total and LDL cholesterol and apoB concentration than ezetimibe and the association simvastatina/ezetimibe was more efficient than both monoterapies according to these parameters ($p<0.001$). Simvastatin/ezetimibe association therapy reduced *SELPLG* and *ITGAM* mRNA expressions compared to the baseline and the ezetimibe treatment ($p<0.05$), but not when it was compared to simvastatin monotherapy ($p>0.05$). *ITGAL* presents a greater reduction in individuals using simvastatin plus ezetimibe when compared with any other stage of the therapeutic protocol ($p<0.01$). Simvastatin, ezetimibe or their association did not modify *SELL* and *ITGA4* mRNA expressions.

Conclusions: Cholesterol-lowering interventions modulated the gene expression of adhesion molecules in PBMC from HC individuals. Although ezetimibe (10mg/day) and simvastatin (10mg/day) monotherapies did not reduce the gene expression of the studied markers, their association seems to give more benefits in attenuating the inflammation process by reducing in a larger extent the expression of monocyte adhesion.

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Evaluation Of The Mlpa (Multiplex Ligation-Dependent Probe Amplification) Technique For Detection Of Numerical And Structural Chromosomal Abnormalities

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Background: Chromosome abnormalities are a frequent cause of congenital diseases and a major cause of mental retardation, affecting approximately 1% of all live births. Among these aberrations, the trisomy of chromosomes 13, 18 and 21, and the sex chromosome aneuploidies counts with 60-80% of the all anomalies.

Alpha. The G-band karyotype (Giemsa) has been considered the gold standard test for measuring aberrations, however this method requires a long-time for the execution and analysis. Aiming to reduce the turn around time of tests results for the detection of aneuploidies, Alpha

the MLPA test (Multiplex Ligation-Dependent Probe Amplification - reaction multiplex probe-dependent ligase reaction) was evaluated.

Methods: This method is a multiplex PCR method that detects abnormal copy numbers of up to 50 different genomic DNA or RNA sequences, which is able to distinguish sequences differing in only one nucleotide. We used 236 samples previously determined by the G-band karyotype to evaluate the MLPA method, using the SALSA MLPA P095 Aneuploidy probemix kit, that detects the number of copies aberrations in 45 specific sequences in chromosomes 13, 18, 21, X and Y. The results are shown in Table 1.

Results: Comparing the results between the two techniques, 99.15% (234/236) of agreement was found. Disagreement was linked to the samples in which the MLPA results were inconclusive, and this corresponds to 0.85% (2/236) of the samples.

Conclusions: In conclusion, MLPA is a fast, simple and reliable test for the investigation of aneuploidies of chromosomes 13, 18, 21, X and Y.

Table 1: Comparison of results obtained by G-band karyotyping and MLPA P095 kit

Karyotype (result)	MLPA (samples confirmed / total number of samples
Trisomy chromosome 21	38/38
Trisomy chromosome 13	2/2
Trisomy chromosome 18	6/7
48, XXX + 18	1/1
45, X	7/8
47, XYY	3/3
47, XXY	8/8
46, XX	71/71
46,XY	98/98

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Influence CYP2C9, CYP2C19 and VKORC1 gene polymorphisms on warfarin therapeutic requirements in Brazilian individuals

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Background: Warfarin therapy is complex by the wide interindividual variation in response and dose requirements for adequate anticoagulation that is monitored by maintaining the internation normalization ratio (INR) within a narrow therapeutic range. Investigations have established that environmental and genetic factors explain approximately half of the interindividual variability of warfarin dose requirements. Althought it has been identified influence of several genes in warfarin response, polymorphisms in the genes cytocrome P4502C9 (*CYP2C9*) and vitamin K episode reductase complex 1 (*VKORC1*) involved in warfarin pharmacokinetic and pharmacodinamic, respectively, has been strongly associated in several populations. The purpose of this study was to evaluate the influence of polymorphism in the gene cytocromo P4502C19 (*CYP2C19*), *CYP2C9*, and *VKORC1* on warfarin dose requirements in a cohort from the Brazilian population.

Methods: One-hundred and two patients under warfarin therapy were selected from the Dante Pazzanese Institute of Cardiology, Sao Paulo, Brazil. Clinical data were recorded and blood samples were obtained. DNA was extracted from leucocytes using the DNeasy blood kit (Qiagen, MD, USA). *CYP2C9* c.430C>T (*2 allele, rs1799853) and c.1075A>C (*3 allele, rs1057910), *CYP2C19* c.681G>A (*2 allele, rs4244285) and *VKORC1* -1639C>T (rs9923231), c.*134G>A (rs7294) and c.173-136C>T (rs9934438) polymorphisms were identified by allelic discrimination using TaqMan® real time PCR. Beta coefficient (B) and standar erros (SE) were obtained

from univariate linear regression models and multiple linear regression models adjusted for relevant covariates in order to evaluate the influence of clinical variables and genotypes on warfarin response.

Results: Minor allele frequencies in our population for *CYP2C9* c.430C>T, *CYP2C9* c.1075A>C, *CYP2C19* c.681G>A, *VKORC1* c.*134G>A, *VKORC1* -1639C>T and *VKORC1* c.173-136C>T polymorphisms were 0.13, 0.05, 0.14, 0.33, 0.39 and 0.39, respectively. Among clinical variables, sex, obesity and chronic kidney disease (CKD) were associated with variation in warfarin dose requirements ($p<0.05$). In the univariate linear regression, male sex and presence of obesity were associated with an increment of 6.125 (B=6.125, SE=2.185, p=0.005) and 7.821 (B=7.821, SE=3.327, p=0.021) in the weekly warfarin dose, whereas patients with CKD need a lower weekly dose (B=-7.566, SE=3.625, p=0.039). Polymorphisms *CYP2C9* c.1075A>C, *VKORC1* c.*134G>A, *VKORC1* -1639C>T and *VKORC1* c.173-136C>T modified the warfarin response. Presence of the less frequent allele was related with a higher dose of warfarin in the case of *VKORC1* c.*134G>A (B=7.401, SE=2.103, p=0.001) and with lower weekly dose for *CYP2C9* c.1075A>C (B=-10.642, SE=3.903, p=0.008), *VKORC1* -1639C>T (B=-6.183, SE=2.238, p=0.007) and *VKORC1* c.173-136C>T (B=-8.015, SE=2.187, $p<0.001$). After multiple linear regression analysis, all polymorphisms previously associated survive to correction by relevant covariates ($p<0.05$).

Conclusions: Effects of clinical variables on warfarin response are similar to those described in other populations. Polymorphisms in genes involved in pharmacokinetics and pharmacodinamics of warfarin have a great impact in dose requirements of individual from the Brazilian population.

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Evaluation of Clinical Indication for HFE Gene Mutation Screening in the Brazilian Population

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Background: Hereditary haemochromatosis (HH) is an iron metabolism disorder associated with excess iron accumulation, especially in the heart, liver, and pancreas. The vast majority of patients who have inherited forms of iron overload are either homozygous for the C282Y or compound heterozygotes for the C282Y/H63D mutations in hemochromatosis (*HFE*) gene. According to the American Association for the Study of Liver Diseases (AASLD) published in 2011, patients with suggestive symptoms, physical findings, or family history of hereditary hemochromatosis (HH), should be subjected to a combination of transferrin saturation (TS) and serum ferritin concentration evaluation. *HFE* gene mutation analysis should be performed only in those with either TS≥45% or ferritin levels higher than the upper limit of normal.

Objectives: To evaluate retrospectively physicians' compliance with the recommendations for genetic testing and the impact of the guidelines on HH diagnosis.

Methods: Data were acquired from laboratory records of patients subjected to *HFE* gene mutation screening from January to December 2011. When available, biochemical iron overload was considered when: TS≥45% and ferritin levels were higher than 290 µg/l for women and 320 µg/l for men. Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) were calculated. Informed consent was obtained for all patients and the results were made available only to the patients.

Results: A total of 152 patients were subjected to *HFE* gene mutation testing during the evaluated period. Prevalence of C282Y homozygosity and C282Y/H63D compound heterozygosity were 4.6% and 2.6%, respectively. Information on TS or ferritin levels was not available for 40.1% of the patients. The frequency of individuals who have positive genetic test results was 4.9% when no iron overload information was present, and 8.8% in the group of patients where at least one of the biochemical parameters was investigated. Overall, raised ferritin levels were present in 74.2% and TS≥45% in 26.3% of the genotyped sample. No indication of mutation testing was detected in 7.7% of the evaluated patients. Subjecting individuals with either raised TS or ferritin levels to *HFE* gene mutation screening has sensitivity and NPV of 100%. Specificity and PPV have been estimated to be 7.5% and 10.9%. On the other hand, if only individuals with both iron overload parameters abnormal are considered for genetic testing, specificity and PPV increased to 52.8% and 19.4%, while sensitivity and NPV remain 100%.

Conclusions: Our retrospective analysis of laboratory records demonstrated that a high number of patients (40%) genotyped for *HFE* gene mutations had no previous information regarding biochemical iron overload or a first-degree relative with previously identified mutation, as proposed by AASLD guidelines. In addition, 7.7% of the patients presented normal TS and ferritin levels and were in fact unnecessarily genotyped. Moreover, using abnormal levels of both clinical parameters as a first approach to select individuals for genotyping seems to increase the specificity and PPV for diagnosing HH, while maintaining the sensitivity and PNV and reducing the overall cost of genetic screening.

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The SLC6A4 Long A>G polymorphism enhances the clinical sensitivity of SSRI poor-responder genotyping

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Background: Selective serotonin reuptake inhibitors (SSRIs) are the second most commonly prescribed medication class in the United States. Variation in treatment response to antidepressant therapy has been linked to genetic polymorphisms in cytochrome P450 genes and the promoter region of the serotonin transporter gene *SLC6A4*. *SLC6A4* variants reduce transporter expression, increasing synaptic serotonin levels and reducing the efficacy of SSRI therapy. A short (S) allele is characterized by a 43-bp promoter deletion that halves expression of the serotonin transporter. An additional polymorphism in the long (L) allele (A>G; rs25531) decreases transporter expression, producing a poor responder phenotype similar to that of the S allele. To date no published studies have indicated the frequency of the Long G (L_G) allele. The risk of adverse events associated with SSRIs such as paroxetine and fluoxetine is also correlated with CYP2D6 metabolizer status. The objective of this study was to measure the utility of testing for the *SLC6A4* L, A>G polymorphism to identify SSRI-poor responder genotypes.

Methods: Buccal swab and saliva samples were submitted to PGXL Laboratories for DNA extraction and clinical genotyping. DNA extraction was performed using the Qiagen EZ1 DNA Tissue kit. *CYP2D6* genotyping was performed with the Luminex xTAG™ version 2 assay. CYP2D6 phenotypes were classified as extensive, intermediate, poor, or ultra-extensive metabolizers. The S, L_A and L_G alleles of *SLC6A4* were analyzed by a PCR-RFLP assay validated by PGXL Laboratories.

Results: *SLC6A4* genotypes were determined for 1,014 specimens with 864 specimens matched to *CYP2D6* results from a US-based population with testing requests for psychiatric and non-psychiatric purposes. *SLC6A4* allele frequencies were 0.49 L_A, 0.43 S, 0.08 L_G. Poor responder *SLC6A4* genotypes totaled 254 (25.0%) and included L_G/L_G (n=5, 0.5%), S/L_G (n=64, 6.3%), and S/S (n=185, 18.2%). Of the population with both *SLC6A4* and *CYP2D6* genotypes, 7.3% of patients (n=63) were classified as *CYP2D6* poor metabolizers, consistent with reported Caucasian genotype distributions. Detecting the *SLC6A4* L_G allele revealed an additional 6.8% (n=69) of patients who would have been otherwise misclassified as SSRI responders in the absence of testing for the L A>G polymorphism. CYP2D6 genotyping alone identified 7.3% of patients as being at risk for SSRI treatment failure; *SLC6A4* S/L genotyping identified 18.4%, *SLC6A4* L_A/L_G identified 24.9%, and combined *CYP2D6* and *SLC6A4* L_A/L_G genotyping identified 30.4% of the tested patients as at-risk.

Conclusions: We conclude that combining *SLC6A4* L_A/L_G with *CYP2D6* genotyping improves clinical sensitivity for identifying patients at risk for SSRI therapeutic failure by 12.0% versus *SLC6A4* S/L testing alone.

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Association study of E-selectin, MMP9, Light, Ltα, VCAM 1, ICAM1 LGALS2 and NF κB gene polymorphism with mRNA expresssion and serum protein soluble with acute coronary syndrome.

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Background: Acute coronary syndrome (ACS) is a clinical manifestation resulted from atherosclerosis that is responsible to a high morbid and mortality. Recently. atherosclerosis was considered a chronic inflammatory disease with a progressive accumulation of lipids and fibrous elements in arteries. The aim of this study was determine frequency polymorphism in *E-SELECTIN, MMP9, LIGHT, LTα, VCAM1, ICAM1, LGALS2* and *NFκB genes and their association with* mRNA expression in peripheral blood leukocytes and serum concentration of soluble protein searching a new marker of ACS.

Methods: Ninety and three patients were selected in emergency room at Institute Dante Pazzanese of Cardiology with acute myocardial infarction (47), unstable angina and in individuals who showed no significant atheromatous process in coronary

angiography (46) were included. both genders with ages between 45 and 90 years . The genotyping were performed by PCR followed by pyrosequencing The genes expressions were studied by TAQMAN® System and proteins soluble forms were used measured by LUMINEX® System.

Results: The frequency of genes polymorphisms were *LIGHT Lys178Glu (*GG = 91% and AA + AG = 9%), *LIGHT 111+36 C>T (*CC = 68% and CT + TT = 32%), *LTα -9-198 A>G* (TT = 45% and CC + TC = 55%), *LTα Thr60Asn* (CC = 46% and AA + CA = 54%), *MMP9 Gln279Arg* (AA = 57% and GG + GA = 43%), *E-SELECTINA Hys468Tyr* (CC = 75% and TT + CT = 25%), *NFκB 8+10029 G>A* (GG = 37% and AG + AA = 63%), *LGALS2 6+3279 C>T* (GG = 31% and GA + AA = 69%), *VCAM Asp693Asp* (CC = 83% and CT + TT = 17%), *ICAM 332-499 C>G* (GG = 29% and CC + CG = 71%). Preliminary results showed no allelic association in all genes studied with ACS. However, this study showed that AA genotype of *LTα Thr60Asn* and CC genotype of *LTα -9-198 A>G were* associated with higher total cholesterol. The genotype CC of *E-SELECTINA Hys468Tyr* showed associated with higher concentrations of urea, the CC genotype of ICAM *332-499 C>G* showed associated with higher concentrations of total cholesterol, LDL and APO B also AST activity. The AA genotype of LGALS2 *6+3279 C>T*, AA genotype of *NFκB 8+10029 G>A* and CC and CG genotype of *ICAM 332-499 C>G* were associated with increased mRNA expression of ICAM, NFκB, LGALS2 and LTα respectively. The GG genotype to *ICAM 332-499 C>G* was associated with their increased serum soluble proteins and GA genotype to *LGALS2 6+3279 C>T* was associated with increased MMP9 serum soluble protein. In this preliminary results showed that some genotypes can be associated with ACS suggested by increased gene expression and serum soluble proteins and increased concentration of lipids related atherosclerosis. Also increased LTα gene expression in leucocytes showed a marker of ACS.

Conclusions: Increased LTα gene expression in leucocyte can possible be a new marker ACS. ICAM, NFкB and LGALS2 genes polymorphism increased mRNA expression showing inflammation marker. LGALS2 polymorphism increased MMP9 soluble protein expression suggesting association with plaque instability.

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The most common mutations among Brazilian Cystic Fibrosis patients

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Background: Cystic Fibrosis (CF) is the most common autosomal recessive disorder within caucasian population, and is caused due to mutation/s in the CFTR gene. The most common mutation in CFTR worldwide is del508 and cystic fibrosis genetic analysis consortium revealed that this mutation is responsible for approximately 66% of all CF chromosomes in the world. The objective of the study was to describe the frequency of mutations among Brazilian patients suspect of having Cystic Fibrosis and from whom a genetic test was ordered.

Methods: Cystic Fibrosis Genotyping assay (Celera, Alameda, CA) was used to evaluate 99 Brazilian samples. This test analyses the 23 mutations and polymorphisms defined by the ACMG plus the following mutations: S549N, S549R, V520F, 3876delA, 394delTT, 2183AA>G, R347H, 1078delT e 3905insT and probe for two reflex tests.

Results: Of the 99 samples analyzed: 9 patients had only one mutation detected (7 del508, 1 F508FC, 1 W1282X) and 5 had 2 mutation (del 508 was present in 4 of them, 1 G452X, 1 poli 5T, 12789+5G>A and one was G542X + R334W).

Conclusions: The mutation del508 was present in 11.1% of the population tested and in 78.6% of the patients who presented mutations.

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Performance verification of automated Real Time HCV quantification assays (Roche Cobas Ampliprep/Cobas Taqman and Abbott m2000) compared to COBAS TaqMan 48

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Background: Hepatitis C is an infectious disease caused by the Hepatitis C virus (HCV), which mainly affects the liver. About 80% of exposed patients develop a chronic infection. For those patients, HCV treatment protocols require viral load monitoring for antiviral therapy management. Therefore, the quantification of HCV viral load represents a key parameter for therapy evaluation and different standardized quantification assays are commercially available. According to Matsuura et al (2009), the automated Roche Cobas Ampliprep/Cobas Taqman (CAP/CTM) and Abbott m2000 HCV methods are considered to be more effective at predicting sustained viral response compared to the manual Cobas Amplicor HCV Monitor test, v.2.0, thus providing a higher clinical value for the management of therapeutic responses to chronic hepatitis C. In order to improve the quality of HCV test provided by our laboratory, we evaluated the performance of automated systems CAP/CTM and Abbott m2000 in comparison with the Cobas TaqMan 48 which was routinely used in our service. We had previously replaced the Cobas Amplicor Monitor by Cobas TaqMan 48 which allows automated Real-Time amplification and detection, but still needs a manual extraction.

Methods: The analysis of the automated processes CAP/CTM and Abbot m2000 included linearity, intra and inter-assay precision and accuracy. Linearity was determined using dilution series of a high viral load sample, while intra and inter-assay variations were determined using three pools of samples (high, medium and low) and a negative plasma sample obtained from the blood bank.

Results: The regression linearity showed an excellent correlation of R=0.997 for CAP/CTM and R=0.999 for Abbott and both systems had an intra and inter-assay precision with coefficient of variation less than 10%, as expected. Accuracy was established using 30 serum samples previously quantified by Cobas TaqMan. The mean of the log differences obtained for CAP/CTM and Abbott m2000 was 0.05 log and 0.29 log, respectively.

Conclusions: Considering that Cobas TaqMan and CAP/CTM method are provided by the same manufacturer, we expected a better accuracy value for CAP/CTM but, in our hands, the performance of the three methods was similar. Based on these results, the use of fully automated system appears as a good option, as one can obtain the same results but with less handling when compared to the other system that needed sample manual extraction.

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Characterization of the INFINITI® Platform for Familial Mediterranean Fever (MEFV) Genotyping Application (FMF-D) and Its Prevention of Genotyping Errors Caused by Potential Allelic Dropout

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Several known mutations in the *MEFV* gene can cause the autosomal recessive, autoinflammatory syndromes, Familial Mediterranean Fever (FMF). *MEFV* genotyping can assist in diagnosing FMF and may also help in determining the appropriate colchicine therapy. It is critical to include essential/relevant genetic variants among hundreds of known mutations in an effective genotyping panel. However, certain analytical limitations in PCR-based assays may result in genotyping errors sometimes creating ambiguity in determining disease causing mutations (e.g., whether E148Q is or is not a disease causing mutation?). Unlike a rather noticeable double allelic dropout (ADO), a single ADO is a silent error and can result in as the incorrect assignment of homozygosity. The Autogenomics INFINITI® platform for *MEFV* genotyping application (FMF-D, *RUO*) is a film-based microarray which includes the 13 most prevalent *MEFV* mutations. This application has been critically determined at addressing potential ADO impacts upon the challenging E148Q variant detection. Genomic regions covering major *MEFV* variants: [M694V-(*c.2080A>G*)]; [M694I-(*c.2082G>A*)]; [V726A-(*c.2177T>C*)]; [M680I-(*c.2040G>C/A*)]; [E148Q-(*c.442G>C*)]; [R202Q-(*c.605G>A*)]; [P369S-(*c.1105C>T*)]; [F479L-(*c.1437C>G*)]; [I692del-(*c.2074_6delAAT*)]; [K695R-(*c.2084A>G*)]; [A744S-(*c.2230G>T*)]; [R761H-(*c.2282G>A*)] were amplified in a multiplex PCR reaction. Without post PCR cleanup (e.g., SAP/Exo treatment), target amplicons were used as templates for subsequent allele-specific primer extension (ASPE, signal amplification). Extension primers were then captured via hybridization to the oligonucleotide-addressed microarray. All post PCR procedures from ASPE to microarray scanning/analysis were streamlined and automated using the INFINITI Analyzer® and yielded 2 samples results per 20 min after an initial 3.5 h processing interval for a maximum 24 samples per INFINITI® run. The developed application provides a simple and effective tool for *MEFV* genotyping.

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Performance evaluation of automated Real Time HBV quantification assay of Roche Cobas Ampliprep/Cobas Taqman (CAP/CTM) and Abbott m2000 compared to an "in-house" method

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Background: Hepatitis B virus (HBV) is a member of the Hepadnaviridae virus family which can cause acute or chronic hepatitis. According to World Health Organization, an estimated two billion people have been infected with HBV worldwide and more than 350 million have chronic long-term liver infection. Molecular diagnostic assays for the accurate detection and quantification of HBV DNA are very important for patient management. The Molecular Pathology Department of a private hospital in São Paulo, Brazil has provided an "in-house" Real Time PCR assay for HBV viral load for 5 years. However, due to the increasing demand for HBV molecular tests in our laboratory, there was a need to replace our method by an automated process with similar or better performance. Two automated systems (Roche Cobas Ampliprep/Cobas Taqman - CAP/CTM and Abbott m2000 HBV test) were evaluated to verify the possibility of changing our "in-house" method with a method which is not only faster but also allows us to meet the increased demand with a better quality.

Methods: The commercial tests were performed according to manufacturer's instructions. "In-house" Real time PCR was performed in an ABI7500 instrument using TaqMan Probes and primers described before. Performance evaluation included accuracy, linearity, intra and inter-assay precision. Accuracy was tested comparing the results of 35 samples previously quantified by our "in-house" method. Linearity was determined using dilution series of a high viral load sample, while intra and inter-assay variations were determined using three pools of samples (high, medium and low) and a negative plasma sample obtained from the blood bank.

Results: Accuracy tests resulted in a log difference of 0.33 and 0.5 log for Abbott and CAP/CTM, respectively. The regression line of linearity presented an excellent correlation of R=0.996 for CAP/CTM and R=0.979 for Abbott, and both systems had an intra and inter-assay precision with coefficient of variation less than 10%, as expected.

Conclusions: Based on the results obtained, our evaluations demonstrated that both systems had a great performance and are able to replace our manual method.

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Monitoring molecular response to CML patients: standardization of results in international scale.

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Background: The Chronic Myeloid Leukemia (CML) is commonly caused by a translocation of chromosome 9 and 22, leading to fusion of the BCR and ABL genes. The product of this fusion gene is a very important target for quantitatively monitoring the disease being performed by Real Time PCR (RT-PCR). However, the different protocols and methodologies hamper the proper comparison of results from distinct laboratories. Therefore, it is mandatory to pursue standardization of results in international scale to facilitate interpretation and decision therapy as well the monitoring of minimal residual disease. The aim of this study was to evaluate the kit BCR-ABL Mbcr IS-MMR (Ipsogen, France) through a comparison with a quantitative methodology developed in-house based on RT-PCR. Both techniques are specific for detection of BCR-ABL related to p210.

Methods: For both methods 44 samples were tested - 38 from patients and 6 from the program of CAP external quality control. In addition, all results were further confirmed by Nested PCR, method that is considered to have a higher analytical sensitivity.

Results: Using the kit BCR-ABL Mbcr IS-MMR kit, positive results were obtained in 81.82% (36/44) of the samples while the in-house quantitative RT-PCR provided 68.18% (30/44) positivity and the Nested-PCR method showed that 84.09% (37/44) of samples were positive.Considering the results provided in log base, treatment response was defined as a decreased in 3, 0 log when compared with previous results for in house methodology. In 80% (20/25) of the positive samples, 3,0 log decrease was not observed using the in house RT-PCR methodology revealing that no treatment response was obtained. With the commercial BCR-ABL Mbcr IS-MMR Kit, the absence of therapy response was observed in 40.91% (18/44) of the samples. It is possible to discriminate the treatment response in complete, major, minor and minimal molecular response when using the international scale unit. From the 59.91% (26/44) considered to be responsive to treatment tested by BCR-ABL Mbcr IS-MMR Kit, complete, major, minor and minimal molecular response was evidenced in 20,45% (9), 11,36% (5), 11,36% (5) and 15.9% (7), respectively.

Conclusions: The results demonstrate that (i) BCR-ABL Mbcr IS-MMR kit is more sensitive than the in house quantitative method and (ii) it has the advantage of providing results on an international scale without the need to calculate correction factors, allowing the standardization of results among different laboratories.

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Molecular fetal sex determination in early gestation ages shows more accurate results than an ultrasound approach

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Background: Fetal sex assessment by detecting Y chromosome sequences in maternal blood has been used routinely in prenatal care and it is associated to some clinical indications including risk of X-linked disorders and some fetal ultrasound findings.

It is an early and non-invasive method usually performed in the first trimester of pregnancy with fetal genetic material amplified from maternal plasma.The test for fetal sex determination is performed by Real-Time PCR detecting fragment of the DYS14 gene present in Y chromosome, chosen during the test standardization due to be in multiple copies, ensuring the analytical sensitivity of the test.

Objective: The purpose of this study was to verify the correlation of the results of molecular method for fetal sex and ultrasound findings. Samples from 5143 pregnant women were tested for fetal sex determination using the molecular method (May - November 2011).

Results: Out of these, 15 (0.29%) reported divergent results between the molecular determination and ultrasound findings, being 12 (12/15 - 80%) characterized as females by the molecular test and 3 (3/15 - 20%) present the Y chromosome fragment amplified (male). The gestational age mean of these women was 9.4 weeks (standard deviation of 1, 35). Blood was recollected from these patients in order to confirm the initial results after 27 days (standard deviation of 10, 3). One patient did not agree on the resubmission of the test. The results of molecular method in the remaining 14 were reproducible in 100% of samples.

Results: The results, described herein, demonstrated that the molecular method provides more accurate sex determination than ultrasound during very early gestational age (8 to 10 weeks of pregnancy).

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Frequency of mutated alleles related to Fragile X Syndrome among children in a large laboratory in Brazil

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Background: Fragile X Syndrome (FXS) is a genetic disorder related to trinucleotide repeats (CGG) located on the 5' region of the FMR1 gene in the X chromosome being the most frequent cause of inherited mental retardation. FMR1 gene is responsible for the production of fragile X mental retardation protein (FMRP), which binds to mRNA to encode synaptic-related proteins. The following types of alleles are observed: normal (6-44 CGG repeats), gray zone (45-54 CGG repeats), premutation (55 a 200 CGG repeats) and full mutation size (>200 CGG repeats).

Patients who present gray zone results are generally asymptomatic but women, both clustered in the gray zone or with permutation have higher risk of premature ovarian failure (POF). Fragile X-associated tremor/ataxia Syndrome (FXTAS) is more frequent in men older than 50 years. The hallmark of Fragile X Syndrome is FMR1 gene silence. Boys are affected more severely than girls, presenting significant intellectual impairment and other co-morbidities. The aim of the study was to evaluate the frequency of mutations among Brazilian individuals from whom a Fragile-X test was requested. All samples were tested using ASR Fragile X test (Celera, Alameda, CA) by fragment analysis technique.

Results: From Oct 2011 to Feb 2012, 201 samples were tested (27 female - 13.4% and 174 male - 86.6%). The majority of samples (91.5%) were from children (184/201). Out of these, 20 (11%) were girls and 164 (89%) boys. Full mutation was detected in 5 boys (2.5%) and gray zone was present in 2 boys (1.0%). Among the female individuals, normal heterozygous results were obtained in 81.5% (22/27) of the samples and in 18 (51% - 5/27: 3 adults and 2 children), only one X chromosome could be detected. In those cases, the results can be attributed to normal homozygous or abnormal allele full mutated undetectable due to a test limitation, since the ASR Fragile X test is able to detect up to 230 CGG repeats.

Conclusions: Five patients with full mutation (2.5%) were detected in a short period of time in this Brazilian population with a biased configuration due to the fact that all individuals had the test requested previously and therefore should have a clinical indication.

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FUT2 Gene Variant: A Biological Marker for Plasma Vitamin B12 levels

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Background: Low plasma levels of vitamin B_{12} have been clinically linked to cancer, cardiovascular disease and neurodegenerative disorders and are usually related to reduced intestinal absorption, rather than dietary deficiency. Common variants in the fucosyltransferase 2 (*FUT2*) gene, which determines the human secretor (Se) blood group by mediating the formation of H type 1 and 2 antigens, have been shown to be highly associated with levels of vitamin B_{12} in three independent genome-wide association studies.

Objectives: To evaluate the influence of the rs492602 genetic polymorphism in *FUT2* gene on plasma vitamin B_{12} and homocysteine levels, using a large and ethnically diverse population-based sample of São Paulo, Brazil.

Methods: The population-based survey used a probabilistic three-stage cluster sample of Sao Paulo inhabitants to represent the population according to gender, age (20-80 years), and socio-economic status. A total of 1010 individuals were subjected to *FUT2* gene polymorphism and 31 genetic ancestry informative markers genotyping and plasma vitamin B_{12} and homocysteine levels quantification. The study protocol was approved by the Research Ethics Committee of the Universidade Federal de Sao Paulo (CEP 0593/06) and all volunteers read and signed the informed consent form.

Results: Valid genotyping data was obtained for 935 subjects (518 women and 417 men; mean age 42.25±14.45 years). The frequency of genotype groups were: GG (32.41%), GA (51.44%) and AA (16.15%). No deviation from Hardy-Weinberg equilibrium was found (p>0.05). Our results confirmed the association of plasma vitamin B_{12} with rs492602 genetic variant ($p = 2.71 \times 10^{-06}$). The presence of the A allele in homozygosity was associated with higher vitamin B_{12} concentrations, when compared to G allele carriers (GG+GA genotypes) (mean difference= 102.17±21,64 pg/mL). A general linear model was applied to verify the effect of possible factors and covariates that might confound the associations of the AA genotype and vitamin B_{12} concentrations. After adjustment for age, sex, body mass index and European ancestry, the associations between the genetic marker and vitamin levels remained highly significant ($p = 1.01 \times 10^{-06}$). No significant association between homocysteine levels and vitamin B_{12} concentration, as well as with the *FUT2* gene polymorphism was found in our sample (p>0.05).

Conclusions: Our data confirms the previously described association between common genetic variants in the *FUT2* gene and plasma vitamin B_{12} levels in a large and multi-ethnic epidemiological sample. Since H antigens are known to influence the adhesion of pathogens such as with *Helicobactor pylori* to the gastric and duodenal mucosa, the hypothesis underlying the current results is that the increased vitamin levels may be a consequence of lower susceptibility of AA genotype carriers to *H. pylori* infection, which will lead to a reduced risk of vitamin B_{12} malabsorption. The findings highlight this and other polymorphisms in the *FUT2* gene as potential biological markers for vitamin deficiency and clinically related phenotypes.

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Differential Expression of Cyclooxygenase-2 in Triple-Negative Hormone Receptor Phenotype Breast Cancers: An Additional Marker in the Quest for Personalized Medicine

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Background: Increased COX-2 expression has been shown to play an important role in carcinogenesis in human breast cancer, predicts aggressive clinical course and provides a potential target for treatment with COX-2 inhibitors. Most studies have used immunohistochemistry (IHC) as sole determinant of protein overexpression using formalin-fixed, paraffin embedded (FFPE) tissues. But, IHC has its limitations due to many artifacts related to fixation, epitope access, etc. Our aim was to explore differential COX-2 expression by real-time polymerase reaction (RT-PCR) on RNA extracted from grade 3 breast cancer FFPE tissues archived in excess of 10 years.

Methods: From a cohort of 343 grade 3, breast cancers from 1995 to 2009 (174 triple negative tumors (TNT), 56 Her2+, 113 ER+), 19 cases (9 TNT, 5 Her2+, and 5 ER+) were randomly selected for this pilot project based on morphological similarity, availability of tumor blocks with >75% tumor content and acceptable RNA quality. Total RNA was isolated using the standard column extraction method, reverse transcribed, and analyzed by RT- PCR on the Human Breast Cancer Signaling Array (Qiagen, PAH-131) on a Roche Lightcycler 480. The array contained 84 key genes commonly involved in the dysregulation of signal transduction during breast carcinogenesis and 5 house keeping genes.

Results: Total RNA extractions from FFPE tissues yielded 1.6-11.9ug RNA in 19/22 samples. Housekeeping genes consistently amplified with cycle thresholds (CT) of 26.6-37.3. COX-2 gene was the most differentially expressed gene, when expressed, had a CT range of 12.8-20.3. After normalization of the mRNA expression of COX-2 gene, two distinct populations with strong positive (4 cases, 44%) and negative expression (5 cases, 56%) were noted in the TNT cases. This stark differential expression was not observed in Her2+ or ER+ cohorts.

Conclusions: Considerable progress has been made to personalize breast cancer treatment in regards to hormone receptor phenotype, but further stratification for prevention and targeted therapy is needed. Here, we describe two sub-populations within histologically aggressive triple negative breast cancers in regards to COX-2 expression in an on/off fashion which may be used as potential targets for chemoprevention and treatment using COX-2 inhibitors. This pilot study also highlights 86% success rate from over a decade old archived FFPE tissues opening the door for future retrospective studies.

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Association between TGF-α and MSX-1 polymorphisms and presence of non-syndromic cleft lip and/or palate

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Background: Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is a common birth defect with unclear etiology. Both genetic and environmental factors may contribute to NSCL/P. Early studies suggested several potential candidate genes for clefting. TGF-α and MSX1 genes have emerged as leading candidates that are associated with risk of orofacial clefts. The aim of this study was to evaluate known SNPs as well new variants in the TGF-α and MSX1 genes by High Resolution Melting technique (HRM).

Methods: PCR followed by High resolution melting analysis and PCR real time assays (TaqMAN were performed to search new mutations and genotyping on 145 NSCL/P (DNA samples) detecting the known SNPs and new variants.

Results: In the TGF-α gene 7 polymorphisms were identified and confirmed by sequencing (rs11466221, rs11466267, rs2166975, rs3771523, rs538118, rs34214089, rs1058213. Three polymorphisms (rs11466295, rs11466287, rs11466282) have being described for the first time in patients with oral clefts. One new variant was found in this study by HRM technique in 3'UTR region presenting a low allele frequency (0.69%). Analysis of alleles adjusted to gender and family history confirms the difference between genotypes for the A allele of rs538118 (p=0.013) with male patients and rs11466267 deletion TATA (p=0.013) and SNP rs2166975 (p=0.017) with a family history. Logistic regression was not significant statistically. In the MSX1 gene were identified three polymorphisms (rs1095, rs2229262, rs3821949), two of them (last) were described the first time in studies with oral clefts. The linkage disequilibrium analysis for TGFα and MSX1 alleles showed a formation of a haplotype block between polymorphisms in the exons 1 and 2 of TGFα gene ($r^{2=}0.93$), indicating that these polymorphisms are transmitted in block.

Conclusions: PCR-HRM was a simple and cost effective procedure useful for high-throughput mutation screening and genotyping. Several polymorphisms were identified by PCR-HRM, including a new variant in the 3'UTR region of TGFα gene in patients with oral cleft. This study intends to continue trying to understand genetic factors associated to NSCL/P in the population of Rio Grande do Norte/Brazil.

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Extraction and Molecular Screening of Decade-Old mRNA from Archived Breast Cancer Tissues

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Background: Since the explosion of molecular techniques in pathology over recent years, a common goal has been to develop retrospective studies where patient outcomes are known. Abundant formalin-fixed paraffin embedded (FFPE) material is available at any institution generally archived for decades. Extraction of amplifiable mRNA from old blocks has been a challenge and has been sporadically reported. Our aim was to test mRNA quality extracted from archived FFPE blocks from breast cancer patients, where the inherent fatty nature of the tissue impedes optimal fixation.

Methods: Tumor FFPE tissues from breast cancer cases were retrieved between years 2000-2001. 34 cases were randomly selected for this pilot project based on morphological similarity and availability of tumor blocks with >75% tumor content. Total RNA was isolated (Recover-All Nucleic Acid Isolation Kit, Ambion), reverse transcribed (RT First Strand kit, Qiagen), and analyzed by real-time PCR on a Roche Lightcycler 480. Total RNA quantity was assessed on Nanodrop machine. Samples with more than 1ug of RNA yield were considered adequate for validation test with HPRT1 gene as primary screening housekeeping gene. Then the samples were run on Human Breast Cancer Signaling Array (Qiagen, PAH-131) that contained 84 key genes commonly involved in the dysregulation of signal transduction in breast cancer and 5 house keeping genes (Beta2 Microglobulin, GAPDH, HPRT1, ACTB, RPL13A). Positive calls were set at an arbitrary cycle threshold (CT) of 40 cycles.

Results: Total RNA extractions yielded 65 ng to 18.75 ug RNA in 34 samples. 26/34 cases (77%) yielded more than 1ug of RNA and all showed successful HPRT1 gene amplification. 19/26 cases were run on the cancer signaling array. All cases had the housekeeping genes consistently amplified with CT of 26.6-38.7. Eighty-four breast cancer associated genes showed CT value ranging from 12.8-39.8 with most arrays yielding at least 65% positive calls.

Conclusions: We have developed a protocol for the extraction and gene expression screening of decade old mRNA. While the cycle thresholds determined during the screen indicate either low relative expression or simply low recovery, the efficacy as a screening tool is readily apparent. Moreover, the process can go from paraffin block to usable screening mRNA expression data in a matter of a few hours. Applying this type of technology to additional breast data sets and other neoplasms will undoubtably increase the feasibility of using molecular techniques to retrieve valuable retrospective information currently locked away in every institution's FFPE storage archives.

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Serum Brain-Derived Neurotrophic Factor, Nerve Growth Factor and Neurotrophin-3 Levels in Dementia

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Background: Neurotrophins, the most well-known trophic factor of the nervous system, are a family of polypeptide growth factors that control the death and survival, growth and differentiation of neurons. In vivo and in vitro studies have demonstrated that neurotrophic factors are involved in a variety of pathways stimulated by excitotoxic damage; ischemia and apoptosis- mediated traumatic brain injury

Methods: Brain-Derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF), and Neurotrophin-3 (NT-3) were determined in patients with probable Alzheimer's dementia receiving cholinesterase inhibitor (CEI) treatment (the AD+CEI group; 17 males and 15 females, the mean age was 74.2 ± 7.4 years, the duration of the treatment was 4.1 ± 2.5 years); patients with vascular dementia receiving CEI treatment (the VaD + CEI group; 13 males and 14 females, the mean age was 69.2 ± 10.2 years, the duration of the treatment was 4.7 ± 2.9 years); newly-diagnosed patients with probable AD without medication (the AD group; 11 females and 11 males, the mean age was 73.8 ± 7.7 years) and age-matched healthy individuals were enrolled in this study (control group; 9 females and 11 males, the mean age was 72.1 ± 12.1 years) .Dementia patients were received donepezil as CEI treatment. The serum levels of BDNF, NT-3 and NGF were measured using an enzyme-linked immunosorbent assay kit according to the manufacturer's instructions.

Results: NGF levels were detected to be significantly higher in the control group than in AD group (p<0.001). BDNF and NT-3 levels in AD group were not significantly different from control group's levels. NGF levels in AD + CEI group were significantly higher than in AD group (p<0.05). A positive correlation between BDNF and Mini Mental State Examination (MMSE) scores (r: 0.422, p<0.01) in AD group and a negative correlation between BDNF and MMSE scores in the AD+CEI group (r: -0.357, p<0.005) were obtained

Conclusions: We suggested that serum BDNF levels vary depending on the degree of dementia in Alzheimer Disease and the decreased serum NGF levels may be associated with the presence of dementia. We also suggested that CEI therapy with donezepil was effective on serum BDNF levels in AD patients. On the other hand, the cause of these changes are still unclear and the question: "to what extent changes in serum neurotrophic factor levels influence the pathology of the brain?'' is yet to be answered. Future studies should be planned to include larger number of patients and psychotic, exercise and depression -related changes in patients.

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A-176

Development of PCR-reverse blot hybridization assay for identification of bacterial and fungal pathogens in sepsis

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Background: Sepsis is a major cause of morbidity and mortality worldwide. The aim of this study was to develop a test for the rapid diagnosis of causative agents of the blood infection. The test uses molecular genotype technology including PCR amplification and subsequent reverse blot hybridization assay (PCR-REBA) which is based on DNA amplification with biotinylated primers followed by hybridization to membrane bound probes. The test was developed to identify major causative agents of sepsis such as fungus, mycobacteria, gram-negatives, and gram-positive cocci including *Staphyolococcus aureus*, and for rapid detection of mecA and van genes.

Methods: The strip of membrane developed in this study included several pan-probes which can identify all gram positive bacteria, all gram negative bacteria, all mycobacteria, and all fungus. In addition, for gram positive cocci treatment, MecA, VanA and VanB gene probes were included. In order to develop the PCR-REBA, reference strains including 24 gram-positive bacteria, 43 gram-negative bacteria, and 16 fungal microorganisms were used.

Results: To assess the performance of the PCR-REBA developed in this study, evaluation using clinical isolates was carried out. In short, DNA samples extracted from 118 clinical isolates grown on sheep blood agar and MacConkey agar, and a total of 40 positive blood cultures from BACTEC 9240 system (Becton Dickinson, USA were included in the study. The identification of the clinical isolates was carried out using Microscan® (Siemens Healthcare Diagnostics, Sacramento, CA, USA) and VITEK-2 (BioMerieux, Hazelwood, MO, USA) kit and Microscan® (Siemens Healthcare Diagnostics, Sacramento, CA, USA) were used for identifying blood cultures. Antibiotic susceptibilities were determined by using Microscan® (Siemens Healthcare Diagnostics, Sacramento, CA, USA). Of the 40 blood culture isolates, 24 were identified as 8 *S. epidermidis* [all were methicillin-resistant (MR)], 9 were *S. aureus* (one was MR), 7 were *E.* faecium (one was vanconycin-resistant) by Microscan® (Siemens Healthcare Diagnostics, Sacramento, CA, USA) methods. In short, the results from the PCR-REBA showed that the overall concordance rate between microbiological identification method and the PCR-REBA was 89.7% in Gram positives, 100% in Gram negatives, and 100% in Fungus. The discordant results were mainly derived from the identification results using the Microscan® (Siemens Healthcare Diagnostics, Sacramento, CA, USA) with blood culture samples. The concordance rate of two methods in determining drug resistance was 100%.

Conclusion: In conclusion, PCR-REBA developed in this study is a fast and reliable test for the identification of the gram-positives, all of gram-negatives, fungus, and mecA and van genes directly from positive blood culture bottles and culture isolates. These data indicated that, routine use of DNA strip technology-based assay would be useful for clinical diagnosis in patients with sepsis.

Tuesday PM, July 17, 2012

Poster Session: 2:00 PM - 4:30 PM

Animal Clinical Chemistry

B-01

Effect of piperine on Lipid profile of Non-Transgenic Mice

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Background: Piperine was isolated from *Piper nigrum* popularly known as black pepper. There were many studies earlier about piperine that it is powerful bioenhancer for many drugs especially antibiotics. Piperine extract believed to potentiates drug into several folds. The present study was focused on its individual effect on biochemical parameters like blood sugar and lipid profile before and after the administration of piperine.

Materials and Methods: 30 non transgenic mice were taken for study obtained from animal house of faculty of Medicine,Garyounis university ,Benghazi, Libya. These mice were fed with high cholesterol diet and divided into 2 groups. 20 mice were administered with piperine at a dose of 5mg/kg body weight. Piperince was isolated in Department of Pharmacognosy, Faculty of Pharmacy, Garyounis University, Benghazi and 10 mice were not administered with piperine but fed with high fat diet. These mice were anaesthetized with ketamine and halothane and blood was withdrawn from each mice before study by cardiocentesis. Piperine was administered with high fat diet for 3 weeks one group of 20mice and only high fat diet given to another group consists of 10 mice. Again blood samples were taken after 3 weeks from both groups. Blood sugar,Serum, Cholesterol, Serum triglycerides and Serum HDL cholesterol were measured in the Serum by authenticated methods.

Results: Blood sugar was significantly elevated (p=0.001) after 3 weeks after administration of the piperine whereas it was decreased in other group who were not given piperine. Serum cholesterol was significantly elevated (p=0.0025)3 weeks administration after piperine administration with high fat diet. There was no decrease in the cholesterol levels after when compared with other group where the piperine was not administered. Serum triglycerides levels were significantly decreased (p=0.0005) after the administration of piperine but there was no significant difference in other group. HDL cholesterol was significantly elevated (p=0.0032) after administration of piperine and it was not significant (p=0.6553) in the group where there was no piperine administration .

Conclusions: As per this study the beneficial effect of piperine appears to be lowering triglycerides and increasing HDL cholesterol. Further research may show promising results on HDL raise. This study has shown it does not have any role in reducing blood sugar and total cholesterol.

B-03

The comparative study of the cytoprotective effect of thymohexin and lansoprazole in epinephrine-induced gastric lesions in rats

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Background. Recently significant data has been accumulated on the gastroprotective properties of olygopeptides. Purpose of research was to explore the cytoprotective effect of the hexapeptide of thymohexin (Arg-alpha-Asp-Lys-Val-Tyr-Arg) in experimental gastric lesions (GL) in rats.

Material and methods. The studies were conducted on 50 outbred male rats. Thymohexin (10μg/kg) was injected i.p. 15 min before the induction of GL by epinephrine (2 mg/kg). The control animals were pretreated with saline (i) or lansoprazole (ii, 30 mg/kg). In 24 hours rats were sacrificed and area and macroscopic index of GL were evaluated. Biochemical investigations included the determination of the activity of constitutive and inducible NO-synthase (NOS) isoforms (cNOS and iNOS), NO content as well as activity of lipoperoxidation processes (tiobarbituric acid (TBA) products) and antioxidant defense system (superoxide dismutase (SOD) and catalase activity) in gastric mucosa (GM) and L-arginine level in plasm.

Results. Epinephrine caused severe GL with the mean area of damage 38.2±3.1 mm2, the macroscopic index made up 11±0.3 grade, accompanied by the acute increase of iNOS activity and NO content in GM as well as the activity of lipoperoxidation processes whereas L-arginine concentration in blood decreased ($p<0.05$). Pretreatment with Thymohexin caused 50% decrease both of the GL area and macroscopic index of damage ($p<0.05$), 2-fold decrease of iNOS activity, 31% decrease of NO content in GM ($p<0.05$) which corresponded with the increase of the NO substrate - L-arginine in plasm. The content of TBA products also decreased for 30% ($p<0.05$) and SOD activity decreased 2-fold in GM of thymohexin-pretreated rats compared to the effect of epinephrine ($p<0.05$). In rats pretreated with lansoprazole the area of GL decreased for 75% ($p<0.05$). The tendence to decrease of total NOS, iNOS was also more significant, although L-arginine in blood plasm of thymohexin-pretreated rats was 25% higher. The indices of TBA products, SOD and catalase activity in thymohexin- and lansoprazole-pretreated rats did not differ significantly.

Conclusions. Thymohexin had significant cytoprotective effect in epinephrine-induced GL, probably mediated by its inhibitory effect on iNOS and lipoperoxidation processes, although inferior to that of lansoprazole. Nevertheless taking into account the low toxicity of olygopeptides and therapeutic effectiveness in significantly lower doses, we suggest that thymohexin is perspective for further studies on the prevention of gastric ulceration.

B-05

Beta-sitosterol promotes an anti-inflammatory effect in murine activated neutrophils in a calcium uptake-dependent manner.

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Background: Beta-sitosterol (β-Sit) is a common phytosterol present in several plant species. This sterol has demonstrated anti-inflammatory properties. *Objectives:* The aim of this study was to evaluate the effect of β-Sit upon activities of myeloperoxidase (MPO) and adenosine-deaminase (ADA), and levels of interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) in the inflammation induced by carrageenan, in the mouse air pouch model, as well as its effect on the $^{45}Ca^{2+}$ uptake in activated murine neutrophils.

Methods: β-Sit was isolated from dried barks of *Esenbeckia leiocarpa* that were extracted with *90% EtOH* resulting in a crude hydroalcoholic extract that was partitioned and extracted with EtOAc to yield an alkaloid fraction.This fraction was submitted to solid-liquid partitioning with ether, resulting in polar (Pol), and Nonpolar (Nonpol) subfractions. Nonpol was eluted with $CHCl_3$, β-Sit was identified by comparison with data in the literature. Adult Swiss mice were used in all the experiments. This study was approved by the Committee for Ethics in Animal Research at Federal University of Santa Catarina (Protocol- 23.080.004957/2009-24). The air pouch model was induced as described by Vigil et al. (2008). In the *in vivo* studies, different groups of animals were treated with β-Sit (0.25 to 2 mg/kg,i.p.) 0.5 h before the inflammation induction, and its effect upon activities of MPO and ADA, and levels of IL-1β and TNF-α was evaluated 24h later. Indomethacin (5mg/kg,i.p.) and dexamethasone (0.5mg/kg,i.p.) were used as reference anti-inflammatory drugs. In another set of experiments, activated neutrophils were harvested from the air pouch and treated with β-Sit (10^{-9},10^{-8},10^{-7}, and10^{-6} M) by 1, 5 or 15 min for evaluation of calcium (Ca^{2+}) uptake. Significant differences between groups were determined by Student's *t* test or two-way-analysis of variance (two-way-ANOVA) complemented with Bonferroni test. $P < 0.05$ was considered significant

Results: β-Sit inhibited the activities of MPO by 39.5±14.8, and ADA by 48.3±9.5, levels of IL-1β by 53.1±0.1, and TNF-α by 64.3±9.5. This compound also promoted a time-and dose-dependent increase of the calcium uptake in activated neutrophils that was reversed by nifedipine, BAPTA-AM, LY294002, and colchicine ($P < 0.05$).

Conclusions: β-Sit showed an important anti-inflammatory effect, since it inhibited activities of MPO and ADA, and levels of IL-1β and TNF-α. This effect seems to be mediated by the calcium uptake in activated neutrophils in a time- and dose-dependent manner through L-type voltage dependent calcium channels, intracellular calcium, PI3K, and microtubules modulation.

B-06

Evidence of anti-inflammatory effects of Rosmarinus officinalis L. in the mouse model of pleurisy induced by carrageenan

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Background: Rosmarinus officinalis L. is a native plant from Mediterranean region, cultivated in Brazil and it is used in the folk medicine for treatment of inflammatory diseases.

Objectives: The aim of this study was to evaluate the anti-inflammatory effect of the crude extract (CE) of R. officinalis, and it derived fraction: Ethyl acetate (AcOEt) and isolated compounds: Betulinic acid (BA) and Carnosol (CA), upon leukocytes, exudation, myeloperoxidase (MPO) and Adenosine-Deaminase (ADA) activities, and nitric oxide (NOx) levels using a murine model of pleurisy induced by carrageenan (Cg).

Methods: Dried R. officinalis aerial parts were underwent extraction through the steam technique to remove the essential oils. This substance was macerated with ethanol at room temperature to obtain the CE without oil, which was partitioned with ethyl acetate solution to obtain the AcOEt fraction. The AcOEt fraction was separated, purified by crystallisation, yielding the isolated compounds: betulinic acid (BA) and carnosol (CA). Swiss mice, 18-20g, were used throughout the experiments. Pleurisy was induced by intrapleural (i.pl.) injection of 0.1 mL of carrageenan (Cg, 1%). The inflammatory response was analyzed after 4 h. Different groups of animals were treated 0.5 h prior pleurisy with: CE (10-100 mg/kg), AcOEt (10-50 mg/kg), BA (1-10 mg/kg) or CA (1-10 mg/kg) administered by intraperitoneal route (i.p.). The animals were previously challenged (1h) with Evans blue dye (25 mg/kg, administered by intra-orbital route) to evaluate the exudation. To analyse the MPO and ADA activities and NOx levels different groups of animals were pretreated (0.5h) with CE (50 mg/kg), AcOEt (25 mg/kg), BA (2,5 mg/kg) or CA (2,5 mg/kg). The inflammation was analyzed after 4h. This study was approved by the Committee for Ethics in Animal Research at Federal University of Santa Catarina (Protocol- PP00632). Statistical differences between groups were determined by ANOVA complemented with Dunnett's test. Values of P<0.05 were considered significant. **Results:** CE (50-100 mg/kg), AcOEt (25-50 mg/kg) BA (2,5-10 mg/kg) and CA (2,5-10 mg/kg) inhibited leukocytes (% of inhibition (CE: 58.3±2.4% to 61.0±2.0%; AcOEt: 32.0±4.1% to 32.8±2.6%; BA: 20.6±2.1% to 44.3±3.6%; CA: 37.2±6.5% to 45.3±4.6%), neutrophils (CE: 60.7±2.1% to 61.0±2.2%; AcOEt: 31.8±4.6% to 32.5±2.9%; BA: 19.9±2.4% to 43.6±3.9%; CA: 35.8±6.2% to 44.6±4.5%)) and exudation (CE: 31.5±3.7% to 46.0±3.3%; AcOEt: 20.1±1.4% to 22.3±2.7%; BA: 13.6±3.8% to 22.8±5.7%; CA: 20.0±8.8% to 22.8±3.8%) (P<0.05). CE (50 mg/kg), AcOEt (25 mg/kg), BA (2,5 mg/kg) and CA (2,5 mg/kg) inhibited MPO by 61.5±6.6%; 63.6±4.9%; 52.7±4.7% and 42.7±3.7% and ADA activities by 34.4±5.6%; 39.6±5.7%; 44.4±5.3% and 45.1±8.9% and NOx levels by 50.7±4.3%; 65.5±6.2%; 26.0±10.8% and 29.6±5.9%, respectively (P<0.05).

Conclusions: R. officinalis showed important anti-inflammatory activity by inhibiting leukocytes and exudation. These inhibitory effects were associated with decrease of MPO and ADA activities, and NOx levels.

B-07

NT pro-BNP Method Comparison in Cynomolgus Monkeys with Congestive Heart Failure

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Background: Brain- natriuretic peptide (BNP) is increased significantly with heart failure. BNP is a 32-amino acid polypeptide secreted primarily by the left ventricle when the heart is unable to pump blood efficiently (e.g. in cases of heart failure). At the time of release, inactive N terminal pro-brain natriuretic peptide (NT pro-BNP) is cleaved from the precursor peptide pro-BNP and is co-secreted in quantities directly proportional to its biologically active counterpart BNP and in close correlation with the severity of heart failure.

Methods: An experiment was conducted to compare commercially available NT pro-BNP immunoassay kits. Two human assay kits manufactured by Mesoscale Discovery (MSD) and Millipore, respectively and a third assay kit specifically designed for use in monkeys manufactured by Wuhan EIAab Science Co. were included in the comparison. The amino acid sequence of NT pro-BNP shares approximately 90% homology between non-human primates (NHP) and humans. Ten cynomolgus monkeys were used to obtain a normal range of values for NT pro-BNP. Serum samples were collected and assayed using the MSD kit /instrument, the Millipore kit / BioPlex instrument, and the EIAab kit /Triturus instrument.

Results: The average baseline level of NT pro-BNP for the ten naive monkeys was 0.00ng/mL (MSD), 0.004ng/mL (Millipore); 16.34ng/mL (ELISA). To provide monkey serum with elevated NT pro-BNP, four cynomolgus monkeys were tachypaced into congestive heart failure. A pre-paced, post surgery serum sample was drawn for NT pro-BNP and separated into three aliquots. The animals were then paced at 240 bpm for 4 weeks with serum samples obtained at 2 and 4 weeks of pacing. Each sample was analyzed in all kits. In the human kits, a result of 0.00ng/mL was observed for all samples indicating a lack of cross reactivity between the human and NHP peptides for these assays. Therefore the baseline result of 16.34ng/mL from the naive monkey study was used for comparison with the congestive heart failure study. Using the monkey specific ELISA kit, NT pro-BNP levels ranged from 7.62-22.2ng/mL for the pre-paced samples with an increase to 12.0-34.5ng/mL for the 4-week paced samples. Individual NT pro-BNP results were well correlated with severity of heart failure symptoms in the paced monkeys and echocardiographic determination of decreased cardiac function and cardiac dimensions. In the two monkeys with the most severe symptoms and cardiac failure, the level of NT pro-BNP was twice that of naive monkeys. The remaining two animals demonstrated changes in their echocardiograms but were asymptomatic at 4-weeks. NT-pro-BNP was 0.7-1.5× levels of naive monkeys.

Conclusions: Despite 90% sequence homology between humans and monkeys, good cross reactivity was not observed. These results clearly demonstrate that non-human primate assessments of NT pro-BNP must be carried out using monkey-specific assay kits. In the heart failure animals, NT pro-BNP levels were well correlated with severity of heart failure, further validating the ELISA assay kit. Due to inherent variability of NT pro-BNP among monkeys, pretest values are necessary to interpret results adequately.

B-08

Metformin Attenuates Neointimal Formation in Fructose-induced Insulin Resistant Rats: the Pathological Role of Methylglyoxal in Stenosis

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Background: Insulin resistance is an important risk factor for the development of diabetes, atherosclerosis, and cardiovascular disease. Methylglyoxal, a very reactive metabolite of glucose is highly elevated in diabetes and associated with the development of diabetic atherosclerosis. We hypothesized that elevated methylglyoxal accelerates the neointimal formation in insulin resistance. This study was to investigate the pathological role and underlying molecular mechanisms of methylglyoxal in neointimal formation induced by balloon catheter injury in fructose-induced insulin resistant rats.

Methods: Sprague Dawley (SD) rats were fed with high fructose diet (60%, Harlan) for 4 weeks for the development of insulin resistance. Rats were then underwent balloon catheter injury on the left carotid artery. A group of rats with insulin resistance were treated with metformin (300 mg/kg/day). Rats were sacrificed and the neointimal formation was then determined at 1 and 4 weeks after balloon injury. Blood sample was collected after 12 hours fast for biochemical analyses. Signalling Molecules and inflammatory markers were measured by Western Blotting or real-time PCR. Carotid artery reactivity was studied. Histological and morphometric analyses were performed to determine the neointimal hyperplasia. Neointimal cell proliferation was assessed by positive BrdU staining. Apoptosis was detected using TUNEL method.

Results: Following fructose diet for 4 weeks and the development of insulin resistance, plasma glucose and insulin levels were highly increased compared to the chow controls (P<0.05). Plasma total cholesterol, LDL-C, VLDL-C, and triglyceride levels were significantly elevated compared to the chow controls (P<0.05-0.01). Plasma levels of glucose, insulin, and lipids were significantly improved following treatment with metformin (P<0.05-0.01). Plasma methylglyoxal levels were significantly increased in fructose-fed rats compared with that with normal chow (P<0.05). Treatment with metformin significantly reduced plasma methylglyoxal levels (P<0.05). The neointimal hyperplasia of the carotid arteries was enhanced in insulin resistance and treatment with metformin dramatically attenuated such phenomena as compared to the controls determined 1 week or 4 weeks after balloon injury (P<0.01 and P<0.05, respectively). The intima/media ratios were also reduced at 1 and 4 weeks. Treatment with metformin induced a significant reduction in the percentage of BrdU-positive cells in the neointima compared with that received vehicle controls (P<0.01). Iinsulin receptor substrate-1 (IRS-1), adenosine monophosphate-activated protein kinase (AMPKa), and their corresponding phosphorylation forms were markedly inhibited in the liver in fructose-fed rats. Treatment with metformin improved the expression of these molecules. Real-time PCR demonstrated that the expression of inflammatory

markers JNK and NFKB were dramatically increased in insulin resistant rats. Treatment with metformin significantly reversed such increased expression. Treatment with metformin attenuated phenylephrine-induced vasoconstriction but increased methacholine-induced vasorelaxation of the balloon injured carotid arteries compared with that of untreated rats.

Conclusions: Insulin resistance facilitates the formation and development of neointimal hyperplasia. Treatment with metformin, a scavenger of methylglyoxal significantly attenuates the neointimal formation via inhibition of smooth muscle cell proliferation, improvement of insulin signalling pathway, and inhibition of inflammation. This finding may provide a potential therapeutic approach.

B-09

Effects of alpha lipoic acid on ifosfamide-induced central neurotoxicity in rat brains

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Background: Ifosfamide(IFOS) is a cytotoxic alkylating agent commonly used in the treatment of a great number of childhood cancers such as ewing sarcoma, osteogenic sarcoma, mesenchymal tumors, malignant germline tumors, some forms of Wilms tumor. Ifosfamide may cause for a series of more specific, potentially life-threatening toxicities: hemorrhagic cystitis, nephropathy, encephalopathy and cardiac toxicity. Alpha-lipoic acid, also referred to as thioctic acid,(pentanoic acid, ALA) is a derivate of octanoic acid. ALA, which has a strong antioxidant effect, is a naturally occurring sulphydryl compound found in virtually all plant and animal species. It is a cofactor covalently attached to a lysine residue forming an essential lipoamide, which is involved in mitochondrial energy metabolism. We aimed to evaluate a) whether α-lipoic acid can reduce ifosfamide- induced central neurotoxicity in rat brains b) the changes of malondialdehyde (MDA), as an indicator of lipid peroxidation, protein carbonyl (PC) levels, as a protein oxidation and catalase activity, as antioxidant enzyme, in brain tissue after administered α-lipoic acid.

Methods: Totally, 26 Wistar rats(12-day-old), weighing 20-30 g were divided into the following groups: Group 1) Control(n=6): untreated Group 2) Ifosfamide(Haloxan) (n=7): 500mg/kg ifosfamide was injected i.p.(24. hours) Group 3) Alpha lipoic acid(Thioctaoid 600,Meda Pharma,Germany) (n=6): 200 mg/kg ALA was administered p.o with the aid of gavages (0.hour and 23. Hours) Group 4)Ifosfamide + Alpha lipoic acid (n=7): In this group 200 mg/kg ALA was administered p.o with the aid of gavages (0.hour and 23. hours) and 500mg/kg ifosfamide was injected i.p. (24. hours).We analyzed PC, MDA and catalase activity in the brain tissue. Protein measurements were analyzed in homogenate and supernatant according to the method of Lowry. The tissue MDA level was determined by a method based on the reaction with thiobarbituric acid at 90°-100 °C. The activities of catalase was analyzed spectrophotometrically by Aebi's method. The protein oxidation was analyzed with the tissue protein carbonyl content by the method based on the reaction of the carbonyl group with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone.

Results: Ifosfamide significantly reduced CAT activity compared to control and ALA groups($p<0.05$). The MDA level and protein carbonyl content in brain tissue was increased significantly in IFOS group in comparison with the other groups ($p<0.05$). ALA treatments prevented significantly the increase in MDA level($p<0.001$) and PC content($p<0.05$) in brain tissue, whereas ALA could not significantly increase catalase activity in brain tissue.

Conclusions: ALA might be an important alternative treatment method for neurotoxicity side effect of chemotherapy in the future. However, functional outcome assessment should be analyzed with different dose regimens and longer administration of ALA in the further studies to achieve better outcome.

B-10

Protective effects of caffeic acid phenethyl ester on ifosfamide-induced central neurotoxicity in rats

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Background: Ifosfamide, a structural analog of cyclophosphamide, is an alkylating chemotherapy agent used for a wide range of solid and hematologic malignancies. Caffeic acid phenethyl ester (CAPE) is an active component of honey bee propolis extracts and has been used in folk medicine for many years. Previous studies have demonstrated that CAPE prevents lipid peroxidation induced by ischaemia-reperfusion injury in renal tissue, spinal cord and brain. The exact pathophysiological mechanisms responsible for the development of ifosfamide-induced encephalopathy are not known, oxidative stress caused by some antineoplastic and chemical toxic agents is known to be an important factor for neuronal toxicity.The aim of this study was to establish the protective effect of caffeic acid phenethyl ester (CAPE) against the Ifosfamide-induced central neurotoxicity in rats and the changes in oxidant-antioxidant status of brain tissue.

Methods: Thirty five wistar rats (12 days old) were used in the experiments.They were randomly divided into five groups. There were seven rats in each group. The first group was used as a control The other groups, CAPE, Ifosfamid, CAPE+ Ifosfamid and ethanol. Control untreated rats (n = 7); Group 2 was treated with i.p. CAPE alone 10 µmol/kg (n = 7); group 3 was treated with single i.p. injection of IFOS (500mg/kg) (n = 7); group 4 was treated for 2 days with i.p. administration of CAPE (10 µmol/kg) beginning from 1 day before single i.p. injection of IFOS (n = 7), group 5 was treated with serum physiological and 10% ethanol . At the 24th hour of IFOS-treatment, brain tissues were removed for analysis. The levels of tissues protein carbonyl content (PC), malondialdehyde (MDA) as well as the activities of catalase (CAT) were determined. Catalase activity was determined according to Aebi's method. The tissue MDA level was determined by a method based on the reaction with thiobarbituric acid (TBA) at 90-100 ^{0}C. The carbonyl contents were determined spectrophotometrically by a method based on reaction of carbonyl group with 2, 4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone. Protein measurements were made in the samples according to the Lowry method.

Results: The brain CAT activity was lower in IFOS group than the other groups ($p < 0.05$). The levels of MDA and protein carbonyl content in brain tissue were higher in IFOS group than the control, CAPE, ETHANOL and IFOS plus CAPE groups ($p < 0.05$). There was no significant difference among MDA and protein carbonyl content of control, CAPE, ETHANOL and IFOS plus CAPE groups ($p>0.05$).

Conclusions: This study revealed that pretreatment with CAPE might protected brain tissue against IFOS-induced central neurotoxicity. CAPE may prevent these brain lesions with its antioxidant and anti-inflammatory action. CAPE could be an effective course of therapy to enhance therapeutic efficacy and to lessen IFOS toxicity in clinical chemotherapy

B-11

The role of RNase L in IBD

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Background The inflammatory bowel disease (IBD) is a disease that causes chronic and relapsing inflammation in the intestine. It is well-known that cytokines and chemokines are mediating and promoting inflammation in the intestine. 2-5A dependent RNase L (RNase L) is one of the key enzymes involved in the molecular mechanisms of interferon functions against viral infection and cell proliferation. In addition, the role of RNase L in the regulation of proinflammatory gene expression and cell apoptosis has been well established in the last decade. Thus, we hypothesized that RNase L regulates the expression of proinflammatory genes, mediates the inflammatory responses and apoptosis in epithelial cells of intestinal mucosal surface. Lack of RNase L attenuates the disease.

Method To induce acute colitis, age and gender matched RNase L $^{+/+}$ and $^{-/-}$ mice (10mice/group) were given drinking water with or without 2.5% dextran sulfate sodium for 7 days. For induction of chronic colitis by DSS, these mice (10mice/group) received four cycles of DSS treatment. Each cycle included treatment with 2.5% DSS for 7 days, followed by a 10-day interval with normal drinking water.

Survival and change in body weights of both mouse types (18 mice/group) were determined by feeding 3% DSS in drinking water. At the end point, the intestines were removed after euthanasia. Intestinal tissues were examined histologically and/or immunohistochemically for evidence of immune cell infiltration and function, and colitis. The expression of specific proinflammatory genes in the intestinal tissues from both types of mice such as TNF-α, COX-2, IP-10, and IFN-γ were quantitatively determined by real-time PCR after treatment with DSS. The expressions of these genes were confirmed by ELISA.

Results RNase L is expressed in nearly all types of mammalian cells from mouse to man. However, tissue distribution analysis of RNase L revealed that the level of RNase L in the intestine was 5-fold higher than in the spleen and thymus, and was 10 to 30-fold higher than that in other tissues. Interestingly we found that the expression of Cox-2 and TNF-α was about 2- and 5-folds higher in RNase $L^{+/+}$ borrow derived macrophages than that in RNase $L^{-/-}$ BMMs after treatment with LPS. Mice lacking of RNase L developed signs of bowel disease (bloody stool) significantly slower than that wild type mice upon treatment of DSS (3% in drinking water). Further, 100% of the wild type mice died on day 15 during the DSS treatment, whereas all RNase L deficient mice were surviving at least 17 days post-treatment. At the endpoint of (day 20) of the experiment, there were 40% RNase $L^{-/-}$ mice survived. The intestinal mucosa of RNase $L^{+/+}$ mice showed prominent features of ischemic colitis. There was near total loss of villous architecture, atrophy and necrosis of glands, and hyalinization of the lamina propria. However, the intestinal tract from RNase $L^{-/-}$ mice showed preserved normal villous architecture, mostly unremarkable mucosa, with focal glandular necrosis.

Conclusion RNase L plays an important role in the development of IBD through regulating the expression of proinflammatory genes and lack of RNase L attenuates the disease.

B-13

Can Benchmarking be used to monitor global analytical performance of laboratories?

M. M. Menises[1], M. Blanco[1], G. Jimenez[2], P. Esteban[1], P. Domecq[1], J. Oyhamburu[2], P. Pagano[1]. [1]*Aleman's Hospital, Buenos Aires, Argentina,* [2]*Italian's Hospital, Buenos Aires, Argentina*

Background: Benchmark using patient samples could be used to monitor the analytical performance of laboratories: both precision and bias. Since the frequency could be decided by laboratories to adequate to lot reagents and calibrators switches, it seems a reasonable tool to use with Proficiency Testing (PT). It is supposed bias between laboratories, even using instruments belonged to different manufacturers, should be acceptable according to clinical criteria and Biological Variation (BV). AIMS: to describe the experience in Benchmarking (Ben) in Clinical Chemistry between two laboratories with different instruments (Beckman vs. Roche), to evaluate Ben's ability to assess the analytical performance of our laboratories in comparison with PT and to decide whether the bias is acceptable

Methods: We analyzed Glucose (Glu), Calcium (Ca), Colesterol (Col), Creatinine (Crea) and Alanine Amino Transferase (Alt) simultaneously in two laboratories with Beckman and Roche in a set of 3 specimens (excess patient samples) in triplicate once a week during 6 months (august 2011- January 2012). We evaluated if there was bias (using Bias Plot) and correlation (r); and calculated the mean bias (%B) and imprecision (%CV). We compared the Ben %B and %CV with BV and %B CAP 2010-2011 (Roche vs. Beckman). Besides we evaluated the PT performance of the laboratories.

Results: According the confidence interval of the estimated difference (Bias Plot), our laboratories have bias in all the analytes except Col; and although Crea and Glu have bias very closed to BV requirement and Ca is above; we still judge all of them as acceptable. Surprisingly CAP (Roche vs. Beckman) 2010- 2011 doesn't show bias in Glu. We have a poor correlation in Ca but CAP has all the correlation coefficients above 0.997. Both laboratories have imprecision below BV requirements except Ca because of the extremely stringent requirement. Neither of the laboratories had failures in PT. Both Ben and PT reflected an acceptable analytical performance of our laboratories.

Conclusions: Ben allows monitoring both bias and imprecision using the frequency needed by laboratories. It evaluates the global analytical performance of laboratories in addition with internal quality control and PT. We believe the Ben %Bs observed can be considered acceptable since the instruments belong to different manufacturers and in some cases they are different methods (Ca: cresolphtalein complexon vs. ion select electrode dilution). The differences observed between Ben and Cap about bias and correlation may be due to different reagents and calibrators lots used by the majority participants of CAP and our laboratories that belong to latinamerica.

B-14

Comparison Of "Benchmarking" And "Quality Control" To Evaluate Bias With Lot Reagent And Calibrator Switches In Clinical Chemistry

M. M. Menises[1], M. A. Blanco[1], G. Jimenez[2], P. Esteban[1], P. Domecq[1], J. Oyhamburu[2], P. Domecq[1]. [1]*Aleman's Hospital, Buenos Aires, Argentina,* [2]*Italian's Hospital, Buenos Aires, Argentina*

Background: Analytical Benchmarking using patient samples (BE) allows assessing bias between laboratories (BLab). It could also be suitable to verify that results for patient samples are consistent when changing lots of calibrators and reagents (Bias related lot: BLot). The shifts of BLab should reflect BLot. Quality control (QC) materials should be convenient to evaluate BLot but noncommutability with clinical patient samples may preclude their use. AIMS:To determine if lot reagent and calibrator switches introduce bias in patient samples: Blot, assuming BE as the Gold Standard procedure (GS)and To evaluate the ability of QC versus BE to monitor BLot.

Materials And Methods: We analyzed glucose (Glu), calcium (Ca), cholesterol (Col), creatinine (Crea) and alanine amino transferase (ALT) in 2 laboratories using different instruments (Beckman vs. Roche). A set of 3 specimens (excess patient samples) and 2 QC (Randox and Roche) in triplicate once a week during 6 months (August 2011- January 2012) were assayed simultaneously. We evaluated BE and QC Randox with Deming Model if BLab changed with the lot reagent and calibrator switches (BLot). We used 2- sample t Test to analyze Blot with QC Roche. Besides we compared the sensibility and specificity of QC to evaluate BLot assuming BE as GS.

Results: According BE, ALT, Glu, Ca and Crea had Blot. QC Randox showed no correlation between the two laboratories. The sensibility and specificity of QC Roche to evaluate Blot were 0.39 and 0.69 respectively.

Conclusions: Benchmarking using patient samples can be used to monitor bias due to lot reagent and calibrator switches. Quality control materials cannot be used to evaluate bias between laboratories because of the poor correlation. Besides QC Roche has neither suitable specificity nor sensibility to be used to monitor BLot. The majority analytes evaluated showed Blot except Col. Every Blot should be evaluated using patient samples.

Tuesday PM, July 17, 2012

Poster Session: 2:00 PM - 4:30 PM

Automation/Computer Applications

B-18

After an assay platform change, a reassessment of a significant reference range change for total bilirubin based on stability of patient results distributions

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Background: Our hospital chemistry laboratory recently converted to use of Roche Cobas c501 analyzers (new) after long-standing use of Beckman Synchron LXi analyzers (previous). For most assays, exact numerical correspondence of results between new and previous assays allowed for direct transfer of the previous reference range to the new assay. Total bilirubin was an exception. Results between new and previous assays were highly correlated (r = 0.997) but with slope (m) significantly different from unity (m = 0.88; n = 58, range: 0.1-16 mg/dL). On the basis of a reference range study (n = 40), a new reference range of 0.1-0.9 mg/dL was established. Because this range differed considerably from the previous reference range (0.1-1.2 mg/dL) and from the manufacturer's study reference range (<1.2 mg/dL), our objective was to reassess the reference range change by comparison of pre- and post-conversion patient results distributions. We first examined the stability of patient results distributions for total bilirubin in periods before and after conversion to the new technology. We then examined whether the percentage of within-reference range results post-conversion was consistent with that of the previous assay results.

Methods: Month-by month patient results for total bilirubin from January-May, 2011 (6 months pre-conversion) and July-October, 2011 (4 months post-conversion) were retrieved from the LIS. These results were then edited to include first-or-only results for individual patients. Numbers of such results for each month ranged from n = 6400 to 7977. Stabilities of the distributions pre- and post-conversion were assessed by calculation of the inter-month variations of the medians and the percentages of within-reference range results. The consistency of the reference range change relative to the prior reference range was assessed by comparison of the percentages of within-reference range results between the pre- and post-conversion results distributions.

Results: First-or-only patient results distributions both pre- and post-conversion were expectedly smooth, continuous, non-normal distributions skewed toward elevated bilirubin results, with means significantly greater than medians. Both pre- and post-conversions distributions were highly stable as assessed by inter-month variations in medians (pre: median = 0.63±0.016 mg/dL (CV=2.6%); post: median = 0.43±0.0064 mg/dL (CV=1.5%)), and in percentage (N) of within-reference range results (pre: N = 88.0±1.2 % (CV=1.4%); post: N = 87.9±0.42 % (CV=0.48%). There was an essentially exact correspondence of pre- and post-conversion N's. This result for N affirmed the correctness of the new reference range, relative to the previous reference range, and relative to the numerical shift observed between pre-and post-conversion results distributions demonstrated by the shifts in medians.

Conclusions: First-or-only patient results distributions for total bilirubin were highly stable across monthly intervals for both the previous and the new assays. In this context, unchanged percentages of reporting of normal and abnormal results across new and previous assays confirmed the correctness, from a clinical perspective, of a significant reference range change for the new assay relative to the previous reference range. Patient distribution analysis was an efficient means of reassessment of the reference range given distribution stability and the large numbers of total bilirubin test results available for comparison.

B-19

Setup and verification of an automated plasma hemoglobin assay utilizing the VITROS 5.1 chemistry platform

J. Olson, W. Castellani. *Penn State, Hershey, PA*

Introduction and Objectives Plasma hemoglobin (PHgb) level is a direct marker of red cell damage and is useful in monitoring processes that cause intravascular red blood cell lysis. At our institution, red blood cell damage caused by extra corporeal membrane oxygen (ECMO) therapy is followed by serial plasma hemoglobin level assay. The current manual spectrophotometic method is not available on all shifts and reduces its clinical usefulness. This manual method utilized a direct spectrophotometric reading described adapted from the literature and is based on the difference in the absorbance of hemoglobin at 575nm and 560 nm.

We have adapted this method to the Vitros 5.1 automated chemistry analyzer as a direct spectrophotometric assay and demonstrate its performance characteristics.

Materials and Methods: The VITROS 5.1 (Ortho Clinical Diagnostics) is capable of adapting open-channel methods with spectrophotometric capabilities. The wavelengths of 575nm and 600nm were selected in adapting the existing manual method to this analyzer. The reagent for this assay is 0.9% saline. We chose to use 160ul of saline for the reagent, effectively diluting the 40ul sample by a factor of 5. We used shortest time the analyzer allows and a two-wavelength assay method. A calibration factor was used to adjust for the dilution in the final calculations, which produces the plasma hemoglobin concentration in mg/dl.

Results: The automated assay showed good linear performance when compared to the manual spectrophotometric method over the expected PHgb range. Over a 7-sample range of 11mg/dl to 956mg/dl, the slope was 0.987 and the observed error was 1.3%. The within-run CV for a low specimen (~25 mg/dl) and a high specimen (~230 mg/dl) was 1.1% and 0.2% respectively. The between day CV for a low specimen (~25 mg/dl) and a high specimen (~230 mg/dl) was 1.1% and 0.6% respectively. Measurements of clear, icteric and lipemic samples spiked with the same hemoglobin solution gave clinically equivalent results (clear: 56mg/dl, icteric: 57 mg/dl and lipemic: 58mg/dl). A 120-sample study using visually non-icteric, non-hemolyzed samples gave a 2 standard deviation range of 0-22mg/dl. A correlation study of 7 patient and 5 spiked patient samples demonstrates a correlation of r = 0.987 between the automated method and the manual spectrophotometer.

Conclusions: The method developed is fully automated, allowing testing to occur at any time without specialized technologist training. The method's precision, accuracy and range also compare well with the previous assay, leading to confidence in the assay's ability to produce clinically useful results.

B-20

Automated urinalysis as a screening method for urinary tract infection

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Urinary tract infection (UTI) is very common in clinical practice and usually requires a bacteriologic culture of a urine sample to establish the etiology of the disease. Although a quantitative urine culture is essential for the final diagnosis of UTI, it is a very time-consuming and expensive procedure.

Several screening tests have been proposed for the rapid diagnosis of UTI. Rapid urine screens should provide immediate results for the clinician and eliminate the unnecessary culturing for most of the negative samples. Recently a new automated urine system has been introduced (LabUMat+UriSed, 77 Elektronika Kft, Budapest, Hungary). The LabUMat is an automated urine chemistry analyzer evaluating eleven chemical parameters of LabStrip U11 Plus test strip. UriSed is a walk-away automated urine microscopy analyzer 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 system to predict the outcome of quantitative urine cultures we studied 2075 fresh midstream clean-catch urine samples from children and adults (1 month to 94 years old, 1359 females and 716 males).

Each sample was submitted to quantitative culture and automated urinalysis. More than one technologist analyzed the urine cultures and they had no knowledge of the LabUMat+UriSed results. For a screening of UTI we considered the combination of quantitative determination of bacteriuria, leukocyturia, presence of yeasts and leukocyte esterase positive. In this study, we adopt the cut-off level of 5/hpf for leukocyturia, 12.5/hpf for significant bacteriuria and $\geq 10^4$ CFU/ml in the urine culture. Leukocyte esterase $\geq$2+ was considered positive. The statistical analysis included evaluation of sensitivity, specificity, positive and negative predictive values and accuracy.

The incidence of positive cultures was 16% (n=338). Among these positive samples 28 of them were misclassified as negative by the screening protocol. A clinical correlation followed by a critical analysis of these results showed that twelve of them were considered as negative by the clinical criteria and the patients did not receive antibiotic therapy. The other sixteen samples were considered as positive and the patients were treated for UTI. This group included patients with renal diseases, recurrent UTI, kidney or bone marrow transplant patients and children under 1 year old.

Considering that, a combination of quantitative determination of bacteriuria, leukocyturia, presence of yeasts and leukocyte esterase (2+ and/or 3+) showed high sensitivity (95%) and negative predictive value (98%), with specificity of 64%, positive predictive value of 33% and accuracy of 69%.

Our data suggest that the LabUMat+UriSed is a promising screening method for the diagnosis of urinary tract infection and offers the clinician an improved aid in directing the diagnosis of urinary tract infection, especially if we consider clinical data and patients characteristics.

B-21

Are leucocyte count in urine predictive of Urinary Tract Infection?

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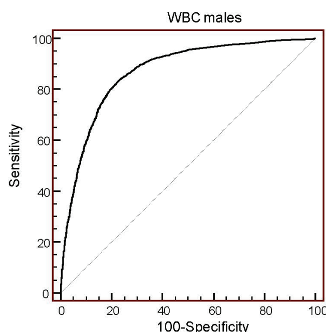
Background: Many clinicians and laboratorians use a diagnostic pathway according to which the presence of leucocytes (WBC) in the urine is confirmed with an urine culture. Different authors reported cut-offs ranging between 20 and 40 WBC/uL. The aim of our study was to assess the correlation between UF-100 and bacteriologic cultures in the diagnosis of UTI .

Methods: We downloaded from the LIS (Noemalife, Bologna, Italy) all the results obtained in the period January-December 2011 in our reference laboratory in samples with the requests of both urinalysis and bacteriologic coltures . The two analyses were carried out respectively with Urysis 2400 (Roche, Milan, Italy) + UF-100 (Sysmex, Dasit, Milan Italy) and HB&L Uroquattro (Alifax, Padova, Italy). We analyzed 10651 samples collected in males an 19833 in females. Sensitivity (SE), Specificity (SP), Positive (LR+) and Negative Likelihood Ratios (LR-), Positive (PPV) and Negative Predictive values (NPV) have been calculated using Medcalc software (Mariekerke, Belgium).

Results: The Area under the ROC curve was 0.78 (all the results), 0.705 (females) and 0.869 (males). SE, SP, LR+, LR-, PPV and NPV in females at the cut-off of 20, 40,60 and 65 WBC were respectively 83.17 (82.1-84.2)- 40.12 (39.3-40.9)-1.39-0.42- 31.4- 87.9; 72.08 (70.8-73.3)- 56.6 (55.8-57.4)-1.66- 0.49- 35.4- 86.0; 64.33 (63.0-84.2)- 65.88 (65.1-66.6)-1.89-0.54- 38.3- 84.9; 62.72 (61.3-64.1)- 67.54 (66.8-68.3)-1.93- 0.55- 38.9- 84.6. SE, SP, LR+, LR-, PPV and NPV in males at the cut-off of 20, 40,60 and 65 WBC were respectively 93.49 (92.5-94.3)- 57.52 (56.4-58.6)-2.2-0.11- 46.1- 95.8; 89.12 (88.0-90.2)- 69.28 (68.2-70.3)-2.90- 0.16- 53.0- 94.3; 85.3 (84.0-86.6)- 74.56 (73.6-75.5)-3.35-0.20- 56.6- 92.9; 84.49 (83.1-85.8)- 75.87 (74.9-76.8)-3.50- 0.20- 57.6- 92.9.

Conclusions: Our study covered a very large population and demonstrated the very low, especially in females, capacity of the commonly used WBC cut-off in predicting of UTI. This data should discourage many laboratorians and clinicians to requests urinalyses.



B-22

Method Comparisons under routine-like conditions, cobas® 8000 modular analyzer series

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Objectives: The analytical performance and functionality of cobas 8000 modular analyzer series was tested intensively under routine-like conditions during five design validation study phases over two years at 14 sites in Europe, US and Australia. Here we report on the outcome for 14 representative assays, comparing the cobas 8000 modular analyzer series results with those generated using the same method on Roche Diagnostics MODULAR *ANALYTICS* and cobas 6000 platforms during routine operation at ten study sites.

Methods: Test results and sampling patterns from the routine laboratory analyzers were electronically captured and the routine samples were then reprocessed on the cobas 8000 platform for up to ~45 methods per experiment. The results for 11 clinical chemistry assays and three immunochemistry assays, each analyzed during routine-like experiments on at least four days at four to nine sites were combined and evaluated using the Bablok/Passing regression procedure.

Results: These real-world comparisons include variables not usually considered during multicenter evaluations. The general chemistry routine (x) results for example are generated on up to 40 individual analytical modules (P, D or c 501) at up to 9 sites using different reagent and calibrator lots on 50 or more days. Similarly, the results on cobas 8000 (y) are generated on multiple modules with two or more different reagent and calibrator lots over about 12 months.

Test, method, Unit	N labs	No. of routine platforms used	n	Range (x) routine	Slope	Intercept	r
Na, ISE mmol/L	9	16: multiple mods	9500	107 - 171	1.01	-2.57	0.895
K, ISE mmol/L	9	16: multiple mods	9661	1.50 - 9.95	0.99	0.05	0.992
CL, ISE mmol/L	8	16: multiple mods	4924	76 - 133	0.95	3.80	0.911
AST pyp, IFCC U/L	5	9: multiple mods	3213	6 - 393	1.04	1.60	0.996
GGT, IFCC U/L	5	10: multiple mods	4369	4 - 1185	1.00	-0.30	0.999
CA ,o-cresol mmol/L	9	16: multiple mods	6395	0.65 - 4.39	1.02	0.00	0.915
Chol , AK mmol/L	9	16: multiple mods	5057	1.09 - 14.9	0.97	0.11	0.994
Crea, Jaffe µmol/L	6	10: multiple mods	8889	18 - 1481	1.01	0.16	0.997
Urea, urease mmol/L	9	16: multiple mods	9487	0.57 - 39.4	0.96	0.02	0.998
GLU, HK mmol/L	9	16: multiple mods	6721	0.39 - 39.4	0.99	-0.03	0.996
CRP, latex mg/L	9	16: multiple mods	5736	0.30 - 349	0.98	-0.03	0.996
TSH, ELECSYS mU/L	4	8: multiple mods	4245	0.01 - 91.7	1.04	-0.01	0.999
fT4, ELECSYS pmol/L	4	8: multiple mods	2331	0.60 - 88.3	0.97	0.17	0.985
PSA, ELECSYS µg/L	4	5: multiple mods	631	0.01 - 96.9	1.00	0.00	0.998

This information is not intended to supersede any assay specific product claims. Routine instrumentation: MODULAR ANALYTICS D, P, E, ISE modules, cobas 6000 c, e modules

Conclusion: The presented findings demonstrate the consistency of results between the new cobas 8000 modular analytics platform and the predecessor platforms from Roche Diagnostics, as well as that between multiple modules integrated within the respective platforms, all operating under routine conditions. COBAS, COBAS C, COBAS E, ELECSYS and MODUALR are trademarks of Roche.

B-23

Validating clinical laboratory tests for use with non-standard body fluids

P. V. Bottini, M. I. Souza, R. C. S. Pozeti, C. R. Garlipp. *University of Campinas, Campinas, SP, Brazil*

Non-standard body fluid (NSBF) is defined as any body fluid other than the body fluid(s) listed by the manufacturer in the product insert as the specimen type(s) suitable for a specific analysis. Examples of a NSBF include pleural and peritoneal fluids. Frequently, laboratory personnel are asked to perform a standard laboratory test for a non-standard body fluid. It's important to remark that most of these tests are measured on routine chemistry or immunoassay analyzers. In this situation these tests may be considered as a laboratory developed test and the method must be validated. For each test or system the laboratory should establish its performance specification, including accuracy, precision, analytical sensitivity and specificity, reportable range of tests results and reference intervals.

In order to fulfill these requirements, we performed a method validation protocol for the most common requested tests in pleural and peritoneal fluids (lactate dehydrogenase, total protein, albumin, glucose and amylase). Validation protocol was based on CLSI documents EP05-A2 and EP15-A2 and included precision, recovery (accuracy and specificity) and linearity (sensitivity and reportable range) studies. For reference range we adopted literature data since NSBF result is usually compared to the same analyte in patient´s serum. CLIA88 and Biological Variation Database (updated in 2012) were adopted as analytical quality requirements. All tests were performed in the Roche Modular Analytics EVO using current reagents and protocols applied for serum analysis (DGKC optimized for lactate dehydrogenase; biuret test for total protein; bromocresol green for albumin; glucose GOD-PAP for glucose and α-amylase IFCC liquid for amylase).

Our results met all the requirements for analytical quality regarding precision (CVs < desirable specifications for imprecision); recovery (80 - 120%) and linearity ($r > 0.99$). For all analytes, sensitivity and reportable range were equal to those observed in serum.

This study showed that with a simple protocol we can validate the use of the most common tests in NSBF and that laboratory personnel can safely use the standard laboratory methods for pleural and peritoneal fluids.

B-24

An Evaluation of Architect c8000 Specific Proteins Assays

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Background: The Abbott Architect c8000 is an automated clinical chemistry analyzer capable of testing specific proteins using immunoturbidimetry. The purpose of this study are two fold i) to compare the analytical performance of specific protein assays on Architect c8000 and compare it with Siemens Vista 1500 and Beckman Immage automated analyzers and ii) to study the impact of testing specific proteins on the turn around times of stats samples due to additional work load.

Methods: Seventeen specific protein assays (IgG, IgA, IgM, alpha-1-antitrypsin, C3, C4, Ceruloplasmin, Haptoglobin, hsCRP, Lp(a), microalbumin, prealbumin, transferring, ASO, beta-2-microglobulin, ApoA, ApoB) were analyzed by Architect c8000 on residual patient serum samples using immunoturbidimetry. Their performance was evaluated according to CLSI guidelines and compared to established methods on Vista 1500 and Beckman Immage. The precision, linearity, detection limit and patient comparison was done by following CLSI guidelines EP5, EP6, EP17 and EP9 respectively. The impact of adding these protein assays on routine and stat turn around times was evaluated by developing discrete event simulation model of Architect c8000 analyzer by using the Medmodel Version 7 from Promodel corporation (Salt Lake City, UT).

Results: Results with highest and lowest values for imprecision and method comparison studies are summarized in the table below. Linearity data showed that all assays were linear across the ranges of their calibration curves. Overall, all of the Architect c8000 assays were in agreement with Vista 1500 and Beckman Immage. No impact on the turn around times for stat samples is noted due to the addition of specific proteins to the chemistry test menu.

Conclusions: Specific proteins assay on Abbott Architect c8000 compared well with the established methods on Siemens Vista 1500 and Beckman Image.

Test		Mean	Imprecision		Method Comparison Architect c8000 Specific Protein Assays					
			Within-Day	Between-Day	Vista 1500			Beckman Immage		
			CV%	CV%	Slope	Intercept	R	Slope	Intercept	R
IgG	L1	896.0	1.0	1.2	1.030	10.46	0.9948	0.994	29.65	0.9719
(mg/dL)	L2	2312.3	4.0	9.6						
hsCRP	L1	0.1	2.0	2.0	0.578	13.15	0.2537	1.114	-0.03	0.9970
(mg/dL)	L2	0.7	0.7	1.2						
Microalbumin	L1	30.0	0.7	1.1	0.164	6.31	0.0385	0.967	0.49	0.9774
(μg/ml)	L2	89.6	1.0	1.2						
ApoA	L1	84.7	0.7	1.0	0.920	6.82	0.9928	0.785	12.60	0.9531
(mg/dL)	L2	268.5	0.7	0.9						
ApoB	L1	40.9	1.5	2.1	0.840	8.44	0.9222	0.631	17.53	0.7034
(mg/dL)	L2	146.8	1.3	3.4						

B-25

Impact of Implementation of Delay Real Time Monitoring in the Hematology Sector of a Private Laboratory in Brazil

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Background: Delay on test results is a major concern in a clinical laboratory. Specifically, some tests which are supposed to be rapidly available to the patient or attending doctor, such as complete blood counts (CBC), demand great effort from laboratory staff to be on time. Furthermore, specific demands tend to create innumerous closing times during the day routine. So, monitoring only "urgent" status on test requests may not be productive. We implemented a software to monitor closing time for each requested complete blood count, in order to reduce delays.

Method: A software was developed by Shift Consultoria de Sistemas (Sao Jose do Rio Preto, Brazil) to monitor delay and near-delay tests. A 42 inches LCD screen was installed in the hematology sector displaying a pie chart where tests within one hour were in yellow status and tests within 30 minutes or delayed were in red. Below the pie graphic a table shows the registry number of each test. We retrospectively reviewed delay statistics for twelve consecutive months, excluding two months of implementation. Analysis was based on percentage of delayed tests.

Results: A total of 621,847 tests, including CBCs and reticulocyte counts were performed during the analysed period, and 3,710 had results after the closing time (0,60%). During the 8 month period before the monitoring started the total number was 504,805 and 2,069 were delayed (0,61%). Two months after the Real Time Monitoring implementation, we had 117,042 tests and 641 were delayed (0,55%), resulting in a 9.91% decrease in relative delays (p=0.023). The mean daily tests, in the second period raised from 2585,8 to 3061,5 (18.40%). There was no change in equipments (four Sysmex® XE2100), sector staff or smear review rules during the two analyzed periods.

Conclusion: Despite a significant raise (18.40%) in total number of exams during the two periods, we still noticed a positive impact after implementing a very simple Delay Real Time Monitoring system, resulting in 9.91% decrease in relative delays in CBCs and reticulocyte counts.

B-26

Automated analysis of blood cells in pleural and peritoneal fluids

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Background: Conventional methods for the cytological analysis of body fluids samples require a manual chamber counting of red and white blood cells and leukocytes differentiation using a cytocentrifuged and stained preparation. Despite being considered the golden standard, it is a time-consuming procedure, which is also subjective and prone to interoperator variability. A promising alternative could be the automated cell analysis of these fluids. However the use of automated analyzers in this situation is not a usual and standardized practice. The aim of this study was to evaluate the performance of the Sysmex XE-5000 hematological analyzer as an alternative to the microscopic analysis of body fluids.

Methods: We studied 150 pleural and peritoneal fluid samples. All samples were sent in an anticoagulant-treated tube and analyzed up to 2 hours after collection. The laboratory routine included manual erythrocytes (RBC) and leukocytes (WBC) total and differential counts (cytocentrifuged air-dried hematological staining of May-Grunwald) and automated total and differential cell counts (Sysmex XE-5000). Paired Student's t-test and simple linear regression (least square method) were used to evaluate the correlation between both methods.

Results: The automated WBC and RBC counts were highly correlated with that of the microscopic reference method ($r \geq 0.95$ in both cases). A good agreement between both methods was also observed for mononuclear cells (r=0.85) and polimorphonuclear cells (r=0.87). Polimorphonuclear cells showed a significant reduction of the percentages obtained by the Sysmex XE-5000 compared to manual method. This reduction is probably due to changes of size and shape of these cells, which are frequently observed in these fluids. Eosinophils are reported separately as a research parameter and demonstrated a good correlation with microscopy (r=0.85). The presence of high fluorescence cells >2.0/100 WBC is visible at the upper border of the scattergram and indicates the presence of macrophages, mesothelial cells or malignant cells.

Conclusions: Automated RBC, WBC and differential leukocytes counts of body fluids show good correlation with the manual method. Considering that body fluids are generally sent for urgent analysis, its laboratory routine requires a skilled personal and microscopic analysis is not available 24h/day in most laboratories, the use of this automated analyzer has the potential of reducing the time to report a preliminary result to the clinician.

B-27

Evaluation of Optical Character Recognition (OCR) technology as a tool to automate the entrance of microscopy data to LIS in the urinalysis department of a large Clinical Analysis Laboratory.

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Introduction: The OCR technology is used after imaging capture and produces conversion of written typefaces into understandable data. This technology has been widely used in the retail and financial sectors as well as government agencies to capture customer or process data and contracts. Its applicability in the manual processes of a clinical laboratory represents an innovation that can bring gains in productivity, quality and work processes. This study aims to show the improvements in process and quality through the implementation of scanning and OCR at urinalysis department.

Methodology: For this evaluation, the process before and after the implementation of this tool was assessed. The urinalysis department performs an average of 130,000 routine & microscopy (R&M) urine / month at the DASA core laboratory in São Paulo, Brazil. In this department, the traditional process has two distinct steps: the first contains automation (physical and chemical analysis) and the second is manual, with all the urinary microscopic being done by technicians. Microscopy represents most of the time of the personal involved, with various steps and activities that rely on manual workforce: microscopic analysis, transcription of the result on worklists, manual insertion of the results into the LIS, and sending the information to the LIS.

This workflow was compared to the new one that consists of scanning the manual filled work lists, to apply the OCR tool that converts the microscopy manual written raw data into electronic files and to send this data to the Laboratory Information System.

Results: The implementation of software for capturing and processing images using OCR technology along with the standardization of worklists using default multiple-choice results has allowed the scanning and conversion of maps into electronic files that could be sent to the LIS according to a predetermined communication protocol.

Based on data extracted from the LIS, the introduction of this technology to the process provided significant improvement, such as:

a) Reduction from 3 to one in the number of FTEs in the process, even with an increase of more than 30% in the daily routine;

b) Reduction from 8 hours to 5 hours of the average turnaround time of the urinalysis routine without increasing the number of employees dedicated to the activity;

c) Anticipation of the end of the routine work in two hours;

d) Elimination of the typing errors in the test results.

Conclusion: In addition to the improvement of productivity coming from the process, other gains that can be attributed to this innovation were: decrease of labor overtime, improvement in the risk of Repetitive Strain Injury (RSI), elimination of the typing results bottleneck and release of the typing employees for the execution of other tasks in the workplace. One can also highlight the fact that this experience enabled the laboratory to expand this model for other existing manual processes that are either multiple-choice or tests with narrative results, since the tool can identify also narrative texts in the work lists.

B-28

Integration of Microbiology Laboratory and Hospital Computerized Systems for Nosocomial Infection Control

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Background: Nosocomial infections are responsible for high patient morbidity and mortality, with increase of hospitalization costs, particularly for that caused by antimicrobial multi-resistant microorganisms. Effective infection control measures include early identification of colonized or infected patients with these microorganisms allowing the implementation of precautions and isolation according to defined guidelines. The microbiology laboratory has an important role in the nosocomial infection control, however, usually there is a delay in the time the results are available by the laboratory and the access to this information by the health professionals at the hospital point of care.

Methods: All culture results from different sites of collections from patients admitted to a 280 beds private general hospital at the city of São Paulo, Brazil, are integrated in a computer network including the laboratory K2 system, the MV ® Hospital system and the epidemiological surveillance and infection control software HEPIC ® . The guideline of the nosocomial infection control service of the Hospital defines the main multi-resistant microorganisms important for precautions and isolation of patients as follow: *Acinetobacter baumannii* resistant to carbapenems (MRAb), *Pseudomonas aeruginosa* resistant to carbapenems (MRPa), *Klebsiella pneumonia* resistant to carbapenems (KPC), *Enterococci* resistant to vancomycin (VRE). The result of any of these microorganism identified at the laboratory is sent through an alert by e-mail direct from the HEPIC ® system to the healthy assistance team at the wards and the infection control team.

Results: From August 2011 to January 2012, 553 alerts were provided to the respective wards and the infection control team. 240 for MRAb , 183 for MRPa, 72 for KPC, 41 for VRE, and 24 for other microorganisms selected by the microbiology laboratory. These alerts allowed the health assistance team to implement the recommended infection control measures including precautions and isolations immediately after the microbiology result was available at real time on line.

Conclusions: The alert of antimicrobial multi-resistant microorganisms by a computerized network integrating the laboratory, the electronic chart system of the hospital and the nosocomial infection control system warrant an early implementation of necessary infection control measures.

B-29

Use of Networked Westgard Advisor Quality Control Software to Reduce False QC Rejections in a Regional Laboratory System.

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Enterprise quality control (QC) data in the Geisinger Medical Laboratories (GML) is generated from 1) eight rapid response labs using Roche mid sized chemistry instruments 2) two hospital laboratories using four Roche c501 instruments and 3) a core laboratory using five “P” Roche modular instruments. All instruments utilize the same lot number of BioRad unassayed serum QC material. All instruments are interfaced via Data Innovations (DI) v 8.10 “middleware” to a single SunQuest laboratory information system (LIS) on a wide area network (WAN). DI “middleware” also integrates BioRad Unity Realtime (URT) software containing the Westgard Advisor sigma statistic module.

QC data from all locations are captured via DI on the URT server and sigma statistics are calculated for all chemistry tests using CLIA total allowable error. A sigma dashboard is plotted monthly and those tests that consistently produce sigmas above 4 are selected for use with less stringent 1-3s Westgard rules in daily QC. The number of monthly QC rejections for selected tests dropped significantly at all three sizes of labs as shown in the following table (baseline month, March 2011 versus most recent month, January 2012):

Test	fail	total	%	fail (3s)	total	%
ALKP	108	2372	4.55%	2	2599	0.08%
K	47	4589	1.02%	17	3058	0.56%
AMY	31	1107	2.80%	11	1613	0.68%
HDL	25	1358	1.84%	10	1211	0.83%
LDL	36	482	7.47%	4	543	0.74%
CK	80	1332	6.01%	38	1741	2.18%
LIPA	17	950	1.79%	6	1341	0.45%
AST	73	2588	2.82%	17	2821	0.60%
TBILI	36	1468	2.45%	2	1399	0.14%
LD	2	409	0.49%	2	714	0.28%
TRIG	120	1411	8.50%	3	1202	0.25%
URIC	2	409	0.49%	0	654	0.00%
ALB	1	519	0.19%	0	500	0.00%
GLU	70	706	9.92%	5	694	0.72%
TOTAL	455	16665	2.73%	105	14927	0.70%
			Reduction	76.92%		

While the reduction of “false” QC rejections with associated QC repeats and troubleshooting averaged 75% for 5 months, peer comparison data from URT did not show trends of increased SDIs or CVRs. Hence it is concluded that identifying tests performing well within total allowable error by Westgard sigma statistics and increasing ranges of acceptability for daily QC from 1-2s to 1-3s will reduce QC-related workload by approximately 75% without degradation of peer compared quality. Decreasing “false” QC rejections also decreases disruption of autoverification and creates a more “lean” automated work flow.

B-30

A Novel Bioinformatic Delta Check Approach to Identify Specimen Inaccuracies

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Introduction: Inaccuracies in specimens of patients may lead to misdiagnosis and inappropriate therapy. The primary purpose of a delta check is to detect misidentified specimens. The traditional delta check procedure compares the change in concentration of an analyte with a delta check limit (DCL) for that analyte. A change greater than the DCL sets a delta check flag for that analyte. This procedure is typically applied to several analytes on a panel. One or more flagged analytes on the panel suggests the possibility that one of the two specimens was misidentified. This procedure is called Univariate (Multianalyte) Delta Check (UDC) and generates many FP flags. Any improvement would be desirable. Here, we hypothesized that a Multivariate Delta Check (MDC) procedure would produce fewer false positive flags than the traditional method.

Methods: A panel of results on a sample can be graphed as a point in space with one axis for each analyte. A subsequent panel of results can be graphed as a second

point in space. If 8 analytes are included, there would be 8 axes in 8-space indicated as MDC8; with 5 analytes MDC5 , respectively. The Multivariate Delta (MD), the distance between these two points, is given by:

$$MD = SQRT(((analyte1_2\text{-}analyte1_1)^2/(SD\ RR_1)) + ((analyte2_2\text{-}analyte2_1)^2/(SD\ RR_2)))$$

The subscript 1 and 2 refer to the results on the first and second sample and the divisor refers to the standard deviation of the reference range respectively.

We calculated the MD for 1035 pairs of patient results from our laboratory on a Basic Metabolic Panel (BMP; Na^+, K^+, Cl^-, CO_2, glucose, BUN, creatinine, Ca^{2+}) and for 1034 pairs of intentionally misidentified BMP results. Afterwards, we calculated the sensitivity and specificity at various MD values and constructed Receiver-Operating Characteristic (ROC) curves. The UDC5 is currently used by our hospital laboratory and includes the following 5 analytes (DCL): BUN (10 mg/dL); Ca^{2+} (1.5 mg/dL); Creatinine (1.5 mg/dL); K^+ (1.2 mmol/L); and Na^+ (8 mmol/L). Here, we compare the sensitivity of the MDC8, MDC7 (minus glucose) and MDC5 (same analytes as current UDC5) at the specificity of the UDC5 (0.862).

Results: The sensitivity and specificity of UDC5 is 0.573 and 0.862, respectively. Areas under the ROC curve for MDC8, MDC7 and MDC5 are 0.823, 0.855, and 0.843, respectively. The sensitivities of UDC5, MDC8, MDC7 and MDC5 at a specificity of 0.862 are: 0.573, 0.545, 0.671, and 0.656, respectively. This novel method can be extended to an unlimited number of analytes. Thus, at the specificity of UDC5, the sensitivity of MDC8 was not as high, but the sensitivity of MDC 7 and MDC5 were higher.

Conclusions: The MDC7 (BMP without glucose) procedure had the highest area under the ROC curve and higher sensitivity at the specificity of the current UDC5 procedure. The MDC method is more efficient than the UDC method used in our laboratory, which results in more true positive flags and fewer false positive flags.

B-31

Evaluation of the DC/RC Flex 900 Automated Decapping and Recapping System

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Background: ARUP's Automated Endocrinology department has a monthly test volume per full time employee (FTE) that is high enough for repetitive motion injuries and blood borne pathogen (BBP) exposure to be a major concern with regards to manual decapping and capping of specimens. Automation can save FTE costs and allow for better allocation of technicians, but savings must be weighed against machine cost, maintenance and depreciation. Several months of planning and research confirmed the need for automation in the laboratory. The DC/RC 900 FLEX instrument (Sarstedt, Inc.) was selected based on its flexibility to accommodate different analyzer racks and tube types, small footprint, and low maintenance costs. Directly attributable cost, savings, and efficiency measures from the 12-months after installation were recorded, analyzed, and compared with the same measures during the 12-months prior to installation.

Methods: In the Capping and Decapping Process Time Study technologists were randomly selected; steps were timed using a stopwatch rounding to the nearest half second. Using the average time from the study and ARUP average wage and reagent costs, a cost/run was determined. Total billed units for each month were used for the 12 months prior and the 12 months after installation of the Flex; the month of installation was not used in the study. Test numbers per year are based a trailing 12 month average. The FTE-hours for each month was calculated by dividing the total number of tubes processed per month by the hourly processing rate determined in the timing study.

Results: During the 24 month study period there was a significant increase in the number of samples received. Without the DC/RC 900 FLEX the FTE-hours required for manual sample processing would have risen from 293/month to 692/month. Instead FTE-hours dropped to 280/month during the same period despite the extraordinary increase. This equates to a cost savings of 2.3 FTEs per year. Additional analysis reveals a 46% reduction in total sample handling costs for the department equaling a \$0.06 savings in processing costs per sample. The high return on investment (ROI) of automating the pre- and post-analytical process with the FLEX means that the total purchase price and operating costs will be recouped in less than 26 months of operation.

Conclusions: Since installation of the DC/RC 900 Flex, the number of tubes processed manually by technicians was reduced by 90%. Without automation at least 2.3 FTEs would have been either newly hired or pulled from other projects in order meet this demand. Instead, with the FLEX there was a net-gain in FTE-hours available to the department. Total savings per run as well as total FTE savings per year was immediately greater than the overall costs of purchase and operation on an annualized basis. The investment in the FLEX automation from Sarstedt showed a highly favorable ROI and internal rate of return. The remarkable reduction in manual sample processing creates a dramatically safer working environment for the technicians in terms of repetitive stress injuries and BBP exposure.

B-32

Text Mining the Laboratory Information System to Automate Monitoring of Result Review Flags for Quality Improvement

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Background: To prevent reporting erroneous results, laboratories frequently use result flags. Result flags, such as delta checks, are used to identify mislabelled specimens, analytical error, or IV fluid-contamination. When accurate results are inappropriately flagged, staff may waste time, money, and effort searching for errors where none exist. At their worst, uneccessary flags cause sample redraws and delay in reporting critical results, risking patient harm. Result review flags are frequently the target of improvement projects. The objective of this study was to develop an automated system to assess the effect of changes to results review flags.

Methods: Laboratory Information System (LIS) data, staff surveys, and existing literature were used to refine delta checks (changes between serial results) and 'R-codes' (flags for results outside the analytical measuring range). Delta checks and 'R-codes' were changed for 50 chemistry analytes (32 delta checks and 39 R-codes) for the main chemistry analyzer (Vista 1500, Siemens). To determine the impact of the changes, Laboratory Information System (LIS) data was analyzed with an automated script written using open-source software ('R', Python, and MySQL). To develop the script, one month of LIS data was used for training. Comment fields were analyzed using combinations of logic, regular expressions, and keyword searches (collectively referred to as text mining) to quantitate and categorize result review flags into four groups: 1). Appropriate Error Detection (mislabelled, analytical error, or contamination), 2). Potential Risk (re-draw from patient where results did not change), 3). Waste (unecessary review of results, repeat of testing, or pages/phone calls to care-providers), and 4). Noise (no documentation of effort or clinical impact). For the review flag impact analysis, LIS data was pulled for two 30 day periods before and after changing the result review flags. The accuracy of the text mining script was verified manually by reviewing LIS data and patient charts.

Results: After revision of result flags, the total number of flags decreased by 57.1% from 22,513 (3.6% of total) to 12,863 (1.9% of total); R-codes decreased from 17,617 to 10,574, and delta checks decreased from 4,896 to 2,289. Before the improvement, there were 21,660 flags classified as 'Noise', 516 flags classified as 'Waste', 9 classified as 'Potential Risk', and 10 errors identified. After the improvement, there were 12,470 'Noise' flags, 239 'Waste' flags, 4 'Potential Risk' flags, and 3 errors identified. Text mining data showed excellent agreement (>99%) with manual review.

Conclusions: The impact of changes to result review flags is ammenable to automation using open source software. Text mining can replace laborious manual review of LIS data allowing for empirical determination of result flags. The ability to rapidly assess the impact of result review flags facilitates quality improvement projects and quality monitoring to help balance erroneous results against wasted effort.

B-34

Implementation and validation of a commercially available moving averages program in the clinical chemistrty laboratory

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Background: Traditional quality control (QC) procedures only provide snapshots of assay performance at fixed points in time. With this strategy detection of a shift in analytical performance may go undetected for several hours potentially affecting several patient samples. A supplemental QC strategy that has been suggested is the continuous monitoring of assay performance through the use of a rolling mean of patient values. This concept of the Average of Normals (AON) or Moving Averages (MA) is not new to the clinical laboratory having first been proposed by Hoffman and Waid in 1965 (Am J Clin Pathol 1965;43:134-41) . One of the likely reasons why the adoption of AON/MA procedures has been slow has been the lack of commercially available software programs that allow the laboratorian to easily implement this powerful quality assurance (QA) tool. In an effort to enhance the QA program in our clinical chemistry laboratory at Dartmouth-Hitchcock Medical Center we have established a MA program from Data Innovations (Burlington, VT) our middleware vendor. The objective of this study was to validate and optimize our user defined MA protocols and the error detection capabilities of the MA software program.

Methods: In order to validate and optimize our user defined MA protocols for total calcium, albumin and total protein we induced an artificial error state by incrementally adding positive or negative bias in a database of patient results for total calcium, albumin and serum total protein values. The datasets with or without the artificially induced error was then imported into the MA software program and monitored for the number of samples affected until detection of the error state.

Results: Initial validation of the software package made it apparent that two separate populations exist for total serum/plasma calcium concentrations in our ambulatory outpatients and inpatients. For this reason inpatient and out-patient patient calcium values are monitored separately. For the outpatient calcium protocol, the addition of a positive or negative error equal to 1.0 mg/dL produced an error detection event within 11 and 15 patient samples, respectively. For the inpatient calcium protocol the induced positive error of 1.0 mg/dL was not detected in this limited data set but an error of 1.5 mg/dl was detected within 25 patient samples. Conversely, an induced negative error of 1.0 mg/dL was detected within 22 samples. For albumin an induced positive error of 0.8 g/dL was detected within 49 samples while a negative error of 0.8 g/dL was detected within 25 samples. For total protein an induced positive error of 0.8 g/dL was detected within 22 samples and a negative error of 1.2 g/dL was detected within 12 samples.

Conclusions: Our initial validation of the MA protocols demonstrates that, as designed, both artificially simulated positive and negative shifts are rapidly detected. However, this validation only represents a snapshot of our protocols and further studies are being currently underway to further assess the error detection capability of these and other MA protocols.

B-35

Hepatitis Testing Improvements Following Switch from Stand-Alone Analyzer to Fully-Automated Chemistry Track System

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Background: Traditionally, hepatitis (Hep) testing is performed in the special chemistry laboratory. However, as more chemistry automation systems offer hepatitis testing, an opportunity exists to consolidate platforms and transfer work to the core laboratory. After implementation of our new chemistry/immunoassay system, we made the decision to move hepatitis testing from manual processing and batch testing in special chemistry to full automation in the core laboratory. This project entails the testing workflow changes and staff training used to make this switch in testing platform. In addition, successful outcomes in turnaround time (TAT) and process steps are described.

Methods: Hepatitis testing (Hep A antibody, Hep B Core antibody, Hep A IgM, HCV antibody, Hep B Surface antigen, and Hep B Surface antibody) was moved from a stand-alone analyzer (Siemens Centaur® XP) to an automated chemistry system (Ortho Clinical Diagnostics enGen ™ and Vitros® 5600). Workflow was analyzed using Lean Six Sigma tools. TAT was captured from the laboratory information system for a four month period of using the stand-alone analyzer versus a four month period for the automation system.

Results: Implementation on the chemistry automation system resulted in several improvements. Process steps were reduced by 57% (14 batch vs. 6 automation). Platform consolidation freed up floor space and reduced technical staff walking distance by 81% (365 inches batch vs. 66 inches automation). The average TAT for all six hepatitis tests from all ordering locations, to include specimens received from remote laboratories, was reduced by 75% (41.66 hours batch vs. 10.21 hours automation). Further stratification of the data denoted site-specific TAT improvements for several hepatitis tests. For example, Hep B Surface antigen testing TAT was reduced by 96% for the emergency department (2,710 min batch vs. 97 min automation).

Conclusions: The switch from hepatitis batch testing in special chemistry to automation testing in our core laboratory resulted in successful outcomes. We were able to decrease the potential for error by eliminating unnecessary process steps. We reduced the physical strain on our technical staff by greatly cutting their walking distance. In addition, we significantly improved our TAT from days to hours.

B-36

Performance Evaluation of the Roche c8000 On-board Quality Control Function

D. A. Dalenberg, D. R. Block, N. A. Baumann. *Mayo Clinic and Foundation, Rochester, MN*

Background: The Roche c8000 modular analyzer series (Roche Diagnostics, Indianapolis, IN) offers an environmentally controlled compartment that accommodates 5 quality control (QC) racks per module. Roche auto QC software allows the user to define assay-specific time intervals for QC and maximum stability time limits. The auto QC function has the potential to improve efficiency within the laboratory by automating the loading and running of QC material and reducing dead volume waste. Objectives: Validate use of the on-board auto QC function for chemistry assays on the Roche c8000 over an 8 hour time period.

Methods: On-board QC stability was evaluated for control materials in Roche false bottom aliquot tubes stored on-board compared to freshly aliquoted control material in Roche Hitachi QC cups. Both the on-board and freshly aliquoted (manual) QC material was analyzed in singlicate on each pipetting unit once per hour for eight hours over 5 days. On-board QC stability was evaluated using 2 or 3 QC levels for betahydroxybutrate (Stanbio beta-hydroxybutrate controls); fructosamine (Biorad Lypocheck Immunoassay Plus controls); c-reactive protein (Biorad Liquicheck Elevated CRP controls); albumin, alkaline phosphatase, alanine aminotransferase, amylase, aspartate aminotransferase, blood urea nitrogen, calcium, creatine kinase, cholesterol, chloride, bicarbonate, creatinine, direct bilirubin, gamma-glutamyltransferase, glucose, high density lipoprotein, iron, potassium, lactate dehydrogenase, lipase, magnesium, sodium, phosphorus, total bilirubin, total protein, triglycerides, and uric acid (Biorad Liquichek Unassayed Chemistry controls). The mean, standard deviation (SD), and %CV, for both the on-board and manual QC were calculated. Initially, two-tailed paired t-test and F-test were used to determine whether the means and standard deviations, respectively, were statistically different between the two methods. If statistically significant differences were found, the means and standard deviations (SD) were reviewed. The average percent difference between the on-board QC and the manual QC at eight hours was calculated. All Levy-Jennings charts were visually inspected for shifts and trends.

Results: The QC mean was determined to be significantly different between auto and manual QC for 18 of 30 analytes, however in all cases the QC mean concentration differences were <3%. Statistically significant differences in SDs between auto and manual QC were found in 8 of 30 assays. In all cases, with the exception of magnesium on level 1 and bicarbonate on level 1 and 2, the SD of the mean was smaller using auto compared to manual QC. At the 8 hour time point, auto QC measurements were within 3% of the manual QC values for all analytes except bicarbonate. For bicarbonate, auto QC at 8 hours was 6-7% lower than manual QC measurement. All assays demonstrated control results within ±2SD of the mean with no observed shifts, and only bicarbonate demonstrated an observed downward trend.

Conclusions: Except bicarbonate, the 8 hour QC material stability for chemistry analytes analyzed on the Roche c8000 using the auto QC function is acceptable and comparable to freshly aliquoted, manually loaded QC. Use of the auto QC software and on-board QC material storage may improve efficiency by automating the process of loading and running QC in high-volume laboratories.

B-37

Control of sample carryover for automated whole blood samples for HbA1c

G. A. Blackwell. *Abbott Laboratories, Irving, TX*

Background: Glycemic control marker Hb A_{1c} has proven to be useful in monitoring a patient's historic overall blood glucose levels and is now used for diagnosis of Diabetes mellitus. Glucose is attached to the Hb molecule within the Red Blood Cell (RBC), a whole blood (WB) sample type is required for Hb A_{1c} analysis. In order to process WB samples, the ARCHITECT *c*8000 & *c*4000 clinical chemistry analyzers will be provided in the future with an automated RBC lysis procedure onboard to avoid inconvenient off-line pretreatment of samples. The sample probe descends to a depth of 70% from the top of the sample, which ensures adequate aspiration of RBCs even if sedimentation has occurred. The increased dive depth and added exposure of the exterior sample probe to red blood cells, requires that the sample probe be washed externally over the entire exposed region to control carryover (c/o) within specification.

Methods: In an automated chemistry analysis platform, the introduction of whole blood samples presents a challenge. The combination of increased surface area

exposure coupled with the heightened concentration of proteins and cellular components inherent with WB potentially presents c/o risk to established serum-based assays. Therefore analyzer probe design and cleaning procedures must be modified in order to process these whole blood samples, The ARCHITECT *c*8000 and *c*4000 clinical chemistry analyzers will incorporate an enhanced sample probe design and associated wash cup to accommodate this RBC exposure. This new sample probe design extends the exterior polished portion of the probe and creates a special wash well within the current wash cup envelope to allow deep probe sampling and washing. To ensure analytical integrity, this new design combination must demonstrate comparable or improved carryover control for the existing sample types and analytes, as well as for WB samples associated with HbA1c. In order to verify this performance, the new design was exposed to serum and WB concentrations containing HBsAg (i.e. “hot” samples) exceeding $1x10^6$ IU/mL concentrations to demonstrate c/o control of 0.1 ppm from sample to sample. These trace levels of carryover are undetectable by the Hb A_{1c} assay, but the specification allows the WB assay to be run on integrated IA-CC Architect systems without concern of sampling sequence (i.e. either IA or CC samples may run first).

Results: Tests of the new probe and wash cup design demonstrated c/o control well below the 0.1 ppm spec for existing serum-analyte sampling operations. In a 12-replicate/sample challenge mode, three instruments and 66 samples each (serum & WB) produced an average c/o of 0.043 ppm for serum and 0.012 ppm for WB samples. For a worst-case single contaminating sample volume of 35 uL, the c/o averages were 0.015 and 0.016 ppm for serum and WB respectively on a total of 75 samples across three instruments.

Conclusions: Feasibility and verification results demonstrate comparable or improved c/o control utilizing the modified HbA1c hardware, with no impact to current sample carryover performance.

Tuesday PM, July 17, 2012

Poster Session: 2:00 PM - 4:30 PM

Endocrinology/Hormones

B-39

The Correlation Between Sex Hormone Binding Globulin And Type 2 Diabetes in Men of African Ancestry

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Background: Epidemiological studies have shown that circulating levels of sex hormone binding globulin (SHBG) have a role in glucose homeostasis in men and women, and could predict the development of type 2 diabetes. I am not aware of any evaluation of circulating SHBG in subjects of African ancestry.

AIM: To investigate the correlation between circulating sex hormone binding globulin(SHBG) and insulin resistance(IR) in type 2 diabetic men of African ancestry.

Methods: Forty eight male subjects with type 2 diabetes were recruited in this cross sectional study by convenient sampling method. Height and circumferences around the waist (iliac crest level) and hip (widest region at the trochanter level) were measured to the nearest 0.5cm, the subject standing. Waist hip ratio (WHR) was calculated from this measurement. Weight (in kilogram) was measured from which body mass index (BMI) was calculated. Fasting plasma concentration of glucose was measured by the glucose oxidase method. Enzyme linked immunoabsorbent assay (Elisa) was used to measure insulin and SHBG. HOMA-2 Calculator software (www.dtu.ox.ac.uk) was used to calculate insulin sensitivity, B-cell function, and insulin resistance

Results: The mean SHBG concentration was 58.2mmol/L. A significant inverse association between insulin resistance and SHBG was observed ($r = -0.353$, $p < 0.015$); positive association between age and SHBG ($r = 0.356$, $p < 0.014$). IR did not correlate with BMI/WHR ($r = -0.043$, $p < 0.772$, $r = -0.068$, $p < 0.646$). SHBG did not correlate with BMI/WHR ($r = -0.189$, $p < 0.203$; $r = -0.126$, $p < 0.397$).

Conclusions: There is an inverse correlation between SHBG and Type 2 diabetes. Further studies to delineate the underlying mechanism on how SHBG contribute to the risk of type 2 DM is necessary as they may provide novel targets for diabetes prevention and management.

Descriptive Characteristics of Subjects	
	Mean(SD)
Age(n =48)	61.8(10.9)
BMI(n =48)	25.9(3.8
WHR(n=48)	1.0(0.1)
FPG(mg/dl) (n =48)	150.8(68.1)
FI(uIU/ml) (n = 48)	13.6(6.5)
SHBG(nmol/L) (n =47)	58.2(41.3
%B (n =48)	80.0(65.5)
%S (n =48)	63.7(35.8)
IR (n =48)	2.0(1.2)

FPG= fasting plasma glucose, FI=fasting Insulin, BMI=body mass index,WHR = waist hip ratio, SHBG= circulating sex hormone binding globulin level,%S = insulin sensitivity,IR = insulin resistance,%B = pancreatic beta cell funtion

B-40

The Reference value of Thyroid Stimulating Hormone (TSH) determined by fluoroimmunoassay in paraguayan newborns

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Background: The term "reference values" is widely accepted and applied internationally and can be expressed as the establishment and use of data relevant to the interpretation of medical observations. Thyroid stimulating hormone (TSH) plays a role in early detection of one of the few preventable causes of mental retardation, congenital hypothyroidism. The reference values used in the program for prevention of cystic fibrosis and mental retardation (PPFQRM) were those recommended by the commercial test used (<9 Normal, 9-18 borderline, >18 iU/mL hypothyroidism) using filter paper with blood samples from newborns at 2-6 days of life.

Methods: We performed a cross-sectional, observational study with 20,168 dried blood samples from fullterm newborns at 1-7 days of life to determine the reference value for TSH in the NB population of Paraguay using the measures of central tendency and dispersion: mean, median,percentiles, and frequency distribution in order to describe the variable under study.

Results: A TSH of between 0.01--1.0 µU/ml was found in 22% of samples, 1.1_2 µU/ml in 25% of samples, from 2.1--3.0 µU/ml in 19%, between 3.1_4 µU/ml in 12%, from 4.1_5 µU/ml in 8%, and more than 5 µU/ml in 14%. TSH values found were: mean: 2.74, median: 2.22, mode: 0.01, and standard deviation was 2.14 µU/ml. For the 75th percentile: 3.26 µU/ml; 95th: 6.68 µU/ml; and 99th: 9 µU/ml.

Conclusions: Based on these observations a cutoff point for TSH was confirmed equivalent to 10 µU/ml.

B-41

Vitamin D levels and insulin resistance in male type 2 diabetics in south-west Nigeria

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Background: Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. The International Diabetic Federation estimates that Nigeria has a national diabetes prevalence of 3.9%.The objective of this study was to compare 25-OH Vitamin D, fasting C-peptide and insulin resistance levels in respondents with diabetes mellitus and healthy controls.

Methods: This is a comparative cross-sectional study that looked at vitamin D and C-peptide levels of male subjects with type 2 diabetics and healthy controls. 80 proven type 2 diabetes male patients attending University College Hospital, Ibadan, were included in the study. 49 apparently healthy men residing in Ibadan and its environment served as controls. Respondents with plasma creatinine levels greater than 1.5mg/dl were excluded from the study. Only respondents with fasting plasma glucose 60-99mg/dl were included in the control group. The serum C-peptide assay was done using enzyme linked immunosorbent assay (ELISA) . Serum 25-OH vitamin D level was determined using high performance liquid chromatography (HPLC). Plasma glucose was assayed using Randox Glucose (GLUC-PAP) kit.

Results: This study has shown a significantly lower mean 25-OH vitamin D levels of 36.55ng/ml in the diabetic group compared 42.96ng/ml in the control group (p= 0.001). Vitamin D status is categorized into 3 groups namely: Vitamin D deficient (< 20ng/ml), vitamin D insufficient(20 - 29.99ng/ml), vitamin D sufficient (greater than 30ng/ml. All four individuals with vitamin D deficiency were diabetic. There is a significant correlation between 25-OH vitamin D and BMI in the control group (p= 0.032). In the diabetic group, 73.8% had sufficient vitamin D, 21.2% had insufficient vitamin D, 5% had vitamin D deficiency. 90% of the control group had sufficient vitamin D levels while only 10% had insufficient vitamin D levels

Conclusions:. There is a high degree of positive association between vitamin D deficiency/insufficiency and diabetes mellitus.

Comparison of measure variables between the diabetic and control groups			
VARIABLE	DIABETICSN = 80MEAN (SD)	CONTROLSn = 49MEAN (SD)	P-VALUE
AGE (years)	61.66 (10.4)	54.50 (11.1)	<0.001*
C PEPTIDE (ng/ml)	3.56 (1.69)	3.26 (2.07)	0.376
VITAMIN D (ng/ml)	36.55 (11.3)	42.96 (10.1)	0.001*
BODY MASS INDEX (BMI)	24.59 (5.5)	24.88 (3.5)	0.740
HEIGHT (m)	1.70 (0.07)	1.71 (0.06)	0.437
WEIGHT (kg)	73.35 (13.1)	72.92 (13.2)	0.856
HOMA2 %INSULIN SECRETION	42.423 (28.4)	66.35 (51.2)	<0.001*
FASTING PLASMA GLUCOSE (mg/dl)	152.10 (69.7)	90.14 (8.9)	<0.001*
HOMA2IR	3.09 (1.4)	2.40 (1.5)	0.014*
HOMA2 %BETA CELL FUNCTION	111.4 (97.6)	167.5 (73.4)	0.004*

B-44

Could Gender Dimorphism Explain The Controversial Association Between Retinol-Binding Protein-4 (RBP4) And Cardiometabolic Risk Factors In Obese Subjects?

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Background: Retinol-binding protein 4 (RBP4) RBP4 is an adipokine discovered as a factor that affects insulin sensitivity in mice but data from clinical studies in different populations are conflicting and controversial. As recent reports suggest that adipose tissue RBP4-mRNA expression is regulated by leptin and gender specific, the aim of this study was to evaluate gender specific differences in the associations of RBP4.

Methods: Fasting RBP4, leptin, insulin, glucose, full lipid profile, ALT, LH, FSH, estradiol (E2), testosterone, SHBG, and Free androgen index (FAI) were determined in 191 (82 males 109 females) apparently healthy first degree relatives of Type 2 diabetic patients with mean (SD) age of 28 (8) years. Insulin resistance was assessed with the homeostasis model (HOMA-IR). Subjects were classified by the IDF criteria for the Metabolic syndrome (MetS). Linear and binary regression analyses were used to determine the associations of RBP4 with other variables, insulin resistance (IR) and MetS.

Results: Mean RBP4 (26 versus 23 µg/ml) was significantly higher in males but leptin (43 versus 19 ng/ml) was significantly higher in females despite similar anthropometric indices. Mean RBP4 was significantly higher in females with IR (23.4 versus 20.1 µg/ml) but not in males (26.1 versus 25.5 µg/ml). RBP4 was not significantly correlated with anthropometric indices and leptin and showed gender dimorphisms in correlations (r) with other variables - in males significant correlations with only FAI (r=0.7), Triglycerides (Tg) (r = 0.4), creatinine (r = 0.4) and ALT (r = 0.2); in females, significant correlations with only age (0.2), E2 (r=0.3) and Tg (0.3). RBP4 showed stepwise increase with increasing number of MetS criteria and was mildly but significantly associated with MetS in males (Odds Ratio (OR) = 1.091; 95% CI = 1.008 - 1.181) and females (OR = 1.037; 95% CI = 1.001 - 1.197). RBP4 was significantly associated with IR in females (OR = 1.17; 95% CI = 1.07 - 1.23) but not in males (OR = 0.98; 95% CI = 0.90 - 1.07).

Conclusions: We conclude that leptin and anthropometric indices are not related to circulating RBP4 which shows gender dimorphisms in its associations with obesity related cardiometabolic factors. These may, in part, account for the controversies on the potential roles of RBP4 in the development of insulin resistance in obese humans.

B-46

QuantoseTM: A Pre-Diabetes Diagnostic That Correlates with Multiple Risk Factors for Metabolic and Cardiovascular Diseases

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Background: Insulin resistance (IR) is a recognized risk factor for type 2 diabetes (T2D) and cardiovascular disease (CVD) and there remains an unmet medical need for a practical measurement of insulin sensitivity. Circulating blood levels of the metabolites α-hydroxybutyrate (AHB), linoleoylglycerophosphocholine (L-GPC), and oleate were recently shown to correlate with insulin resistance in a nondiabetic, healthy population as measured by the hyperinsulinemic euglycemic clamp, the gold standard for measuring insulin sensitivity. The fasted plasma levels of these three molecules along with BMI form the basis of Quantose™, a novel algorithmic diagnostic test for identifying high-risk, insulin resistant patients and predicting their risk of progression to T2D. The Quantose test was found to accurately identify insulin resistance and predict incident T2D over 5 years in large population based studies.

Method: The potential broad utility of Quantose as a marker of risk was evaluated in a US based random population (n=69) spanning normal HbA1C values (20) to pre-diabetes (22) and T2D (27). Of clinical interest, Quantose identified one-third of patients within the *normal* range of HbA1C as insulin resistant and, presumably, at higher risk for developing T2D. Regression analyses were used to correlate Quantose insulin resistance scores with a number of analytes and biomarkers of metabolic and cardiovascular risk measured in fasted plasma samples (all p-values <0.05).

Results: Quantose scores correlated with insulin (r=-0.42), HbA1C (r=-0.35), free fatty acids (r=-0.42), and lipoprotein particle size, including VLDL (r=-0.49), LDL (r=0.33), and HDL (r=0.40). Intriguingly, Quantose scores also correlated with vitamin D (r=0.35), the CV risk index total cholesterol/HDL (r=-0.37), and the inflammation markers hsCRP (r=-0.31) and MPO (r=-0.49). Quantose scores decreased (worsened) in individuals as they met a composite of risk factors such as the criteria for the Metabolic Syndrome (e.g., triglycerides ≥150 mg/dl, glucose ≥110 mg/dl, etc) further establishing Quantose as a valid risk index. Additionally, each of the three Quantose analytes (AHB, L-GPC, Oleate) correlated with markers linked to different mechanisms leading to CVD. While AHB was associated with traditional markers of glycemic dysregulation such as glucose (r=0.50) and HbA1C (r=0.48), L-GPC correlated more prominently with hsCRP (r=-0.21), a marker of inflammation and MPO (r=-0.27), a marker of atherosclerosis. Furthermore, free oleic acid correlated highly with markers of dyslipidemia such the IR proxy, triglycerides/HDL ratio (r=-0.84), the ApoB/ApoA1 ratio and small LDL particle number (r=0.52 and 0.44, respectively).

Conclusions: Quantose, a measure of insulin resistance, may have clinical utility in further identifying patients at risk of CVD in addition to traditional risk factors due to its broad association with multiple markers of mechanisms leading to cardiovascular diseases.

B-47

Incidence Of Hyperglycemia In The Nigerian Elderly

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Background: Hyperglycaemia has been recognized as one of the important diseases of the elderly and demonstrated to be increasingly common with old age. The issues surrounding treatment decisions for older people are complex and involve many more factors than age alone. Diagnosis of hyperglycemia early enough in the elderly could help improve their health as well as alleviate the associated co-morbidities. This study was designed to assess the incidence of the different types of hyperglycemia in the Nigerian elderly.

Subjects and Methods: The glucose result records of 287 elderly individuals, aged 60 years and above, done in chemical pathology laboratory, University College Hospital, Ibadan, Nigeria were categorized as normal, impaired fasting glucose(IFG), impaired glucose tolerance(IGT) and diabetes(DM). The plasma glucose concentration was determined colorimetrically using glucose oxidase method. Impaired fasting glucose was defined as fasting plasma glucose between 100 and 125mg/dl, impaired glucose tolerance as two hours post prandial between 140 and 199 mg/dl and diabetes as fasting plasma glucose > 126mg/dl or two hours post prandial > 200mg/dl. Subjects excluded from this study include patients on diabetic treatment and those with missing data on age and hospital diagnosis.

Results: Twenty-four subjects had impaired fasting glucose, fifteen had impaired glucose tolerance, twenty had these two combined while forty-four had plasma glucose values characteristic of diabetes. Thus, accounting for 8.4,5.2,7.0 and 15.3 % of the population studied respectively.

Conclusions: Our results suggest that a significant proportion of the elderly are prone to hyperglycemic disorders. In view of the strong incidence of hyperglycemia in this population it is recommended that screening for diabetes at primary health level be strongly advocated in the elderly thereby controlling diabetic complications.

B-49

Evaluation of the Liaison 25 OH Vitamin D TOTAL Assay on the new DiaSorin Liaison XL.

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Background: Vitamin D has been known for decades for its critical role in increasing the efficiency of dietary calcium absorption and preventing rickets in children. However, recent research has uncovered other important relationships between vitamin D and bone health as well as new information on its pleiotropic action. These new evidences have fuelled the global demand for 25-OH vitamin D (25(OH)D) testing. Clinical laboratories are under pressure to seek automated reliable platforms for 25(OH)D analysis to keep up with the demanded volume. Recently, DiaSorin (Stillwater, MN) launched a new immuno-assay analyzer called Liaison XL. This analyzer presents some interesting features compared to the former Liaison. Nevertheless, according to the ISO 15189 Guideline, we needed to validate the 25(OH)D assay prior to use it in our daily routine.

Methods: We evaluated the precision with a modified protocol based on CLSI EP-5A2: 6 serum pools were assayed in three replicates per day on five different days. We established the functional sensitivity and the accuracy profile of the method. Finally,

we compared the results obtained with our current Liaison on 201 patients spanning the whole rang of measurement and studied the lot to lot variation, on 118 patients and two different lots.

Results: The LOQ was established at 2.5 ng/L. Repeatability and intermediate precision were <5% from 21 to 69 ng/mL. At 10 ng/mL, they were respectively of 6.5 and 7.1%. 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 10.1 - 63.9 ng/L studied range. The Bland Altman plot shows a good agreement between the 2 instruments with a mean bias of 2.5±6.2 ng/mL. The regression equation between the two methods is Liaison XL = 0.92 X Liaison + 1.2. The mean difference between two different lots was 6.6% and the Bland Altman plot showed a mean difference of 1.4±2.3 ng/mL.

Conclusions: The DiaSorin Liaison XL is a friendly and easy-to-use instrument that possesses a lot of interesting new features compared to the former Liaison. From an analytical point of view, the performances of this new instrument are really improved.

B-50

Performance evaluation of Abbott Architect Intact PTH

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Background: Parathyroid Hormone represents a key analyte in the evaluation of bone metabolism. Its measurement is particularly relevant in patients with renal insufficiency and in the follow-up of the pharmacological treatment of osteoporosis. Due to the great heterogeneity of the circulating forms its measurement is particularly critical. Aim of the work is the evaluation of the analytical performances of Abbott Architect Intact PTH.

Methods: Precision was evaluated following the CLSI document EP5-A2 on two serum pools and 3 control materials. Method comparison: the leftover, anonimized EDTA plasma samples from 49 dialysis patients and 70 osteoporotic patients were measured in parallel with Liaison® Diasorin N-tact ™ PTH and Abbott Architect Intact PTH. The results were obtained in 5 different analytical runs.

Results: the various components of imprecision defined by ANOVA are shown in the table. Method comparison. The non parametric regression equation (Passing - Bablok) gave the following Results dialysis patients: Abbott = 2.0888 Diasorin - 8.6; osteoporotic patients: Abbott = 1.5629 Diasorin - 6.4. The parametric coefficients of correlation were r=0.9.7756 and r=0.97312 respectively.

Conclusions: the Abbott test showed excellent precision allowing to reach the optimal quality specification (0.25 CVw). The correlation with Diasorin was good, but we obtained substantially different results; in particular the results of dialysis patients were more than two times higher than Diasorin, so the decision limits for treatment have to be changed accordingly. The difference in the slope in the two comparisons may be due to different cross reactivity for the 7-84 fragment, known to be present in higher concentrations in patients with chronic renal insufficiency.

Abbott Architect PTH Precision					
	Low Pool	Medium Pool	Ctrl Low	Ctrl Medium	Crtl High
Mean (pg/mL)	47.6	110.9	9.8	65.3	256.3
CVduplicates	3.6%	3.0%	3.5%	2.4%	2.6%
CVwithin run	3.1%	5.5%	2.8%	3.6%	2.2%
CVbetween day	--	3.4%	1.6%	1.3%	1.4%
CVbetween run	1.8%	5.1%	1.3%	3.2%	1.3%
CVoverall	3.9%	6.8%	4.1%	4.2%	3.2%

B-51

Estimating reference intervals based on a hospitalized population.

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Title: Estimating reference intervals based on a hospitalized population

Introduction: Traditionally, the optimum approach to establish reference intervals for any particular analyte is based on the evaluation of a healthy population. However, for many clinical laboratories, it can be difficult to recruit at least 120 healthy individuals per group studied each time there is a need to establish a new reference interval. Some alternative methods have been suggested and include use of a hospitalized population instead of healthy volunteers (Hoffmann RG. Statistics in the practice of medicine, JAMA 1963; 185:864-73).

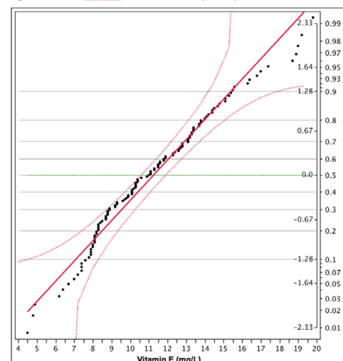
Objectives: To estimate and verify reference intervals for Ionized Magnesium, Vitamin E and Vitamin A using the Hoffmann technique.

Material and Methods: Using in- and out-patient samples from a high complexity 240-bed hospital, we estimated the reference intervals employing the Hoffmann method. Ionized Magnesium testing was performed with the Nova 8 Analyzer (Nova Biomedical, Waltham, MA) according to manufacturer specifications. Vitamin A and E measurements were performed by reverse phase High Performance Liquid Chromatography with UV detection. The data analysis was performed on the statistical software JMP 8.

Results: The following 2.5-97.5th percentiles determined the reference intervals as: Ionized Magnesium: 0.44 - 0.59 mmol/L (n= 51); Vitamin A (subjects ≥ 20 years old): 23.7 - 84.5 µg/dL (n= 126); Vitamin E (subjects ≥ 20 years old): 5.0 - 19.0 mg/L (n= 123). Figure 1 shows the plot for Vitamin E.

Discussion and Conclusions: The reference intervals found are very similar to those reported in the literature employing a healthy population. The Hoffmann approach for the estimation of reference intervals is a valuable alternative to clinical laboratory professionals who wish to assess intervals in their own hospital population.

Figure 1. Normal Quantile Plot for Vitamin E (n= 123).



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Comparison of two analytical methods for hemoglobin A1c (HbA1c) measurement

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Background: Hemoglobin A1c (HbA1c) is widely used to assess long-term glycemic status in patients with diabetes mellitus and is directly correlated with the risk of developing diabetic complications. Several analytical methods are currently used for measuring HbA1c in blood samples. The purpose of this study was to compare two automated HbA1c tests that use different measurement principles, evaluating the correlation between the two methods as well as the practicality of performing the tests.

Methods: Six hundred and twenty two whole blood samples were analyzed using two automated **Methods:** high performance liquid chromatography (HPLC, ADAMS A1c HA-8160 analyzer; A. MENARINI Diagnostics, Italy) and an immunoturbidimetric assay (Tina-quant Hemoglobin A1c Gen. 3, cobas 6000 analyzer; Roche Diagnostics, Switzerland). Both methods were calibrated in accordance with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Reference Measurement Procedure. A database of results was generated using Microsoft Excel and the correlation between the two methods was assessed by least squares and Passing-Bablok linear regression analyses (R program v.2.11.1). The daily start-up time of the two instruments used, daily maintenance tasks and determination of throughput were also recorded.

Results: HPLC analysis using the HA-8160 analyzer reported the following HbA1c concentrations: mean±standard deviation, 32.81±3.38 mmol/mol (5.15±0.31%); median, 32.24 mmol/mol (5.10%); range, 22.41-49.73 mmol/mol (4.20-6.70%).

The immunoturbidimetric assay using the cobas 6000 analyzer reported the following HbA1c concentrations: mean±standard deviation, 33.44±3.24 mmol/mol (5.21±0.30%); median, 33.34 mmol/mol (5.20%); range, 23.50-50.82 mmol/mol (4.30-6.80%). There was strong correlation between the results generated by the two test methods using both the least squares (intercept 0.05460; slope 0.97844) and Passing-Bablok (intercept 0.10; slope 1.00) regression methods. The time spent daily for the start-up of the HA-8160 analyzer was 25 minutes, including manual switch-on, replacement of reagents and implementation of quality control procedures. Completion time, including the corresponding washing and recharging of reagents was 15 minutes. Maintenance tasks for the cobas 6000 analyzer at the beginning and end of the day are automated processes so do not require additional operator time. In addition, quality control procedures are integrated into the system and performed as part of the test run. Throughput for the HA-8160 analyzer was 20 samples/hour compared with 100 samples/hour for the cobas 6000 analyzer. The HPLC method also includes time-consuming manual analysis of each chromatogram to exclude the presence of hemoglobin variants, a step that is not required by the more specific immunoturbidimetric method.

Conclusions: We found excellent correlation between the HPLC and immunoturbidimetric methods for measuring HbA1c levels in whole blood, and therefore a change of method would not affect the analytical and clinical monitoring of a patient with diabetes. The advantages of the immunoturbidimetric method are optimization of the daily start-up time and fewer maintenance and service actions. These factors lead to reduced hands-on operator time, faster processing of HbA1c tests and a reduction in the unit cost per test.

B-53

Plasma Orexin-A Levels Of Patients With Major Depression

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Background: Main clinical signs of depression include disturbances of sleep and appetite, as well as changes in emotional states. Orexin-A is a neurotransmitter produced in dorsal and lateral hypothalamus and is a modulator of sleep, appetite, energy consumption, reward and emotional states. The aim of our study was to investigate orexin-A levels, as well as leptin and ghrelin levels, which are important in synthesis of orexin-A, in major depression patients.

Methods: In our study, orexin-A levels of unipolar major depression patients during both acute and post-treatment period were compared with orexin-A levels of 44 age and sex matched healthy controls. In addition, the relation between orexin-A levels and leptin and ghrelin levels was also investigated. Plasma orexin-A, ghrelin and leptin levels were analyzed by enzyme immunoassay method. Self-report and Clinician-Applied versions of Inventory for Depression and Symptomatology Scale were used to evaluate patients' depression levels. Statistical analyses were performed using Paired Samples T-test, T-test for independent samples and Pearson Corelation analyses tests.

Results: Patients' pretreatment orexin-A levels were significantly higher than control group ($p<0.05$). Pretreatment leptin levels were significantly lower than control group ($p<0.05$), while ghrelin levels were significantly higher ($p<0.05$). Orexin-A, leptin and ghrelin levels of patients' during pre and post-treatment phases did not differ significantly.

Conclusions: Results of this study may show that; orexin-A may be play role in pathogenesis of major depression and usage of orexin-A levels as a biochemical marker may support the diagnosis of major depression. We believe that; "orexin resistance" may be responsible for high orexin-A levels and thus low leptin and high ghrelin levels in major depression.

B-54

Concentrations of choline and choline compounds in human breast milk and their relations to maternal serum hormones

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Background: Choline is a vital amine which plays a fundamental role in structural and functional integrity of cell membranes, transmembrane signaling, lipid and methyl group metabolisms, cholinergic neurotransmission, body growth and normal neuronal development. In humans, breast milk is the main nutritional source of choline for breast-fed infants particularly during the first six months.

Methods: This study assessed the contents and concentrations of choline compounds in breast milk at different lactation stages and their relations to serum hormones in 159 breast-feeding women. Breast-feeding women were enrolled either in the first three days [expressing colostrum (N = 67)], days 4-14 [expressing translational milk (N= 47)], or days 22-180 [expressing mature milk (N = 71)] post-partum. Free choline, phosphocholine, glycerophosphocholine and phospholipid-bound choline were extracted from whole milk. Free choline content of the dried aqueous phase was assayed by high-performance liquid chromatography-electrochemical detection system (HPLC-EC). Phosphocholine and glycerophosphocholine were first hydrolyzed enzymatically to free choline by using specific enzymes, namely, alkaline phosphates for phosphocholine and glycerophosphocholine phosphodiesterase for glycerophosphocholine. The resulting free choline was assayed by HPLC-EC. Phospholipid-bound choline content of the dried organic phase was measured by an enzymatic colorimetric method.

Results: Free choline, phosphocholine, glycerophosphocholine, phospholipid-bound choline and total choline concentrations were 42±8, 157±25, 76±9, 201±4 and 476±37 μmol/L, or 139±9, 594±48, 200±5, 1250±84 and 2184±120 μmol/L or 112±8, 491±25, 1179±50, 197 ± 4 and 2020±70 μmol/L in colostrum, or translational milk or mature milk, respectively. Concentrations of free choline, phosphocholine, glycerophosphocholine and total choline, but not phospholipid-bound choline, in colostrum were much lower than those in translational milk, or in mature milk. Free choline, phosphocholine, glycerophosphocholine and total

choline concentrations in colostrum were positively correlated with the serum prolactin and adiponectin, and inversely correlated with the serum cortisol and leptin concentrations. Free choline concentration in translational milk was positively correlated with the serum prolactin and inversely with the serum cortisol. Translational milk phosphocholine, glycerophosphocholine and total choline concentrations were inversely correlated with the serum prolactin, progesterone and cortisol levels. Mature milk free choline concentrations were positively correlated with the serum prolactin levels, and inversely correlated with the serum estradiol and cortisol levels. Concentrations of phosphocholine, glycerophosphocholine and total choline in mature milk were inversely correlated with the serum estradiol, progesterone and insulin levels. In addition, mature milk phospholipid-bound choline concentrations were inversely correlated with the serum insulin levels.

Conclusions: In conclusion, these data show that the contents of choline compounds in human breast milk vary considerably throughout lactation possibly due to hormonal status of the mother.

B-57

Short Synacthen test: 30 or 60 or both?

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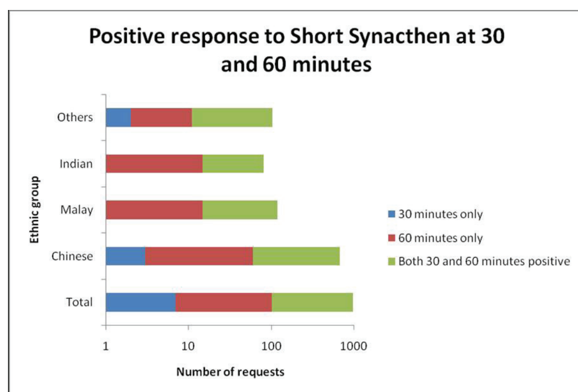
Background: High dose short synacthen test(HDSST) using 250-μg Synacthen is commonly used to screen for adrenal insufficiency. However, it requires 3 timed blood collections, and some have recommended either the 30 minutes or 60 minutes cortisol concentration for diagnosis. Ethnic differences may contribute to different physiological responses to Synacthen, hence we looked at cortisol concentrations at 30 minutes compared to 60 minutes in HDSST in different ethnic groups in Singapore.

Materials and Methods: We did a retrospective review of 1268 HDSST done from 2009 to 2011. 18 requests were excluded due to incomplete data. Using the cutoff of 550nmol/L at either 30 minutes or 60 minutes or both as normal adrenal response(A), we identified those with positive results at 30 minutes only(B) or 60 minutes only(C).

Results: The mean age was 62 years old, and female:male ratio was 0.83:1. The ethnic distribution was 69% Chinese, 13% Malays, 8% Indian and 10% other ethnic groups. 983 requests(79%) showed normal adrenal response using A. Using B or C, the percentage of normal adrenal response were 71% and 78% respectively. 79%, 72% and 78% of Chinese patients had positive response using A, B or C respectively. In Malay patients, it was 73%, 65% and 73% respectively. In Indian patients, it was 84%, 69% and 84% respectively. In patients in other ethnic groups, it was 81%, 74% and 79% respectively.

Conclusions: In HDSST, using only the 60 minutes cortisol concentration is comparable to using either 30 minutes or 60 minutes or both cortisol results to screen for adrenal insufficiency. This is particularly so in the Indian patients and less so in Malays and Chinese. Therefore, cortisol measurements at baseline and 60 minutes are sufficient in HDSST to screen for primary adrenal insufficiency.



B-58

Simultaneous Determination of Free T3 and Free T4 in Human Serum Using Equilibrium Dialysis Followed by LC-MS/MS

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Background: Production of thyronine (T_4) and triiodothyronine (T_3) is regulated by thyroid stimulating hormone (TSH), which is produced in the pituitary gland. Increased serum levels of T_3 and T_4 will result in decreased TSH production, and vice versa, in what is generally considered to be a closed-loop feedback system. While T_4 is predominantly a prohormone for T_3, both moieties actively bind to cell receptors and provoke an increase in the basal metabolic rate for most tissues in the body. To fully assess thyroid function, it is important to measure the concentration of free, circulating T_3 and T_4 relative to the concentration of the fraction that is bound to thyronine-binding globulin, transthyretin, and albumin. In this report, we present a method for the simultaneous determination of free T_3 (FT_3) and free T_4 (FT_4) in serum based on equilibrium dialysis (ED) followed by LC-MS/MS measurement.

Methodology: Using a split dialysis cell separated by a 5,000 molecular weight cut-off membrane, 125 µL serum was dialyzed against 1.325 mL of an in-house prepared buffer for 17.5 hours at 37 °C. The buffer solution (900 µL) was then decanted and supplemented with 100 µL of dialysis buffer containing C^{13}-labeled T_3 and T_4 isotopes. The dialysate (400 µL) was then injected onto a Phenomenex Kinetex C-18 column (4.6 mm i.d. x 50 mm length) operated at a flow rate of 1 mL/min, with 0.1 % formic acid in water as mobile phase A and 100% methanol as mobile phase B. The HPLC gradient was as follows: 1) 1 min isocratic elution at 30% mobile phase B, 2) 5 min linear ramp to 95% mobile phase B, and 3) 1 min isocratic elution at 95% mobile phase B. For both T_3 and T_4 species, negative ionization electrospray was employed to measure the iodine fragments at m/z=127.1 Da.

Results: Sample analysis was accomplished in 7 minutes for both analytes. The LOQs for FT_3 and FT_4 were determined to be 2 and 4 pg/mL, respectively. The upper limit of linearity for both analytes was 128 pg/mL. Total CVs were 5.5% to 7.3% for FT_3 at 3 concentrations (4, 8, and 16 pg/mL). Total CVs were 3.4% to 5.9% for FT_4 at 3 concentrations (8, 17, and 67 pg/mL). The reference interval for FT_3 was determined to be 3.0 - 7.3 pg/mL, and the reference interval for FT_4 was determined to be 11 - 31 pg/mL. No carryover or interferences from bilirubin, fatty acids, or hemoglobin were observed.

Conclusions: Both FT_3 and FT_4 can be measured simultaneously by this method in 7 minutes using only 125 µL of serum. This ED followed by LC-MS/MS method offers excellent precision and accuracy for both FT_3 and FT_4 measurements with a linear range that spans 2-128 pg/mL. Overall this method would be useful for any clinical laboratory performing FT_3 and FT_4 analysis.

B-59

Insulin and insulin-like growth factor binding protein-1 (IGFBP-1): Reference change values and intra-individual variation in individuals with a varying glucose tolerance

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Background: Serum concentrations of insulin-like growth factor binding protein-1 (IGFBP-1) vary reciprocally with those of insulin and correlate strongly with measurements of insulin resistance. It is appropriate to investigate variation in consecutive fasting plasma insulin (FPI) and IGFBP-1 measurements in an effort to inform decision making concerning the management of individuals with insulin resistance. The aim of this study was therefore to evaluate intra-individual variation (CVi) and reference change values (RCV) for FPI and IGFBP-1 in healthy individuals and to compare them with values derived from individuals in other glycaemic categories.

Methods: FPI and IGFBP-1 concentrations were measured in 42 fasting subjects subcategorised by glucose tolerance (WHO criteria) as follows: normal glucose tolerance (NGT), n=15, impaired fasting glucose (IFG), n=9, impaired glucose tolerance (IGT), n=9 and type 2 diabetes (DM), n=9.

Results: The CVi and RCV for serum IGFBP-1 increased from 19.7% and 59.9% in NGT up to, 47.5% and 133.9% in diabetic subjects respectively. For FPI the CVi and RCV increased from 24.2% and 68.5 in NGT up to 39.8% and 111.2% in diabetic subjects respectively (Figure 1). CVi and RCV values for both analytes were observed to increase progressively through the glycaemic categories from NGT to diabetes.

Conclusions: RCV values for both IGFBP-1 and FPI are higher in individuals with deteriorating glucose tolerance compared to NGT individuals. Our findings support the contention that glycaemic category-specific RCV values should be used when interpreting apparent changes in serum IGFBP-1 and FPI levels.

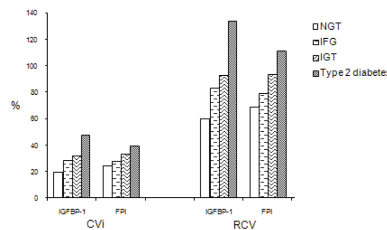

Figure 1: Percentages of intra-individual variation (CVi) and reference change value (RCV) for insulin and IGFBP-1 in different glycaemic categories. Key: NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

B-60

Levels of free thyroxine in euthyroid subjects are negatively associated with insulin resistance

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Background: The physiological role of thyroid hormones on insulin sensitivity in euthyroid subjects as well as the consequences of insulin resistance on thyroid function are still poorly understood. In order to evaluate the relationship between insulin resistance determined by homeostasis model assessment (HOMA-IR), and thyroid function in a sample of Brazilian euthyroid individuals a cross-sectional study was carried out.

Methods: This study was conducted from March 2009 to January 2010 including 267 euthyroid subjects (mean age of 34.5±11.2 years-old, 68,5% female). Thyroid stimulating hormone (TSH), free thyroxine (FT4), anti-thyroid antibodies (anti-thyroglobulin and anti-thyroperoxidase), fasting glucose and insulin levels, HOMA-IR, and thyroid ultrasound were assessed. The subjects were grouped into tertiles according to increasing levels of HOMA-IR (group 1: Δ 0,23 - 1,32; group 2: Δ 1,33 - 2,32; group 3: Δ 2,33 - 8,50). Mann-Whitney test was performed to compare means of TSH and FT4 in each tertile (significance level of 5%, p <0.05, SPSS[a]).

Results: Among the 267 euthyroid evaluated individuals, the mean HOMA-IR was 2.11±1.45. TSH levels (mUI/mL) were not statistically different among HOMA-IR tertiles (1.65±0.90 vs. 1.68±0.80 vs. 1.74±0.80; p>0,05), while the FT4 (ng/dL) values were progressively lower with increasing HOMA-IR, reaching statistically significant difference between the average first and last tertiles (1.23±0.20 vs. 1.21±0.16 vs.

1.15±0.20; p <0.001).

Conclusions: As previously demonstrated in other populations, those findings of FT4 negatively associated with insulin resistance in Brazilian euthyroid subjects may suggest an increased risk of metabolic syndrome in individuals with low normal thyroid function, or a possible adaptive response to the former condition.

B-62

Evaluation of the enzymatic BM test HbA1c and simultaneous plasma glucose assay from same sample on JCA-BM 6010/C

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Background: A novel enzymatic HbA1c assay was introduced for use in an automated chemistry analyzer. HbA1c and plasma glucose can be measured from same tube with this unique method. We evaluated the analytical performance of this enzymatic HbA1c test and simultaneous plasma glucose test on JCA-BM6010/C.

Methods: Enzymatic HbA1c test (BM Test HbA1c) and plasma glucose test (BioMajesty glucose hexokinase FS*) on JCA-BM6010/C(JEOL Ltd., Tokyo, Japan) were evaluated for precision, linearity, and carry over, and interferences. Two kinds of plasma glucose test methods on JCA-BM6010/C, i.e. routine plasma glucose analysis and simultaneous plasma glucose test with HbA1c from same sample tube were compared. Total 40 specimens from 24.60 to 471.51 mg/dL were used for this comparison.

Results: HbA1c coefficients of variation(CVs) of within-run imprecision for low and high levels were 0.6% and 0.4%, respectively. CVs of within-laboratory imprecision for low and high levels were 1.2% and 0.8%, respectively. The linearity was excellent with R^2 = 0.9986 in the range of 5.0 % - 15.2 %. The carryover rate was 0.02%. Triglycerides, bilirubin, glucose, BUN did not affect HbA1c. CVs of within-run imprecision for low and high levels for simultaneously assayed plasma glucose were 1.4% and 1.5%, respectively. CVs of within-laboratory imprecision for low and high levels were 1.9% and 2.8%, respectively. The linearity was excellent with R^2 = 0.9999 in the range of 31.85 mg/dL-262.33 mg/dL. The carryover rate was 0.53%. Simultaneous measurement of plasma glucose was well correlated with routine plasma glucose assay(R^2=0.9999).

Conclusions: Enzymatic BM Test HbA1c on JCA-BM6010/C showed excellent precision and linearity and minimal carryover rate. Simultaneously assayed plasma glucose analysis was well correlated with routine plasma glucose test method. They performed excellent and may useful for any clinical application, diagnosis and monitoring of diabetes.

B-63

Comparison of HbA1c assay methods among enzymatic HbA1c test on JCA-BM 6010/C, Bio-Rad Variant II Turbo HPLC method, Tosoh HLC 723 G8 HPLC method, and AutoLab immunoturbidimetry method

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Background: Hemoglobin A1c (HbA1c) is widely used for the monitoring of glycemic control in diabetes mellitus patients. Various methods can be applied for the determination of HbA1c levels. We compared HbA1c values measured using a enzymatic JCA-BM6010/C with the BM Test HbA1c reagent with those of two HPLC analyzers and a immunoturbidimetry reagent using automated chemistry analyzer.

Methods: Total 463 specimens with HbA1c levels from 3.9 %(19.1 mmol/mol) to 14.4 %(133.9 mmol/mol) were used. JCA-BM6010/C(JEOL Ltd., Tokyo, Japan) with the BM Test HbA1c reagent , Variant II Turbo(Bio- Rad Laboratories, CA, USA), Tosoh HLC 723 G8(Tosoh Corporation, Tokyo, Japan), and AutoLab Hemoglobin A1c(IVD Lab Co., Korea) immunoturbiimetry reagent on Hitachi 7600 -110(Hitachi, Tokyo, Japan) were evaluated.

Results: Coefficients of variation (CVs) of low and high level of JCA-BM6010/C with the BM Test HbA1c were 1.2 % and 0.7 %, and those of other methods were 0.94% and 0.57% for Variant II Turbo , 1.9% and 1.2% for Tosoh HLC 723 G8 , and 1.5 % and 2.4 % for AutoLab Hemoglobin A1c immunoturbiimetry reagent, respectively.

Highly significant correlations of HbA1c levels between JCA-BM6010/C with the BM Test HbA1c and other methods, Variant II Turbo (R=0.990, P<0.001), Tosoh G8 (R=0.995, P<0.001), and AutoLab(R=0.986, P<0.001).

Conclusions: JCA-BM6010/C with the BM Test HbA1c reagent showed excellent correlation with the other widely used methodological instruments, Variant II Turbo, Tosoh HLC 723 G8, and AutoLab Hemoglobin A1c immunoturbimetry reagent. It may be used for the diagnosis and the treatment monitoring of diabetes.

B-64

Basic performance of thyroid assays on STACIA®

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Background/Purpose: The thyroid stimulating hormone (TSH) which is secreted from hypophysis and the thyroid hormones (free thyroxine (FreeT4) and free triiodothyronine (FreeT3)) of which their secretions are regulated by TSH play important roles on the thyroid function management. TSH and thyroid hormones assays are useful in a diagnosis of thyroid disease such as thyroid tumor or the thyroid function abnormality or the confirmation of the curative effect. In this study, we evaluated the performance of newly developed rapid and high sensitive TSH and thyroid hormone assay using fully automated clinical analyzer ; STACIA®.

Principles/Methods: The STACIA® can measure seven different types of assay principles on one instrument.

(1) Chemiluminescence enzyme immunoassay (CLEIA)
(2) Coagulation time
(3) Chromogenic substrate
(4) Latex agglutination
(5) Immunoturbidimetry
(6) Biochemistry
(7) Electrolyte (option)

All results are available within 21 minutes and throughput is 270 tests per hour with fully random access. STACIA TSH, FreeT4, and FreeT3 assays are one-step CLEIA method. All measurement is conducted with an STACIA analyzer using an appropriate STACIA reagent kit. TSH assay uses two monoclonal antibodies which recognize different epitopes of antigen. FreeT4 assay is competitive analogue assay using T3-Alkaline phosphatase(ALP), and FreeT3 assay is competitive homologue assay using T3-ALP.

Results: For TSH, Precision at various levels of Serum TSH gave CV less than 15%. Correlation against Elecsys® (Roche) was y = 0.96 x - 0.46 r = 0.97 and ADVIA Centaur® XP (Siemens) was y = 0.96 x + 0.36 r = 0.99. The LoQ was 0.01mIU/L which is equivalent to 3rd generation TSH assay criteria. The dynamic range was 0.01 to 100mIU/L. For FreeT4, Precision at various levels of Serum FreeT4 gave CV less than 20%. Correlation against Elecsys® (Roche) was y = 1.03x + 0.15 r=1.00. Analytical sensitivity was 0.1ng/dL. The dynamic range was 0.1 to 8ng/dL.

For FreeT3, Precision at various levels of Serum FreeT3 gave CV less than 20%. Correlation against Elecsys® (Roche) was y=1.03x + 0.03 r=0.99. Analytical sensitivity was 0.7pg/mL. The dynamic range was 0.7 to 30pg/mL.

Conclusions: The STACIA TSH, FreeT4 and FreeT3 assays showed rapid, precise and reliable assay for the assessing the thyroid function. Since these three thyroid disease markers are tested simultaneously so often in the hospitals, STACIA® analyzer should be suitable for routine clinical use. STACIA® adds three thyroid disease markers to the measurable item and gets closer to completion of the integration. STACIA® is the new all in one clinical laboratory platform for a central laboratory and the satellite laboratory, emergency use or pre-treatment use.

B-65

Analytical and clinical performance of the PATHFAST® Testosterone assay

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Background: There is a growing awareness of infertility and the requirement for its medical treatments are enormously expanded today. We developed the fertility marker panel for the quantitative determination of human chorionic gonadotropin, follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol, and progesterone on PATHFAST®. In this study, we suggest the clinical usability of rapid measurement of testosterone, the main androgen hormone in point-of-care testing (POCT) settings.

Methods: All testosterone assays were performed using PATHFAST® analyzer. PATHFAST® Testosterone is a chemiluminescent enzyme immunoassay based on MAGTRATION® technology. It is possible to measure up to 6 multi-items, so that the testosterone assay can be simultaneously performed with other fertility marker panel in one batch. The assay data can be reported in 26 minutes. The calibration

frequency is every 4 weeks for any one lot. Whole blood sample can be tested on the PATHFAST® as well as plasma and serum samples. The analytical performances were studied by precision, analytical specificity, interference, method comparison, and correlation between whole blood and plasma samples according to CLSI guidelines. The clinical performances of the testosterone assays were studied by using blood samples from patients with infertility treatment.

Results: Analytical performances: Within-run and Total imprecision were ranged from 3.4% to 11.5% and from 5.5% to 13.6%, respectively. Assay correlation with other commercial testosterone tests was determined: y = 1.034x - 0.028, r = 0.983, n = 102 (y: PATHFAST® Testosterone x: Roche Elecsys Testosterone II). There is a good correlation between whole blood and plasma samples: y = 0.994x - 0.020, r = 0.992, n = 58 (y: Li-heparinized whole blood x: Li-heparinized plasma).

Clinical performances: Patients with infertility treatment were enrolled into the study and blood sample was collected from each patient at hospital admission. In this study, it was suggested that testosterone concentration reflected the clinical observation, since testosterone levels showed a significant decrease in hypogonadism. Many male patients are difficult to go to the hospital regularly, so it is possible to diagnose rapidly using the rapid whole blood assay of testosterone.

Conclusions: Including the testosterone assay, 7 fertility maker panel was developed on the PATHFAST® analyzer. The PATHFAST® Testosterone has a suitable analytical and clinical performance for the rapid whole blood assay of testosterone. PATHFAST® Testosterone is considered to be useful and strong system in a small clinic such as physician office laboratory as well as various clinical sites.

B-68

Clinical performance evaluation of VIDAS® Anti-TPO and Anti-Tg assays

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Background: A classical thyroid profile involves first-line testing of TSHus and/or FT4 and/or FT3. Second-line testing for autoantibodies enables to differentiate thyroid autoimmune diseases from other disorders. The VIDAS® Anti-TPO and Anti-Tg assays (bioMérieux) are intended as an aid in the diagnosis of autoimmune thyroid diseases in combination with clinical symptoms and other tests. Indeed, significant levels of these autoantibodies are often found in Graves' (Basedow's) disease and Hashimoto's thyroiditis.

The aim of the study was to evaluate the positive and negative agreements of these 2 assays compared to Architect® (Abbott) when evaluating a thyroid disorder population, including patients meeting a case definition for Hashimoto's thyroiditis or Graves' disease, and samples submitted for routine thyroid disease testing. On the clinically characterized population, the diagnostic sensitivities of these assays were also compared to prevalence described in literature.

Methods: The VIDAS Anti-TPO and Anti-Tg principles combine a two-step enzyme immunoassay indirect sandwich method with a final fluorescent detection (ELFA). Anti-TPO and anti-Tg autoantibodies present in the sample bind with antigens, respectively recombinant TPO and native Tg (solid phase) and with a monoclonal anti-human IgG antibody conjugate to alkaline phosphatase (final detection step).

The study was performed on about 450 samples from a population of patients with thyroid disease or for whom an anti-thyroid antibody assay had been prescribed. Among this population, 267 patients presented with Graves' disease or Hashimoto's thyroiditis. The clinical diagnosis was defined according to the evaluator's routine procedures. Negative and positive status were provided by prospectively testing samples with Abbott Architect Anti-TPO and Anti-Tg assays. Results were analyzed both before and after repeat testing.

Results: The diagnostic sensitivity of the VIDAS Anti-Tg was 85.3% for Hashimoto's thyroiditis and 73.3% for Graves' disease. The diagnostic sensitivity of the VIDAS anti-TPO was 96.3% for Hashimoto's thyroiditis and 87.7% for Graves' disease.

Positive and negative percent agreements of VIDAS Anti-Tg compared to Architect Anti-Tg were : 87.2% [95% CI : 83.0% - 90.6%] and 94.7% [95% CI : 89.5% - 97.9%] respectively. After repeat testing of 39 discrepant samples as above, agreements increased to : 89.1% [95% CI : 85.1% - 92.3%] and 95.0% [95% CI : 90.0% - 98.0%]. Positive and negative percent agreements of VIDAS Anti-TPO compared to Architect Anti-TPO were : 95.4% [95% CI : 92.5% - 97.4%] and 97.6% [95% CI : 93.1% - 99.5%] respectively. After repeat testing of 15 discrepant samples, negative agreement remained 97.6% [95% CI : 93.1% - 99.5%] and positive agreement increased to : 96.3% [95% CI : 93.6% - 98.1%].

Conclusions: According to these results, the new CE-marked VIDAS Anti-TPO and Anti-Tg assays show good performances for the determination of thyroid antibodies for the diagnosis of Graves' disease or Hashimoto's thyroiditis, and high agreement percentages with Abbott Architect ones. Moreover, these kits are reliable, reproducible, easy-to-use and well positioned in the state-of-the-art for this type of test. The US registration of these new CE-marked products will complete the thyroid panel offer on the VIDAS immunoassay systems.

B-69

Analytical Performance of the IDS-iSYS CROSSLAPS® (CTX-I) Automated Immunoassay

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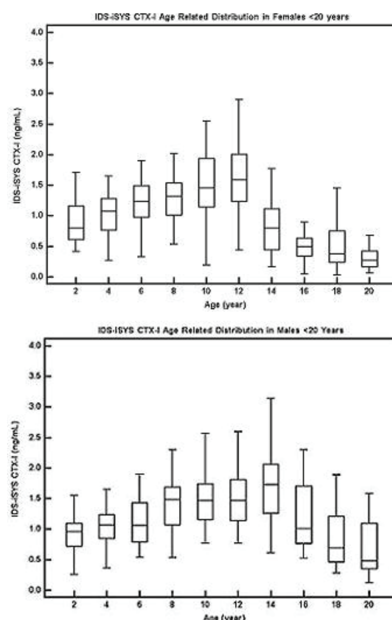
Background: As of late 2010, IOF and IFCC recommended s-PINP and s-CTX as reference analytes for bone turnover markers in observational and intervention studies. We assessed the analytical performance of the IDS-iSYS Crosslaps (CTX-I) automated assay and compared this method to the Elecsys assay. The observed ranges for apparently healthy children and reference intervals for pre-menopausal, post-menopausal women and men were established.

Methods: The accuracy profile was determined with 7 serum pool levels (0.05 - 3.12 ng/mL). The method linearity was verified with two sets of high/low serum samples. A total of 100 remnant serum samples [0.06-4.5ng/mL] was assayed by each method for method comparison. Serum specimens from overnight fasting, apparently healthy Caucasians subjects, normal Calcium, Phosphates, Intact PTH, and eGFR > 60, were selected to establish the reference intervals for males, pre-menopausal and post-menopausal (N = 120). The observed ranges were established with apparent healthy young (ages: 0.3 - 20 years) males (N = 287) and females (N = 295).

Results: The accuracy profile showed that the risk that one result falls out of the ±30% acceptance limit is <5%. The obtained linearity equation was: Y = -0.003 +1.00x with r^2 of 0.998. The Passing Bablok regression was: IDS-iSYS = 1.11 x (Elecsys) -0.02. The 95% reference intervals were: 0.076-1.225 (males), 0.038-1.197 (pre-menopausal) and 0.061-1.361ng/mL (post-menopausal). Figure below illustrated the 95% observed ranges, stratified by every 2 years of ages, for young males and females.

Conclusions: The IDS-iSYS CTX-I demonstrated suitable characteristics as a high throughput, fully automated bone resorption assay for clinical laboratories. The established pre-menopausal, post-menopausal, and males reference intervals will be useful tool for the monitoring of osteoporosis therapeutic treatment. Values from more than 500 children classified according to age and gender would be helpful to clinical laboratories and pediatricians.



B-70

Development of an Assay to Detect Total 25(OH) Vitamin D* on the Beckman Coulter Access® Family of Immunoassay Systems

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Background: Vitamin D is a lipid-soluble steroid hormone that is produced in the skin through the action of sunlight or obtained from the diet. The role of vitamin D in maintaining calcium homeostasis is well established. Increasing evidence also suggests that vitamin D may play a role in decreasing the risk of many chronic illnesses. 25-hydroxyvitamin D [25(OH)D] is the major circulating metabolite of vitamin D in the body and reflects inputs from cutaneous synthesis and dietary intake. For this reason, serum concentration of 25(OH)D is considered the standard clinical measure of vitamin D status. There are two forms of 25(OH)D, 25(OH)D_2 (ergocalciferol) and 25(OH)D_3 (cholecalciferol), both of which should be measured in totality to accurately assess total 25(OH)D levels.

Beckman Coulter is developing an automated assay to measure total 25(OH)D levels in human serum and plasma on its Access and DxI instrument platforms.

Methods: The prototype Access Total 25(OH) Vitamin D assay is a competitive chemiluminescent immunoassay. Sample is added to a reaction vessel with vitamin D binding protein (VDBP) releasing agent and paramagnetic particles coated with monoclonal anti-25(OH)D antibody. 25(OH)D is released from VDBP and binds to the immobilized monoclonal anti-25(OH)D on the solid phase. A 25(OH)D-alkaline phosphatase conjugate is then added and competes for binding to the immobilized monoclonal anti-25(OH)D. After a short incubation, bound materials are held in a magnetic field and unbound materials are washed away. A chemiluminescent substrate, Lumi-Phos 530, is added and the light generated is measured with a luminometer. The light production is inversely proportional to the concentration of 25(OH)D in the sample, which is determined from a stored, multi-point calibration curve.

Results: Feasibility studies have been completed on a prototype assay with the following preliminary performance characteristics:

- A limit of detection (LoD) of 1.8 ng/mL and a limit of quantification (LoQ) equal to 6 ng/mL with a measuring range up to 180 ng/mL.
- Total CVs of approximately 10% for samples at 10 ng/mL and ≤ 5% for samples ranging from 30-180 ng/mL.
- A correlation with LC-MS/MS was performed on 64 samples across the measuring range, yielding a regression coefficient of 0.97.
- 25(OH)D_2 cross reactivity equal to 97% of the 25(OH)D_3 value.

Conclusions: This early evaluation demonstrates that the prototype Access Total 25(OH) Vitamin D assay exhibits good precision and sensitivity with acceptable correlation to LC-MS/MS. In addition, the assay displayed satisfactory recognition of both 25(OH)D_2 and 25(OH)D_3 across the clinically relevant range of the assay. These characteristics demonstrate the potential for this assay to be used in the quantitative determination of total 25(OH)D levels in a routine laboratory environment.

* Pending submission and clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use.

B-71

Development of Reference Method for Measuring Binding of Sex Hormone Binding Globulin (SHBG) to Sex Hormones in Human Serum.

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Objective: To develop surface plasmon resonance (SPR) method to measure the kinetics of SHBG binding to sex steroids in human serum. Relevance: Biological activity of sex hormones (Testosterone (T), Estradiol (E_2) and dihydrotestosterone (DHT)) in the blood is regulated by high-affinity binding to SHBG and to a lesser degree, by weak-affinity binding to albumin (Alb). Genetic variations in SHBG and posttranslational modification of SHBG at the level of glycosylation can affect the binding characteristics of SHBG and therefore, result in a change of sex hormones availability. SPR technology provides an approach to measure SHBG binding kinetics. However, multiple factors related to complexity of human serum must be considered in the development of the method.

Methodology: SPR measurements were performed on the BIACore T-100 using CM4 and CM3 sensor chips (GE Healthcare) with low carboxymethylation levels. To ensure optimal and uniform steroid binding to SHBG, sex steroids were synthesized as 17α-aminocapryl derivatives of T, DHT and E_2 and then immobilized at low immobilization level (50-100 RU) to avoid mass transfer limitation. The functionality of the steroid-coated surface of sensor chip was tested with anti-T, DHT and E_2 antibodies. Kinetic studies were performed using human purified glycosylated SHBG (Cell Sciences) in the concentration range 0.1-100 nM at 37°C at flow rate 45 ul/min with 180 sec of association followed by 300 sec of dissociation in HBS-P+ buffer (GE Healthcare). CM3 and CM4 sensor chips with three different levels of T immobilization (50-100 RU) were used. Serum samples were diluted 1:10 ratio with HBS-P+ buffer for SPR measurements. Association and dissociation data were calculated by the BiaEvaluation program for respective interaction models. **Validation:** Equilibrium dissociation constants (K_D) and on/off (ka/kd) rate constants of purified SHBG binding to immobilized T fit 1:1 binding model with mass transfer for both CM3 sensor: ka=(3.96±0.0078)E+05, kd=(3.47±0.0073)E-04, K_D= 8.76E-10 with Chi²= 0.993 and CM4 sensor: ka=(9.82E±0.11)E+05, kd=(1.074±0.0045)E-03, K_D= 1.09E-9 with Chi²= 0.819. SHBG binding in serum samples showed strong interference due to albumin presence. SPR measurements of SHBG binding in the presence of Alb demonstrated that Alb over the range 5-500 μM increases SHBG responses (10-75% increase) in dose dependent manner. Therefore, Alb depletion prior SPR measurements of SHBG binding is needed.

Conclusions: SPR measurements of SHBG binding to testosterone in human serum require the depletion of Alb below 5 μM (99% of serum content) to ensure specific detection of SHBG binding. With this strategy the development of a SPR method for measurement of SHBG binding to sex hormones in human serum looks feasible.

B-72

Determination of the reference value of the thyroid-stimulating hormone in a population sample of healthy individuals from the city of Fortaleza, Brazil

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Background: Dosage of thyroid-stimulating hormone (TSH) is especially susceptible to inter-individual and intra-group biological variations. Therefore, the proper interpretation of the results is completely linked to the definition of the reference values [[Unsupported Character -]][[Unsupported Character -]] (RV) in an specific population. Ideally, each laboratory center should determine the RV based on representative studies of the target population. In addition, as TSH is the most sensitive screening test for detecting changes in thyroid function, it is mandatory to define more stringent criteria of normality, supported by clinical, laboratory and ultrasound findings. This study aimed to determine the RV of TSH in a sample of healthy individuals, in terms of anatomical and function thyroid, residents in the city of Fortaleza, Brazil.

Methods: This was a cross-sectional study conducted from March 2009 to January 2010 where 432 individuals were evaluated. This evaluation included four elimination rounds, including the completion of a self-administered questionnaire (Stage 1), medical evaluation (phase 2), collection of laboratory tests (phase 3) and thyroid ultrasound (TUS) (Phase 4). For the laboratory evaluation of thyroid function was used the electrochemiluminescence immunoassay for the third generation (Roche ®). Data were statistically analyzed using the Student t test to compare continuous variables, with statistical significance level of 5% ($p < 0.05$). To determine the reference range of TSH were adopted percentiles 2.5% and 97.5% of the distribution curve, as the corresponding lower and upper limits of the RV of TSH.

Results: Of the initial 432 volunteers, 275 participants with clinical and laboratory normal features were selected (normal FT4 and T3 and thyroid antibodies negative). Among them, 125 individuals volunteer were called reference, characterized by normal clinical, laboratory and ultrasound avaliation. The mean TSH in this latter group was 1.78 ± 1.0 mIU / L and the reference interval values [[Unsupported Character -]][[Unsupported Character -]]were between 0.56 to 4.45 mIU /L. The evaluation of TSH in the population of the 275 euthyroid individuals defined after the initial three phases, had a mean and distribution similar to the group of individuals named reference.

Conclusions: The reference values [[Unsupported Character -]][[Unsupported Character -]]for TSH derived from this study, are between the limits 0.56 to 4.45 mIU / L. For the determination of TSH VR, the UST has not been shown to be essential to defining the criteria for sample selection.

B-73

Evaluation of 4 automated assays for the detection of 25-OH Vitamin D

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In 2011 several manufacturers introduced new automated methods for the detection of 25-hydroxyvitamin D in patient samples for the assessment of Vitamin D sufficiency. Despite claims of high sensitivity and accuracy of these assays by the manufacturers, variable performance has been observed in practice.

A study was designed to compare the accuracy and sensitivity of the new assays of Siemens (ADVIA Centaur® Vitamin D Total - (Vit D), Abbott (ARCHITECT 25-OH Vitamin D) and Roche (Elecsys Vitamin D total) to the DiaSorin (LIAISON® 25 OH Vitamin D TOTAL Assay) assay. All immunoassay methods were also compared to an LC-MS/MS reference method (Esoterix Inc. LC-MS/MS Vitamin D, 25-Hydroxy, Fractionated - Total Vitamin D, Vitamin D-2, Vitamin D-3 Test Code 500337).

A total of 101 patient samples were selected from various sites across Germany in order to obtain a panel which spanned the range of the immunoassays. A total of 40 samples with sufficient volume were selected for subsequent LC-MS/MS analysis and when measured by LC-MS/MS, the doses ranged from 12 to 139 ng/mL (30 to 348 nmol/L).

In addition to the samples detailed above, a further panel of samples, selected to contain low 25 OH Vitamin D concentrations, was evaluated on both the LC-MS/MS method and each of the four immunoassays in order to evaluate and compare the detection limits of the immunoassays.

Following collection, the samples were stored at -20°C until analysis on each of the automated instruments and LC-MS/MS. All four immunoassay methods were performed according to the instructions for use provided by each manufacturer. LC-MS/MS assay results were used as an independent reference.

The DiaSorin assay correlates well with LC-MS/MS and demonstrates highly accurate results with a superior lower detection level when compared to other automated methods.

Both Siemens and Abbott assays demonstrated poor sensitivity and clear positive bias for doses <10 ng/mL (25 nmol/L) against LC-MS/MS. Despite the positive bias demonstrated at these low doses for the Siemens assay, the results of this study indicates that the Siemens assay shows an overall negative dose bias across the measuring range when compared against LC-MS/MS.

The Roche assay exhibits considerable variation across the assay measuring range when compared with LC-MS/MS.

The DiaSorin assay remains the most accurate and sensitive method for the measurement of total Vitamin D.

B-74

Evaluation of the Abbott ARCHITECT 25-OH Vitamin D Assay reference intervals in a Turkish adult population by an indirect method

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Background: Abbott Architect 25-OH Vitamin D immunoassay has been recently introduced on the market in Turkey. Our laboratory has been the first laboratory that has studied the maximum test number of Architect 25-OH Vitamin D immunoassay in Turkey. Expected values provided by assay manufacturers are representative data, and may not always be appropriate for different geographical areas and patient demographics. Reference intervals can be evaluated from the cumulative laboratory test results of individuals submitted to laboratory by an indirect method. The aim of the study was to evaluate reference intervals of Abbott Architect 25-OH Vitamin D in our local population and to verify the appropriateness of expected values for our population by using indirect method.

Methods: The cumulative laboratory test data [n=20535/after exclusion n=8079 (18-80 years; 1255 males, 6824 females)] obtained from our information system (March 2011-January 2012) were analyzed. Individuals have been excluded when showing results out of the reference values for parathormone, calcium, phosphorus, creatinine or with bone disease, chronic disease (diabetes, renal failure, hyper/hypoparathyroidism) or having vitamin supplementation. Seasonal and gender related differences were tested using Oracle's built-in Kolmogorov-Smirnov (K-S) function that compares two samples to test whether they are from the same population or from populations that have the same distribution. D values for gender related differences or seasonal differences were calculated as 0.27 (p=1.68x10-72), 0.10 (p=2.05x10-18) respectively. Thus, Vitamin-D reference values had to be calculated separately for males and females. Since K-S test can be applied for only two different samples, and at the same time autumn and spring days in Turkey may be both sunny and gloomy, the function was applied to winter (December-February) and summer (June-August) only. This time D value was calculated as 0.19 which was almost two times stronger (p=1.05x10-32). As a result, we came to the conclusion that Vitamin-D reference values had to be calculated separately for summertime and wintertime. The same function was applied to different age groups as pairs (18-24, 25-44, 45-64 and 65-80) and apart from 65-80, no significant differences were observed in other age groups. Later, these K-S tests were compared with Oracle's built-in Anova test and our conclusions were confirmed. Before histograms were drawn, using Oracle's built-in dbms_stat_funcs. summary, extreme values were detected with sigma=6 and these extreme values were removed from the obtained results. Finally, histograms were prepared and according to Naus's smoothing algorithm (For M=2, nL=2, nR =2 using -0.086, 0.343, 0.486, 0.343, -0.086 Savitzky-Golay coefficients) reference intervals were calculated.

Results: The estimated seasonal reference values were; Female: Summertime 21.10-36.72 ng/mL, Wintertime 10.43-34.51 ng/mL; Male: Summertime 16.53-34.99 ng/mL, Wintertime 12.67-26.61 ng/mL (autumn and spring days were excluded). Female: Whole year 19.64-41.42 ng/mL; Male: Whole year 9.81-29.87 ng/mL (all days of the year were included). The manufacturer's expected values for 25-OH-Vitamin D were 15.7-60.3 ng/mL and 8.8-46.3 ng/mL for summertime and wintertime respectively. For female were 9.4-59.1ng/mL and for male were 9.4-52.4 ng/mL; for all were 9.5-55.5 ng/mL.

Conclusions: Obviously, the upper reference values suggested by the manufacturer do not seem to be appropriate for our local population.

B-75

Evaluation of a 25 (OH) Vitamin D levels in an cohort of 10372 outpatients without known nutritional or bone diseases in Brazil

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Background: The Vitamin D deficiency is an outstanding health problem, that may be involved in pathogenesis of osteoporosis, nutritional diseases, gastrointestinal and cognitive problems, and growth disorders in childhood and adolescence. The assessment of its prevalence is very important for each region over the world, in order to allow the panning of the policies to avoid diseases. The aim of the present study was to assess the concentration of 25-hydroxyvitamin D (25OHD) across different ages and genders, and to compare it to PTH and Calcium levels.

Methods: We searched the database of the local Laboratory Information System to retrieve results of 25OHD (Quemiluminescent method- Diasorin) tests performed on the whole cohort of presumably healthy outpatients distributed in groups A-0-10 years (n=473); B-11-20 years (n=746);C-21-30 years (n=1019); D-31-40 years (n=1784); E-41-50 years (n=1792); F-51-60 years (n=1825); G-71-80 years (n=852); H > 81 years (n=479), who were referred to our laboratory in Brasilia routine laboratory testing ordered by general practitioners, over a period of one year (January- December 2011)

Results: Cumulative results for 25OHD testing were retrieved for 10372 outpatients. A high prevalence of vitamin D insufficiency was found, considering Vitamin D < 20 ng/ml (14 % 0-10 years, 24% 11-20 years, and in groups C,D,E,F,G 20-31% , with a frequency of 40% in patients aged > 81 years. When the cut-off for normal range was 30 ng/ml, the frequencies were (58 % 0-10 years, 69% 11-20 years, and in groups C,D,E,F,G,H 73-79%. No significant differences between females and males were observed for 25OHD values as well as a similar prevalence of mild and moderate 25OHD deficiency. The percentage of subjects displaying mild and moderate 25OHD deficiencies was lower in young children and severe vitamin D insufficiency was found in older subjects (>81 years) in comparison to that observed in the adult population.

Conclusions: The results of this large epidemiological investigation attest that the prevalence of mild and moderate vitamin D deficiency is very prevalent in our region despite solar exposure in our tropical country.

B-76

Analytical performance of the automated ROCHE Vitamin D immunoassay on COBAS 6000.

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Background. Vitamin D plays a pivotal role in the bone metabolism, and hypovitaminosis D has been associated with falls and low bone mineral density. The assessment of serum vitamin D is receiving growing interest as risk factor for several disease, leading to an increased demand for rapid and easy measurements. The introduction of simple automated assays is hence advisable, and several automated methods are now becoming commercially available, although most of these are not suited for the majority of clinical laboratories.

Aim. The aim of this study was to assess the analytical performance of the new ROCHE Vitamin D on COBAS 6000 and compare it with the automated commercially available Vitamin D assays on Liaison and the reference HPLC method.

Methods. Two serum pools were selected for imprecision studies (n=15 for intra-assay and n=10 for inter-assay). Three paired serum aliquots were also assayed simultaneously with HPLC as well as with Roche Vitamin D on COBAS 6000 and DiaSorin Vitamin D on Liaison.

Results. The intra- and inter-assay coefficients of variations of ROCHE Vitamin D at low (12.4 ng/mL) and high (47.6 ng/mL) concentrations were comprised between 6.4-9.0%, and 2.1-4.1%, respectively. The assay was proven linear in a range of concentrations comprised between 7.7 and 59.3 ng/mL, as confirmed in linear regression analysis ($y = 0.99x + 2.6$; r=0.99; p<0.001). Results of serum samples (n=83) compared with HPLC and DiaSorin Vitamin D on Liaison showed median values (2.5-97.5 percentile) of 33.1 ng/mL (14.4-58.6 ng/mL) for HPLC, 32.4 ng/mL (11.2-61.8 ng/mL) on Roche COBAS 6000, and 28.1 ng/mL (6.6-54.3 ng/mL) on Diasorin Liaison. The comparison with the reference HPLC method was r=0.77 (95%CI, 0.67-0.85; p<0.01), bias = -0.84 (95%CI, -2.84 to 1.17), whereas that with the well established Liaison immunoassay was r=0.67 (95%CI, 0.53-0.77; p<0.001), bias = 5.55 (95% CI, 3.10 to 8.00).

Conclusion. We thereby conclude that the analytical performance and the technical features of new ROCHE Vitamin D assay would make it a suitable assay for the rapid quantification of Vitamin D on the COBAS 6000 analyzer, with acceptable performance according to the reference HPLC assay. The use of a single specimen for measuring vitamin D and other bone metabolism markers (i.e. beta-CrossLaps, beta-CTX and parathyroid hormone) simultaneously on Roche COBAS 6000 is an additional advantage.

B-77

Studies on BMD and bone turnover markers: Risk factors for low bone mass in healthy young adults from North Indian.

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Background. Despite availability of adequate sunshine, Indian population has the highest prevalence of low bone mass and bone mineral content. Consequently number of osteoporotic patients in India are rising and estimated to reach about 26 million (2003 estimates) a figure likely to shoot up to 36 million by 2013. Risk factors for osteoporosis have been extensively studied in the west but poorly investigated in India. We studied BMD and bone turnover markers (BTMs) in this group. The aim of this study was to determine the predictors of low BMD and the relationship among bone morphometry, biochemical bone turnover markers (BTMs) and Bone mineral density in young healthy adults aged between 20-35 years.

Methods. Fifty one (28 Males, 23 Females) healthy subjects in the age group of 20-35 were studied. Morphometric, biochemical parameters and BMD (whole body, spine, hip & wrist) were recorded. Anthropometric measurements included height, weight, BMI and waist/hip ratio (WHR). BTMs studied included - serum bone-specific alkaline phosphatase (sBAP), serum collagen cross-linked C-terminal telopeptide (sCTx), serum Osteocalcin and Parathyroid hormone (PTH) using standard ELISA kits.

Results. Out of 51 healthy volunteers, only 11 (21.57%) had normal BMD (7 males, 4 females), Six (11.77%) were frankly osteoporotic (3 males, 3 females), while 34 (66.67%) were osteopenic (18 males, 16 females). Age, weight and BMI were the best predictors of total BMD and BMC at all sites. Serum Crosslaps positively correlated with total bone area(TBA), BMD at Hip and Forearm. Using multiple regressions - age, weight, and BMI were significant predictors of BMD in young adults. Percentage body fat had inverse correlation with BMC, BMD and TBA. Weight and height positively correlated with BMD at femoral neck, inter-trochanter and Ward's triangle. Body weight was best predictor of BMD at femoral neck, Ward's triangle, forearm UD, forearm MID and forearm1/3.

Conclusions: Age, body weight and percentage body fat are important determinants of bone mineral density in younger adults. Within the age group of 20-35 years, only 21% of the population has normal BMD while a majority remains Osteopenic. Among urban Indians BMD starts declining around the age of 30 yrs which is quite early as compared to their western counterparts. Larger studies are necessary to establish or refute these preliminary observations so that the results can then be fruitfully utilized towards development of more effective public health strategies for preventing osteoporosis. Though markers of bone turnover have been advocated to serve as predictors of bone loss in post-menopausal women but extrapolation of this information to predict bone loss in younger population remains largely unknown and elusive.

B-79

A Rapid and Sensitive Method for Measurement of Testosterone in Human Serum by LC-Tandem/Mass Sectrometry.

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Background: Concern has been expressed in recent years regarding the accuracy and specificity of Testosterone (T) measurements by immunoassay in women and children and in men at concentrations below the reference range. In some instances it has been a challenge generating sufficient sensitivity even by more expensive and technically sophisticated equipment such as LC-tandem mass spectrometry (LC/MS/MS). We report a method for the measurement of Testosterone in human serum using 200 µL of serum after protein precipitation and derivatization with O-benzylhydroxylamine capable of reporting T down to a concentration of 2 ng/dL.

Methods: 200 µL of patient serum is combined with 50 µL of D5 Testosterone internal standard (IS) in methanol, 100 µL of 5 % aqueous zinc sulfate and 400 µL of acetonitrile in a 1.5 mL polypropylene microcentrifuge tube. After mix and centrifugation 500 µL of supernatant is added to 50 mcL of 0.1 M O-benzylhydroxylamine in methanol in a glass tube. After incubation at 20 minutes at 56 °C. 10 µL of the solution is injected into the LC/MS/MS (Water Assoc., Acquity™ TQD). Chromatography is conducted at 50 ºC on an HSS C18 column (2.1 by 50 mm analytical column containing 1.8 micron spherical particles, Waters Assoc. Milford MA). Detection utilizes a mass transition of 394.25→90.05 for T and 399.42→91.03 for ^{5}D Internal Standard (IS) after isocratic elution with 75 % methanol/0.1%formic acid using ESI+ mode.

Results: Retention time of T and IS was 3.37 min. No interferences were noted from other androgens and adrenal steroids. No ion suppression was noted in the chromatographic region of interest. Functional sensitivity defined as the lowest concentration demonstrating a CV of 25% was 2 ng/dL. Spiked bovine serum albumin achieved precision of 8.5 % and 3.6 % at concentrations of 15 and 40 ng/dL over 20 days. The method was linear to 2000 ng/dL. We also evaluated instrument response for hydroxyl-, methyl-, ethyl- and benzyloxime derivatives and found the benzyloxime derivative provided approximately a twenty-fold greater response over underivatized T. Results from 120 patient samples were compared to results from a national reference lab and yielded Deming regression statistics as follows: Slope 1.001, Y-Intercept=2.96, SEE=8.54, r=0.9966.

Conclusions: The use of the benzyloxime derivative permits a simple methodology involving a small serum sample, protein precipitation and isocratic chromatography over a short time period. The low limit of detection permits applicability to children and female populations with very low T levels and the potential for simple scale-up in the event of a higher demand.

B-80

Analytical performance evaluation of a novel assay for vitamin D determination by electrochemiluminescence compared with liquid chromatography-tandem mass spectrometry.

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Background: The role of the vitamin D in regulating circulating levels of calcium and phosphorus to ensure normal bone mineralization is well known. Emerging evidence correlates insufficient levels of vitamin D to an increased risk of developing non-

skeletal pathologies: cardiovascular disease, hypertension, cancer, diabetes, multiple sclerosis, rheumatoid arthritis and infectious diseases. It is therefore important to diagnose vitamin D deficiency monitor and supplementation. There are a variety of methods for measuring vitamin D but suffer from a great variability and lack of standardization between them. It has been estimated that one billion people worldwide do not reach the minimum optimal concentration of 30 ng/mL. Objectives: Evaluating the validity of the new reagent for measurement of vitamin D, developed by Roche Diagnostics ®.

Methods: The accuracy of the method was evaluated following the recommendations of CLSI (EP15-A2 protocol) using two pools of serum. For the comparison study were processed same time, 55 serum samples covering the range of measurement by Roche's method and liquid chromatography-tandem mass spectrometry (LC-MS/MS-selected here as the nominal gold standard). Statistical analysis was performed using linear regression Deming, and the graphical representation of the differences according to Bland-Altman.

Results: The total CV% was 2.9% at a concentration of 57,5 nmol/L and 8,3% to 94,5 nmol/L. Correlation of patient specimen results between the Roche's method and LC-MS/MS yielded a Deming slope of 1,021 (CI 95% [0,9183 to 1,123]), and an Y-intercept of -0,1380 (CI 95% [-6,585 to 6,309]).The mean bias, as determined by Bland-Altman analysis, was -1,01895 (CI 95% [-25,8104 to 23,7725]).

Conclusions: The precision is excellent both low and high concentrations. We didn't found systematic differences, ie bias, with LC-MS/MS and electrochemiluminescence. Therefore we can say that the results are interchangeable between them. The electrochemiluminescence method has the advantage that has a high performance power integrated into a general purpose analytical platform avoiding the transfer of samples.

B-81

Analytical Verification of the IDS-iSYS Intact Amino-terminal Propeptide of Type I Procollagen (P1NP) Automated Immunoassay

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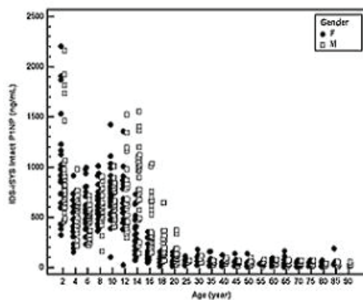
Background: P1NP circulates as different forms: larger intact trimeric P1NP and several fragment monomers. In healthy individual, the circulating P1NP form is predominantly the trimeric intact with almost non-detectable monomers. Under certain conditions, especially in renal impaired and bedridden geriatric patients, the proportion of monomeric form is elevated. The IOF and IFCC recommended s-P1NP and s-CTX are used as reference Bone Turnover Markers (BTMs) in observational and intervention studies. We seek to validate the analytical performance and establish reference intervals for apparently healthy children, pre-menopausal, post-menopausal women, and men for the IDS-iSYS Intact P1NP assay.

Methods: The accuracy profile was determined with 8 serum pool levels (9.4 - 217.2 ng/mL). The method linearity was verified with two sets of high/low serum samples. Serum specimens from overnight fasting, apparently healthy Caucasian subjects presenting normal Calcium, Phosphate, Intact PTH levels and eGFR>60 ml/min/1.73 m², were selected to generate the preliminary reference interval: 132 males, 102 pre-menopausal, 124 post-menopausal, children (0.3 - 20 years, 281 males, 295 males).

Results: The accuracy profile showed the risk that one result falls out of the ±15% acceptance limit was <5%. The obtained linearity equation was: Y = 1.00(x)-0.0004, r^2= 0.996. The 95% reference intervals (ng/mL) were: 12.7-81.5 (males), 11.2-104.0 (pre-menopausal) and 10.0-83.8 (post-menopausal). Similar to other BTMs, Intact P1NP concentration changes with age and menopause; the levels are much higher in children than in adults (Figure below).

Conclusions: IDS-iSYS Intact P1NP assay offers a reliable automated method for clinical laboratories. The pre-menopausal, post-menopausal, and males reference intervals will be useful tool for the monitoring of osteoporosis therapeutic treatment. Values from more than 500 children classified according to age and gender can be helpful to clinical laboratories and paediatricians. Further data are needed to fully assess the distribution of Intact P1NP in children, especially between 8 - 14 years of age.



B-82

Comparison of Five Automated Immunoassay reagent Kit for 25-OH Vitamin D Analysis

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Background: The use of Vitamin D in various research studies is increasing worldwide. Because of the high workload, there is a need for the laboratory to use fully automated analysers with high throughput for the measurement of 25-OH Vitamin D. Currently there were several diagnostic manufacturers have launched automated 25-OH Vitamin D immunoassays including Abbott, Siemens, Roche and DiaSorin. All the four assays are measuring total D2 and D3 25-OH Vitamin D. Our aim is to compare the performance of the four different immunoassays. We also compared the results with the Roche old reagent kit that measure only 25-OH Vitamin D3.

Methods: Imprecision studies for Vitamin D were performed on Roche Cobas E411, Abbott Architect Ci4100, Siemens Centaur XP and DiaSorin Liason by measurement of 3 replicates of 2 QC materials on 5 consecutive days (CLSI EP15-A2 protocol). All the immunoassays are measuring total Vitamin D (D2 + D3). The comparison study for all the 25-OH Vitamin D immunoassays were performed by using 59 samples collected form postnatal women (6 weeks post delivery); all assays are run simultaneously except for the Roche Vitamin D3 kit.

Results: The within run imprecision for the assays ranged from 2.8% to 7.9% and the total imprecision ranged from 2.8% to 22.8%. Imprecision for Architect Ci4100 is the lowest that is 2.8% for within run imprecision and 2.8% for total imprecision (mean= 19.8 ng/mL). The total imprecision CV for Roche Total Vitamin D is very high (22.8%). Comparison study showed that the Vitamin D results performed by using Roche Vitamin D3 kit is significantly lower (mean=10.23±5.65 ng/mL) compared to Roche Total Vitamin D kit (mean=19.49 ±7.46 ng/mL), Abbott Architect (23.29±7.84 ng/mL), Centaur XP (18.36 ± 6.03 ng/mL) and DiaSorin (22.45±7.58 ng/mL). ANOVA analysis showed that the mean for Roche Total Vitamin D is not significantly different from Centaur XP but both mean are significantly lower compared with the mean for Architect and Diasorin.

Conclusion: The performance for the available Vitamin D assays is variable and the laboratory need to be aware of the limitation of the various assays. The limitation of this study is not able to compare the Vitamin D results with the LCMS/MS for the method comparison.

B-83

Comparison of Parathyroid Hormone Assays in Chronic Kidney Disease Patients

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Background: Mineral and bone disorders are common in chronic kidney disease (CKD). Significant effort is put into maintaining circulating calcium, phosphate and parathyroid hormone (PTH) levels within target levels to lower the risk of progression of CKD. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines recommend PTH target ranges as two to nine times for dialysis patients. Such recommendations place a major responsibility on clinical laboratories to ensure that results obtained using different PTH immunoassays are comparable and clinical decision limits are appropriate. The aim of this study is to compare six different commercial PTH assays including one bio-intact PTH(1-84) and determine the degree of divergence between the assays.

Methods: PTH was measured in serum from CKD patients in six commercially available PTH assays: Siemens Centaur iPTH, Siemens Immulite iPTH, Abbott

Architect iPTH, OCD Vitros iPTH, Beckman Access iPTH and Roche Cobas bio-intact PTH(1-84) assay. PTH was initially measured using the Siemens Centaur iPTH assay and excess serum was aliquoted into five different aliquots and frozen at -20°C within 6 hours. Each aliquot was thawed only once within 6 months for measurement of PTH. Between-run imprecision was determined by measurement of PTH over ten days at three levels of pooled serum. Within-run imprecision was determined by measurement of PTH in quadruplicates at three levels of pooled serum. Correlation was obtained for 65 serum samples.

Results: Total imprecision was <5% for the Abbott Architect, OCD Vitros and Beckman Access iPTH assays at low, medium and high levels. Total imprecision was <10% for the Siemens Centaur iPTH assay, the Siemens Immulite iPTH assay and the Roche Cobas PTH(1-84) assay. Although all the PTH assays, including the Roche Cobas bio-intact PTH(1-84) assay, showed high correlation, the concentrations of each sample differed from one assay to another by up to 3-fold. The Roche Cobas bio-intact PTH(1-84) assay measured PTH levels at 45% of the Abbott Architect, Siemens Centaur and Siemens Immulite iPTH assays. However, the bio-intact PTH(1-84) levels were 65% and 70% that of the iPTH levels for the Beckman Access and OCD Vitros iPTH assays respectively.

Conclusions: Although all the PTH assays showed high correlation in CKD patients, there are significant between-method differences in PTH concentrations. The third generation Roche Cobas bio-intact PTH(1-84) assay showed good correlation with second generation iPTH assays.

B-84

Aldosterone Automated Chemiluminescent Measurement in Plasma and Urine

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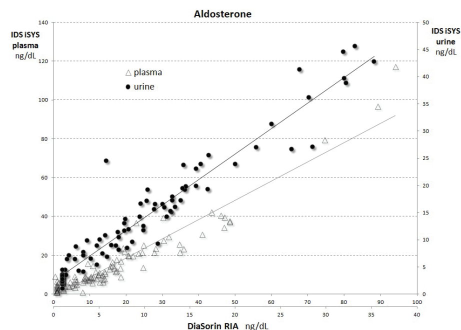
Background: Aldosterone plays an important role in electrolyte balance and blood volume-pressure regulation. Its level is very useful in hypertension as part of the diagnostic protocol to highlight hyper or hypo secretion. Measurement of Aldosterone in urine is needed as a 24 hour collection gives the whole estimation of Aldosterone secretion over the entire day. In this study we evaluated the analytical performance of a new chemiluminescence immunoassay.

Methods: Aldosterone was measured in 88 urine remnant samples and 95 plasma EDTA samples from subjects referred to our laboratory (48.4 ± 18.3 years of age, 93 females, 90 males). Determinations were run with the ALDOCTK DiaSorin RIA assay (Stillwater, US) and the IDS-iSYS Assay (Boldon, UK) on the automated iSYS platform. Aldosterone concentrations ranged from 1.3 to 40.5 ng/dL and from 12.6 to 94.1 ng/dL for urine and plasma respectively. Reproducibility was evaluated according to CLSI EP5-A2 in urine samples with Aldosterone concentrations ranging from 14.1 to 97.7 ng/dL.

Results: the Passing-Bablok regression of iSYS against RIA shows a slope of 1.20 (95%CI 1.11-1.28) and intercept 2.1 (1.08-3.19) for urine and a slope of 0.84 (0.76-0.92) and intercept 3.78 (-6.28-13.57) for plasma samples. Pearson correlation coefficient was 0.951 (P <0.0001) and 0.953 (P <0.0001) for urine and plasma respectively. The obtained Bland-Altman %Mean (± 1.96 SD range) was -39.7 (-99.8 to 20.3) for urine and 5.8 (-77.9 to 89.5) for plasma. CV values from 5.5 to 12.7% within run and from 2.8 and 8.4% between run were observed in the reproducibility evaluation.

Conclusions: the positive correlation of automated IDS with RIA, the good reproducibility of results, the reduced turnaround time and hands on labour gives a significant improvement in the use of Aldosterone measurement in hypertension diagnosis.



B-85

Performance of the IDS-iSYS Intact PTH Automated Immunoassay

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Background: Numerous studies had demonstrated the lack of comparability among PTH assays. In addition, many noted that the differences observed may potentially influence clinical decision making and proved to be problematic for subsequent diagnosis and treatment. This variability might be influenced by a variety of conditions such as the assay used, the population evaluated, vitamin D status, and numerous pre-analytical conditions. In the present study, we set out to evaluate performance characteristics of the newly available IDS-iSYS Intact PTH assay including sensitivity, imprecision, comparison of methods and reference intervals.

Methods: The precision profile and sensitivity were determined with 11 serum pool levels. Serum samples from 218 dialysis (52 peritoneal, 156 hemodialysis) and 169 normal subjects were measured with the IDS-iSYS, ADVIA Centaur and Liaison for method comparison. The Caucasians reference intervals were analyzed according to the CLSI C28-A3 with following inclusion criteria: overnight fasting, apparently healthy, sufficient vitamin D, normal Calcium, Phosphates, and eGFR >60 mL/min/1.73 m^2.

Results: We obtained the following results for the IDS -iSYS Intact PTH method: LoQ <5 pg/mL. The precision profile between 20.3 - 2120 pg/mL was ranging from 0.7 - 8.3%. Table below summarized the Passing Bablok regression against the IDS-iSYS, Pearson correlation coefficient, and 95% reference interval for each indicated Intact PTH test method.

Conclusions: The IDS-iSYS Intact PTH demonstrated suitable performances as a fully automated parathyroid hormone method for clinical laboratories. Although good correlation coefficient were obtained between the IDS-iSYS, Centaur and Liaison Intact PTH assays, this study confirmed the proportional differences among Intact PTH methods. The 95/646 IS has been recommended as the universal standard for parathyroid hormone test, it might not solve the concentration levels variation among intact PTH methods. The variable recognition of PTH fragments such as PTH (7-84) by the assays also contributed to the difference.

Intact PTH Method	Method Comparison (pg/mL)			Reference Interval (pg/mL)	
	Observed Range (pg/mL)	Passing Bablok regression [slope x (iSYS) + intercept]	Pearson Correlation coeff.	Vicenza Range	Manufacturer Range
Centaur	5.8 - 1338	1.37 x (iSYS) + 5.86	0.995 (P <0.0001)	10.9 - 71.0	11.1 - 79.5
IDS-iSYS	6.3 - 1102	-	-	11.5 - 50.3	11.5 - 78.4
Liaison	9.3 - 1001	1.15 x (iSYS) + 9.40	0.934 (P<0.0001)	18.0 - 82.0	17.3 - 72.9

B-86

Evaluation of IGF-I levels in liver cirrhosis: correlation with liver function and prognosis.

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Background: Insulin-like growth factor I (IGF-I) is a polypeptide hormone secreted by multiple tissues in response to growth hormone (GH). Ninety percent of circulating IGF-I originates in the liver and has autocrine, paracrine and endocrine effects, the latter on multiple tissues. It is partly responsible for GH activity, and also has anabolizing effects. There are reports of reduction in their levels in liver cirrhosis. Its value as a prognostic indicator in patients with cirrhosis has not been established.

Objective: To evaluate IGF-I levels in patients with liver cirrhosis, its correlation with liver function and clinical outcome.

Methods: The study included 160 liver cirrhosis patients divided into 3 groups according to severity of cirrhosis (Child-Pugh score): 53 Child-Pugh A, 52 with Child-Pugh B and 55 Child-Pugh C. The mean age was 58 ± 12 yrs. All the patients were accompanied in hepatology division. Serum levels of IGF-I were measured by chemiluminescence method (Immulite 2000, Siemens, USA), using reference values according to age. Patients were followed for a mean period of 20 months. IGF-I levels were correlated with the Child-Pugh score and mortality was evaluated among

patients who had normal or low IGF-I levels.

Results: In cirrhotic patients Child A, B and C, the percentages of IGF-I values below normal were 56.6%, 86.6% and 89.1%, respectively (p <0.001, Child A versus B and C). Mortality among patients with normal IGF-I levels was 15.8 % and with low IGF-I levels was 16.3% in the period of observation (p= 0.92).

Conclusions: IGF-I levels were associated with degree of liver function, but not proven to be a good prognostic indicator in patients with cirrhosis to predict mortality.

B-87

Correlation Between Immunoassays and CE-marked LCMS/MS 25 Vitamin D Method

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Background: New evidences have fuelled the global demand for the 25 vitamin D blood test. Today, these demands are largely met in the clinical laboratory by automated platforms or high throughput LCMS/MS procedure. In this study, we compared the values of seven 25(OH) Vitamin D immunoassays to a CE marked LCMS/MS method.

Methods: Remnant samples from 220 subjects (50.8±20.0 years of age, 152 females, 68 males, and 60 supplemented patients) with LCMS/MS 25(OH)D levels ranging from 3.8 - 103.3 ng/mL were measured in three 25(OH)D

Methods: IDS-iSYS, Architect, and Perkin Elmer LCMS/MS. Subsets of these samples, targeting low concentration levels, were analyzed with ADVIA Centaur, Liaison Total, Liaison XL and Elecsys. The Liaison and Liaison XL tests were the improved assays; the Architect and ADVIA Centaur methods were the adjusted standardization test kits.

Results: The summary table below documented the Passing Bablok regression and Pearson correlation coefficient of the immunoassay versus the LCMS/MS. The obtained Bland-Altman %Mean (± 1.96 SD range) of each immunoassay against LCMS/MS were: -33.2% (7.7 to -74.1) for Architect, -8.2% (39.5 to -55.9) for Centaur, -17.6% (38.6 to 73.7) for Diasorin RIA, -1.4% (31.1 to -31.4) for IDS-iSYS, -27.9% (9.4 to -65.3) for Liaison, -16.7% (22.6 to -56.1) for Liaison XL, and -4.6% (49.9 to -59.1) for Elecsys.

Conclusions: In this assessment, the automated IDS-iSYS 25-Hydroxy Vitamin D performance deemed to be the most comparable method to the CE-marked LCMS/MS 25(OH)D method. There were reasonable correlations but significant differences with other immunoassays. Such variability confounded attempts to define a single "cut point" value as indicating low vitamin D status. For this reason, the Endocrine Society suggested to target higher value than current cut off to ensure that the individual's "true" value is greater than 30 ng/mL.

25(OH)D Immunoassay	N	Observed Range (ng/mL)	Passing Bablok regression [slope x (LCMS) + intercept]	Pearson correlation coefficient
Architect	180	7.6 - 146.2	0.77 x (LCMS) - 1.81	0.875 (P <0.0001)
Centaur	109	7.8 - 37.8	0.70 x (LCMS) + 3.83	0.873 (P <0.0001)
Diasorin RIA	219	6.0 - 64.7	0.55 x (LCMS) + 6.44	0.843 (P <0.0001)
IDS- iSYS	219	5.7 - 110.8	0.93 x (LCMS) + 1.03	0.949 (P <0.0001)
Liaison	104	5.0 - 38.0	0.80 x (LCMS) - 0.82	0.894 (P <0.0001)
Liaison XL	109	5.3 - 39.5	0.87 x (LCMS) - 0.38	0.887 (P <0.0001)
Elecsys	149	5.3 - 62.4	0.86 x (LCMS) + 2.34	0.867 (P <0.0001)

B-88

Correlation between insulin-resistance and nodular thyroid disease in class III obesity patients.

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Background: Over recent years various studies have focused on the association between insulin resistance and thyroid abnormalities. It has been suggested that subjects with hyperinsulinemia may present greater thyroid size and prevalence of nodules.

Objective: The aim of this study was to evaluate the correlation between insulin resistance and nodular thyroid disease in class III obesity patients.

Methods: A cross-sectional study was conducted. Both experimental group subjects and a control group were submitted to thyroid ultrasound and laboratorial analysis for the determination of serum TSH, Free T4, anti-TPO, glucose and insulin for HOMA-IR. The experimental group was composed of patients who were followed in the bariatric surgery program, before the surgical procedure, all with class III obesity (BMI > 40 kg/m²). The control group was selected among healthy volunteers with similar demographic characteristics regarding age and sex, albeit normal-weight patients (BMI <25 kg/m²). The exclusion criteria were the same for both groups: diagnosis of diabetes mellitus, use of drugs which could interfere with insulin sensitivity and known thyroid diseases. All the ultrasound exams were performed by the same radiologist, using a high-frequency Siemens- Acuson X300 multi-frequency transducer (12MHz). Focal lesions were only considered as present when diameter > 3.0 mm. Serum TSH, FT4 and TPO-Ab were measured by chemiluminescent method (Immulite 2000, Siemens, USA). Serum insulin was measured by ECLIA (Modular E, Roche, German, reference range: 2.0 - 23.0 mU/L). The HOMA-IR was calculated through the formula: [Fasting blood glucose (mmol/L) x Fasting insulin (mU/L)]/ 22.5. The statistical analysis was performed with the software SPSS version 13.0 for Windows.

Results: 21 patients and 40 controls, 36.7± 8.8, 35.9± 9.1 years, respectively, were included. Mean BMI was 50.9 ± 7.2 kg/m² (patients) and 22.5 ± 2.3 kg/m² (controls). Mean TSH and FT4 were 2.9 mUI/L and 1.1 ng/dL (patients) and 2.3 mUI/L and 1.0 ng/dL (controls). Means of HOMA-IR were 5.6 ± 3.9 and 1.4 ± 1.1 (p<0.001). Normal ultrasound was found in 47.6% of patients and in 75% of controls, p = 0.30. Nodules were found in 35% and 20%, respectively; p = 0.171. Among the subjects who had nodules, there was no difference in the predominance of nodules bigger or equal to 1.0 cm for fine-needle aspiration biopsy (FNAB). The nodules were on average bigger in the experimental group, despite the fact that such difference was not statistically significant (0.9 ± 0.5 in the control group and 1.4 ± 1.4 in the experimental group). The HOMA-IR values did not differ between subjects requiring or not requiring FNAB (3.13 ± 3.3 vs 2.6 ± 3.0), p=0.70.

Conclusions: In this study, we did not find a significant higher prevalence of nodular disease in class III obesity patients, with no correlation between insulin resistance and nodular thyroid disease in these patients.

B-89

Correlation between insulin resistance, lipid profile and nocturnal blood pressure in women with hyperandrogenism.

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Background: Blood pressure (BP) varies according to the circadian cycle, showing a physiological decline during sleep (dipping pattern). Several studies are showing that the absence of the nocturnal fall of BP (non-dipping pattern) can be linked to worse cardiovascular prognosis and increased insulin resistance.

Objective: To evaluate the metabolic parameters and insulin resistance in women with hyperandrogenism, correlating with the nocturnal fall of blood pressure.

Methods: We assessed 38 women, 18 to 45 years old, with clinical and/or laboratorial hyperandrogenism. As exclusion criteria, we adopted: kidney disease, chronic obstructive pulmonary disease, liver failure, congestive heart disease, type 1 diabetes mellitus, alcoholism, Cushing´s syndrome, thyroid dysfunction, pregnancy, previous history of hypertension and chronic use of corticosteroids. The following parameters were evaluated: presence (dipping pattern) or absence (non-dipping pattern) of nocturnal blood pressure decrease during ambulatory monitoring of blood pressure (AMBP), lipid profile (total Cholesterol, HDL cholesterol and triglycerides assessed by automated enzymatic method, Roche, German; LDL cholesterol calculated by Friedewald formula), fasting insulin and blood glucose (ECLIA and enzymatic method, respectively, Roche, German), HOMA-IR [Fasting blood glucose (mmol/L) x Fasting insulin (mU/L)] / 22.5. The dipping pattern was characterized as the fall of > 10% of the systolic BP during sleep.

Results: The results are presented in the table 1 (mean ± SD).

Conclusions: We found no statistically significant difference in any of the parameters evaluated among patients with or without nocturnal fall of blood pressure.

Table1: Correlation between metabolic parameters and dipping / non dipping patterns			
	Dipping pattern	Non dipping pattern	P value
Age	28.91 ± 5.48	27.40 ± 5.23	0.40
BMI	28.69 ± 7.87	29.82 ± 8.37	0.67
Glucose	89.00 ± 12.81	89.00 ± 22.65	1.00
Insulin	11.58 ± 7.90	10.66 ± 7.43	0.74
Homa-IR	2.45 ± 2.02	2.42 ± 2.99	0.97
Total Cholesterol	188.17 ± 46.16	170.28 ± 53.05	0.29
LDL Cholesterol	95.39 ± 31.59	93.00 ± 45.14	0.85
HDL Cholesterol	53.49 ± 17.86	48.27 ± 10.92	0.32
Triglycerides	111.55 ± 59.44	109.60 ± 75.39	0.93

B-90

Analytical Performance of the Vitamin D Advia Centaur XP Assay

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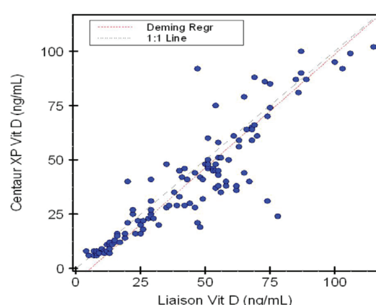
Background: Vitamin D has been known for some time to play a role in proper bone development and maintenance. Recent studies have also attempted to elucidate roles in aging, neurodegenerative disease, cardiovascular disease, infection, cancer, reactive airway disease, demyelinating processes and disease of hair. While many of these associations remain controversial, what does not is the need to accurately measure vitamin D serum concentration. Recently, Siemens Healthcare Diagnostics received FDA approval for their vitamin D ADVIA Centaur XP assay. Because using that assay would minimize specimen handling and work-flow, we investigated the analytical performance of the Advia assay and compare it to our current method, the Diasorin Liason assay.

Methods: We investigated linearity/reportable range, simple precision, analytical sensitivity, and accuracy. Samples used in this validation were commercially available quality controls or calibrators and de-identified human samples. For accuracy, we used 124 serum specimens spanning the calibrated range. These specimens were collected and froze at -30C, then, thaw once at the time of testing, and vitamin D concentration was measured using the Advia and the Liaison assays. Data was analyzed using Microsoft Excel or EP Evaluator.

Results: The assay was linear within the manufacturer's acceptable criteria in the 2.2-121 ng/mL range. Assay recovery from 18.4 to 121 ng/mL averaged 98%. Simple precision at 19.4 ng/mL and 83.1 ng/mL had a %CV of 11.8 and 3.6 respectively. The analytical sensitivity was 5.3 ng/mL. Linear regression showed that Vitamin D concentrations measured by the Advia method were very similar to the values obtained by the Liaison (Advia = 0.906 Liaison + 0.1; see figure).

Conclusions: The analytical performance of the newly approved vitamin D Advia Centaur XP assay is very good. Using this assay in our laboratory would decrease specimen handling and facilitate the work-flow.



B-91

Evaluation of Five Automated Estradiol Immunoassays

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Background: Estradiol (E2) is a steroid hormone produced primarily by the ovaries with small amounts coming from the testes and adrenal cortex. E2 measurement is used for assessing sexual development, fertility disorders, gynecomastia, estrogen-producing ovarian and testicular tumors, and hyperplasia in the adrenal cortex. E2 is also used in monitoring fertility therapy for patients undergoing in vitro fertilization. E2 immunoassay standardization has been a challenge. A study was performed to evaluate performance of five automated E2 immunoassays including comparison to a high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) E2 assay.

Methods: The automated immunoassay methods studied were ARCHITECT (Abbott Diagnostics), Centaur (Siemens Healthcare Diagnostics), DxI (Beckman Coulter), E170 (Roche Diagnostics), and IMMULITE 2000 (Siemens Healthcare Diagnostics). For the DxI the manufacturer's recently released reagent was used with a new polyclonal antibody replacement formulation. Imprecision was evaluated using control material pools (BioRad) at three levels over ten days of testing. Method comparison was performed comparing immunoassay results with an in house LC-MS/MS method using 200 patients. Sample results above or below assay measurement ranges were eliminated from analysis. Results were analyzed using Passing-Bablok regression including slope, intercept, scaled median absolute deviation (SMAD), a measure of median error, and r.

Results: Total imprecision results for ARCHITECT, Centaur, E170, and IMMULITE 2000 were <10% over concentrations of 84.7-281.6, 85.2-565.8, 96.4-435.3, and 83.6-572.6 pg/mL, respectively. DxI total imprecision was <13.4% over concentrations of 115.5-521.3 pg/mL. Method comparison results are summarized in Table 1.

Conclusions: All immunoassays, except DxI, showed excellent imprecision with CV's less than 10%. All Immunoassays had good overall correlation to LC-MS/MS. Three methods (ARCHITECT, DxI, and E170) showed calibration differences in terms of slope with the LC-MS/MS method. ARCHITECT and Centaur showed the least scatter in data in terms of the SMAD statistic. E2 immunoassay harmonization continues to be a challenge.

Table 1. Passing-Bablok Statistics for E2 Method Comparison to LC-MS/MS					
Immunoassay	n	Slope	Intercept	SMAD	r
ARCHITECT	188	0.892	8.98	9.79	0.983
Centaur	180	0.990	6.53	10.16	0.978
DxI	197	1.143	14.49	17.85	0.980
E170	197	1.196	1.54	12.76	0.992
IMMULITE 2000	163	0.995	4.59	17.25	0.966

B-93

Multicenter Evaluation of ARCHITECT 25-OH Vitamin D Compared to LC-MS/MS and DiaSorin Liaison

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Objective: The aim of this study was to evaluate the precision and accuracy of the ARCHITECT 25-OH Vitamin D assay and perform a method comparison with LC-MS/MS and DiaSorin Liaison.

Methods: The evaluation was performed at 10 sites throughout the US. Sites tested between 120 and 299 routine samples on their in-house method and ARCHITECT 25-OH Vitamin D. Three sites performed comparison to LC-MS/MS, six sites performed comparison to DiaSorin, and one site compared to both. Seven sites performed a 5-day precision study with ARCHITECT 25-OH Vitamin D. Nine sites evaluated a DEQAS panel for accuracy. Slope, correlation and intercept were calculated from the method comparison, and percent recovery was calculated from the LC-MS/MS data.

Results: Repeatability ranged from 2.4 - 11.6 %CV at 20 ng/mL, 1.9 - 3.1 %CV at 40 ng/mL, 1.4 - 3.2 %CV at 75 ng/mL. There were 1920 samples tested at 10 sites. The median of the study population was 31.3 ng/mL, and the central 50% was between 23.4 - 41.8 ng/mL. The slopes of ARCHITECT 25-OH Vitamin D compared to LC-MS/MS ranged from 0.91 to 1.12 and correlation coefficients ranged from

0.67 to 0.86. The slopes of ARCHITECT 25-OH Vitamin D to DiaSorin Liaison ranged from 0.89 to 1.20 and the correlation coefficients ranged from 0.81 to 0.93. DEQAS samples 381 - 385 were tested on all three platforms and the mean recovery was calculated compared to the mean LC-MS/MS value reported by DEQAS in the October 2010 report. The mean recoveries of the 5 DEQAS samples ranged from 89.6 - 101.1% on ARCHITECT, 85.1 - 109.3% on DiaSorin Liaison, and 74.0 - 88.0% on LC-MS/MS. The recovery of ARCHITECT 25-OH Vitamin D was also calculated compared to the LC-MS/MS results for the four laboratories that ran LC-MS/MS. Overall mean recovery of all 832 samples tested on both ARCHITECT and LC-MS/MS was 116.8%. Overall mean recovery of 565 samples that contained only vitamin D3, as defined by LC-MS/MS was 111.6%. Overall mean recovery of 61 samples that contained vitamin D2 over 4 ng/mL and had a D2/D3 ratio of 1.0 or greater was 75.2%. The mean recovery of just the vitamin D2 in those samples [recovery = (ARCH value - LC-MS/MS D3 value)/(LC-MS/MS D2 value) x 100] was 65.5%. The mean vitamin D2 recovery for 5 samples that contained a D2/D3 ratio of greater than 10 ng/mL, and a vitamin D3 concentration of less than 5 ng/mL was 64.9% (range = 44.3% to 94.7%).

Conclusions: The ARCHITECT 25-OH Vitamin D assay exhibited good repeatability at 20 ng/mL and excellent repeatability at 40 and 75 ng/mL. The assay recovered the DEQAS samples within 10% of the reported LC-MS/MS method mean and exhibited acceptable comparison to both LC-MS/MS and DiaSorin Liaison. The overall recovery of vitamin D compared to the laboratories respective LC-MS/MS systems was as expected.

B-94

Evaluation of the Cross-reactivity of 25-Hydroxy-vitamin D2 on Six Commercial Immunoassays on Native Samples.

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Background: In serum, 25-hydroxy-vitamin D (25(OH)D) can be found in two forms, namely 25(OH)D2 and 25(OH)D3. We recently published a mathematical method to estimate the 25(OH)D2 recovery without spiking the samples. Since then, new “total” vitamin D immunoassays have appeared on the market (Roche “Total” vitamin D, Siemens Centaur vitamin D “total”, “total” vitamin D on DiaSorin Liaison XL, Abbott Architect Vitamin D). We aimed to study the 25(OH)D2 recovery of these new immunoassays and re-evaluate the cross-reactivity of previously studied assays (IDS iSYS Vitamin D and DiaSorin RIA).

Methods: We only used native samples. First, in a group of 19 individuals presenting 25(OH)D3 levels exclusively and ranging from 6 to 90 ng/mL, approximately, we defined the regression equation of each immunoassay method in comparison with the Perkin-Elmer LCMS/MS method run on a AbSciex TQ 5500 instrument. Then, we established the respective concentration of 25(OH)D3 and 25(OH)D2 in the serum of 11 individuals supplemented with various levels of vitamin D2. For each method, with the help of the regression equation previously obtained, we calculated what would be the “immunoassay-equivalent” of 25(OH)D3 based on the LCMS/MS 25(OH)D3 value. We thus subtracted this amount to the “total” value obtained with the respective immunoassay to obtain the 25(OH)D2 “immunoassay-equivalent” concentration. Finally, we divided this amount by the 25(OH)D2 obtained with the LCMS/MS to calculate the recovery.

Results: See Table.

Conclusions: As we previously shown, IDS Isys and Diasorin RIA, present a 100% cross-reactivity with 25(OH)D2. Among the new “total” immunoassay, Roche Elecsys is the only one to present a 100% cross-reactivity. The DiaSorin Liaison XL presents a slight but significant positive bias whereas Centaur and Architect are respectively clearly over and underestimating 25(OH)D2 when compared with the Perkin-Elmer LCMS/MS.

Cross reactivity (95%CI) of six immunoassays for 25(OH)D2		
Method	Regression equation obtained in Individuals with 25(OH)D3 only (PED3=25(OH)D3 obtained with the LCMSMS)	Mean cross-reactivity (%) for 25(OH)D2 (95% CI)
Architect (Abbott)	1.27 x PED3 - 6.9	61.6 (54.3 - 69.0)
iSYS (IDS)	1.02 x PED3 - 0.57	101(100.6 - 101.6)
RIA (Diasorin)	0.82 x PED3 + 3.75	98 (94.2-102)
Liaison XL (Diasorin)	0.93 x PED3 - 1.69	113 (108-118)
Elecsys (Roche)	0.80 x PED3 + 3.41	101 (97-106)
Advia Centaur (Siemens)	1.13 x PED3 - 4.81	130 (109-150)

B-95

Sclerostin and DKK-1 levels in pre-dialyisis CKD patients.

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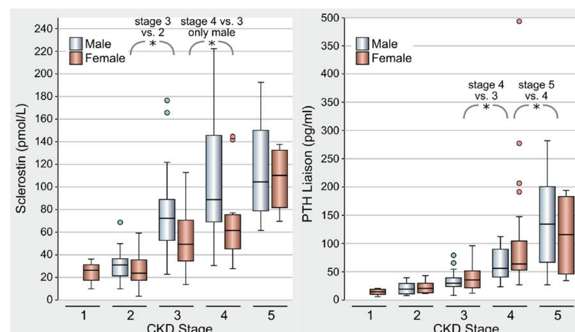
Sclerostin and Dickkopf-1 (DKK-1) are inhibitors of the canonical Wnt pathway, known to suppress bone formation. In dialysis patients, serum levels of sclerostin are elevated and correlate positively with bone mineral density and bone volume and negatively with bone turnover. Little is known about serum levels of sclerostin and DKK-1 in predialysis CKD patients.

We performed a cross-sectional observational study in 149 patients (CKD stage 1-5, 81 men, 60 ± 16 years, 22 diabetic). Serum sclerostin and DKK-1 were measured using ELISA kits (Biomedica, Austria). Other parameters of mineral metabolism included 1-84 PTH (LIAISON assay-DiaSorin, USA), calcitriol (DiaSorin), calcidiol (DiaSorin), and bone specific alkaline phosphatase (BsAP - electrophoretic method). Glomerular filtration rate was estimated using the 4-variable MDRD method.

Mean eGFR was 43 ± 27 ml/min/1.72 m^2 (range 4.1 - 139.2). Overall mean serum sclerostin and DKK-1 levels were 68 ± 45 pmol/l and 38 ± 15 pmol/l respectively. Overall, sclerostin correlated positively with 1-84PTH ($r = 0.312$, $p < 0.001$) and age ($r = 0.474$, $p < 0.001$), and negatively with calcitriol ($r = -0.393$, $p < 0.001$) and eGFR (r -0.616, $p < 0.001$). Multivariate analysis indicated older age, lower eGFR, and gender (male) to be independently associated with higher sclerostin levels. No correlation was found between sclerostin and bone alkaline phosphatase. DKK-1 levels, conversely, showed a positive correlation with eGFR ($r = 0.212$, $p < 0.01$).

Serum sclerostin levels but not DKK-1 levels increase along the progression of renal disease. The finding of positive association between PTH and sclerostin is opposite to what has been observed in other populations (dialysis, primary hyperparathyroidism, general population) and conflicts with experimental data. Whether the increasing sclerostin levels in progressive renal failure protects the bone against the deleterious consequences of high PTH levels or merely reflects decreased clearance of sclerostin remains to be clarified.



B-96

HoSt: The CDC Hormone Standardization Program

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The CDC Hormone Standardization Program (HoSt) is working toward providing accurate and reliable results through the standardization of laboratories measurements of steroid hormones regardless of the method, the measurement procedure, and the laboratory where the analyses are carried out. The need for standardization of steroid hormone measurements has been stated by the clinical and research communities. The CDC responded to this need by starting a project in 2007 to standardize steroid hormone testing and improve the use of these tests. The aim of this effort is to harmonize laboratories such that accurate and comparable measurements are obtained allowing for the improvement in diagnosis, treatment, and prevention of hormone related diseases.

In late 2009 the CDC HoSt Program started providing participants with matrix-based materials with target value assignment in the form of non-pooled sera from single donors obtained following the protocol from CLSI C37-A. Sera prepared according to this protocol have been shown to be commutable in previous studies and were recommended for use in trueness control and calibration studies allowing different methodologies of measurement to participant in the HoSt Program. A set of 40 samples, 20 male/20 female, have been made available to laboratories and manufactures for method evaluation purposes. To date the CDC has supplied 28 sets of target value assigned material to laboratories and manufactures.

In the HoSt certification program, 4 blinded challenges, with 10 samples per challenge are sent to participants over the period a year. Values obtained are used for bias assessment as described in CLSI guideline EP9-A2. At present, 20 participants are enrolled in the HoSt Testosterone Program at varying stages of certification. Participants include clinical, academic, and pharmaceutical laboratories as well as immunoassay manufactures. Currently, an overall mean bias of ±6.4%, based on biological variability, is used in the final assessment. 73% of participants have been able to meet this criterion and laboratories that are successful have been published on the CDC website (http://www.cdc.gov/labstandards/hs.html). Estradiol will be added to the HoSt certification program and executed in the same manner as testosterone with an overall mean bias of ±8.3%, based on biological variability, used for evaluation of participants.

The CDC also works with proficiency testing (PT) programs to provide target value assignment in their surveys linking these programs to the CDC reference laboratory. This allows for the monitoring of measurements in over 2,000 patient care facilities. Improvements in measurements have been observed in the small sample set of mass spectrometry (MS) based assays participating in the NY State PT program. In 2006, prior to the start of the CDC standardization program inter-laboratory variability on 5 PT samples measured by MS ranged from 2.6-19.8% over 54.7-202 ng/dL, in 2011 the inter-laboratory variability of 5 PT samples had significantly decreased with all laboratories reporting a <4.3% difference over 66.4-331 ng/dL. While this first data represents only subset of HoSt participants it shows the impact of the HoSt Program.

The CDC Hormone Standardization Program-HoSt is working toward providing accurate and reliable results for optimal patient care.

B-97

Reference ranges for thyroid function tests derived from healthy subjects with normal thyroid examination and thyroid ultrasound.

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Objective: Establish reference ranges for thyroid function tests based on samples from healthy subjects with normal thyroid examination and negative thyroid ultrasound evaluation. Relevance: TSH is an important assay because it is the primary test of thyroid function used in both disease screening and treatment monitoring. Excessively broad reference ranges run the risk of missing subclinical disease, while inappropriately narrow ranges may trigger unnecessary treatment and follow-up investigations.

Methodology: IRB consented healthy volunteers underwent medical history review and thyroid gland examination and high resolution ultrasound by experienced, board certified endocrinologists. Blood and urine samples were collected for measurement of thyroid function tests and aliquots were frozen at -80°C. Validation: Measurements of TSH, T4, T3, FT4, FT3, anti-TPO, FT4 by dialysis, urine creatinine and urine iodine were made using our routine clinical assays. In February 2012, frozen aliquots of serum were used to re-measure TSH, T4, T3, FT4, FT3, and anti-TPO using the Roche e602 analyzers and TSH was also re-measured on Beckman Coulter DxI. These newer thyroid function tests form the basis of this analysis. A cut-off of 30 IU/mL was used to define positive anti-TPO on the Roche assay. Reference ranges for TSH was based on log-Gaussian statistics and non-parametric analysis for the other tests.

Results: We recruited 99 women (age 21 to 67) and 40 men (age 26 to 66). Positive family history for thyroid disease was found in 35% of women and 18% of men. Thyroid nodules >1 cm were found in 9 women and 1 man. Positive anti-TPO antibodies were detected in 9 women and 1 man. The central 95% range for Roche TSH was 0.7 to 5.3 mIU/L for all 139 subjects. The TSH range in 129 anti-TPO negative subjects was 0.7 to 4.7 mIU/L. The 119 subjects with neither TPO antibodies nor thyroid nodules had a TSH range of 0.7 to 4.5 mIU/L, with 90% CIs of 0.58-0.86 and 3.7-5.5. None of the 20 subjects with anti-TPO antibodies or thyroid nodules had abnormal FT4. In the 84 subjects without TPO antibodies, without nodules and without family history, the TSH range was 0.8 to 3.7 mIU/L. The Beckman TSH values correlated with the Roche TSH results (correlation=0.97), but were lower with a Deming regression slope= 0.94 (excluding one discordant value). The Beckman TSH range for subjects without TPO antibodies or thyroid nodules was 0.7 to 3.6 mIU/L. The central 95% reference ranges derived for the Roche e602 analyzers for subjects without TPO antibodies or thyroid nodules (N=119) were: 5.0 to 11.7 ug/dL for T4, 81 to 200 ng/dL for T3, 0.8 to 1.4 ng/dL for FT4, and 2.4 to 4.1 pg/mL for FT3.

Conclusions: In a carefully selected group of healthy adults, TSH reference ranges remain broad. The reference ranges depend on the exclusion criteria and assays used. We recommend inclusion of the 30% of healthy individuals with a family history of thyroid disease. Using these broader reference ranges will likely reduce unnecessary testing in healthy patients, while better identifying patients with true thyroid disease.

B-98

Measurement of total thyroxine and triiodothyronine in human serum by isotope dilution liquid chromatography tandem mass spectrometry

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Background: Reliable measurements of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) are crucial for the assessment of thyroid function and diagnosis of thyroid diseases. Measurement of T3 and T4 based on current research could provide insight into the diagnosis of several diseases and disorders, including hyperthyroidism, Grave's disease, goiters, and thyroid tumors. Although existing tests to measure total T3 and T4 are widely used in research and clinical diagnosis, inaccurate test results prevent optimal patient care and public health activities. Total serum T3 and T4 measurements are routinely performed using immunoassays, which is an approach prone to specificity issues for many analytes. A sensitive and selective analytical method to measure total T3 and T4 in human serum has been developed and evaluated involving isotope dilution (ID) paired with liquid chromatography tandem mass spectrometry (LC-MS/MS).

Methods: Total endogenous T3 and T4 was simultaneously isolated from serum matrix by an automated solid phase extraction (SPE) using a strong anion exchange followed by chromatographic separation and measurement by tandem mass spectrometry. Internal standards (triiodothyronine-13C6 and thyroxine-13C12) were used for quantification. Measurements were performed using an API4000 triple quadrupole mass spectrometer coupled with a TurboV electrospray ionization source and a Shimadzu HPLC system. The HPLC separation was performed on a C18 reverse phase column with a gradient of water and acetonitrile in 0.1% formic acid. Quantification by selective reaction monitoring (SRM) analysis was performed in the positive ion mode. Two transitions were monitored for each analyte and IS.

Results: This method showed great stability when analytes were protected from light, with minimal conversion of T4 to T3 (<2.0%). With chromatography, T3 and reverse T3 were adequately resolved and 2 transitions were monitored to assure additional compounds did not interfere with value assignments. A linear response was obtained within the clinically relevant calibration range of 25-300 ng/dL for T3 and 2.5-20 µg/dL for T4. Method performance parameter evaluation for T3 and T4, respectively, included a limit of detection of 2.0 ng/dL and 0.01 µg/dL and a limit of quantification of 8.0 ng/dL and 0.04 µg/dL. Recovery of T3 by spiking at 3 levels (50, 150, 300 ng/dL) were 94-104% and for T4 at 3 levels (5.0, 10, 20 µg/dL) 98-100%. Within-day precision was between 1.2-3.8% for T3 at levels (68, 99, and 120 ng/dL) and between 0.1-2.7% for T4 at levels (2.7, 7.1, and 8.9 µg/dL). Between-day precision was between 3.6-15.8% for T3 at the same levels and between 3.1-6.6% for T4 at the same levels. Human serum material was analyzed with this method over a range of 27.0-132 ng/dL for T3 and 2.0-89.6 µg/dL for T4.

Conclusions: A fully automated, accurate, precise, and sensitive ID-LC-MS/MS method was developed for the simultaneous measurement of total T3 and T4 in serum.

B-99

Inter-laboratory comparison study and commutability assessment of estradiol measurements

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Accurate laboratory results are imperative in Estradiol (E2) testing, especially at lower levels found in postmenopausal women. The goal of the CDC Hormone Standardization Program is to improve diagnosis, treatment, prevention and research of diseases and disorders by standardization of hormone measurements. This Inter-Laboratory Study is the first step in this process for E2 measurements.

E2 measurements from 14 laboratories (17 different platforms from clinical, commercial, and research laboratories, including mass spectrometry-based laboratory developed tests -'LDTs'-, immunoassay manufacturers and end users of immunoassays) were compared against a reference method. Participants measured 40 single donor samples from adult male and pre and postmenopausal female donors (range of 2.5-300 pg/mL) in duplicate over 3 different days. The serum material used for this study was produced following a standard protocol developed by the Clinical Laboratory and Standards Institute (CLSI Protocol C37-A). In addition the commutability of E2 standard reference materials (SRM 971) at 2 levels and of 9 commercial testing materials used in external quality assurance programs or as potential calibrator materials were evaluated.

Current suggested criteria based on biological variability for estradiol recommend an acceptable bias of $\pm$8.3% and imprecision of $\leq$11.4%. With the exception of MS6, assays showed an overall positive bias from the target values. 6 out of 17 assays had a mean bias that was at least 3 times greater than the acceptable bias. Imprecision and bias increased with decreasing E2 serum concentrations. Substantial differences in accuracy and assay performance were observed, especially at low E2 concentrations observed in postmenopausal women. The findings of this study highlight the needs for standardizing E2 measurements. The information obtained in this study is used to better define problems with E2 measurements and address them in CDC's Hormone Standardization Program.

	Bias			Imprecision	
Platform MS=Mass Spectrometry IA=Immunoassay	Overall Mean Bias	95% CI of Mean Bias		%CV Range of 40 Samples Measured in duplicate at 3 days (6 measurements)	
MS1	26.6%	18.1%	35.1%	2.9%	41.5%
MS2	12.7%	10.8%	14.6%	3.6%	17.0%
MS3	11.4%	10.1%	12.7%	2.2%	13.7%
MS4	8.3%	7.3%	9.3%	1.5%	20.7%
MS5	15.3%	10.0%	20.7%	6.9%	75.1%
MS6	-1.1%	-3.5%	1.3%	2.4%	56.8%
IA1	33.6%	24.7%	42.5%	2.6%	15.2%
IA2	30.3%	24.0%	36.5%	1.1%	22.0%
IA3	9.3%	4.4%	14.2%	2.2%	14.9%
IA4	0.7%	-2.6%	4.0%	1.9%	21.4%
IA5	15.7%	11.6%	19.8%	1.3%	57.9%
IA6	11.3%	2.1%	20.5%	2.3%	14.1%
IA7	1.2%	-6.7%	9.1%	5.1%	91.7%
IA8	73.9%	59.6%	88.2%	6.3%	87.6%
IA9	230.4%	208.6%	252.2%	4.0%	20.9%
IA10	226.7%	204.9%	248.5%	4.0%	29.8%
IA11	14.4%	12.0%	16.8%	1.2%	13.4%

B-100

Simultaneous quantitation of a comprehensive panel of adrenal steroids in serum by a novel liquid chromatography-tandem mass spectrometry method

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Background: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from deficiency of enzymes responsible for steroidogenesis. The most frequent subtype is 21-hydroxylase deficiency, accounting for more than 90% of CAH cases. Other deficiency subtypes include 11β-hydroxylase deficiency (5-8%), 3β-hydroxysteroid dehydrogenase deficiency (rare, <5%), and 17α-hydroxylase deficiency (very rare, <1%). Identifying the subtype of enzyme deficiency is crucial for diagnosis and therapeutic management. The aim of this study was to develop a liquid chromatography-tandem mass spectrometry method (LC-MS/MS) for simultaneous and sensitive measurement of six adrenal steroids (pregnenolone, progesterone, 17α-hydroxyprogesterone, 11-deoxycortisol, cortisol, and androstenedione) in serum. This panel covers four the subtypes of enzyme deficiency in CAH.

Methods: Patient serum samples (200 µL) was mixed with a methanol solution (100 µL) containing isotope-labeled internal standards then extracted with methyl-tert butyl ether (2 mL). The organic layer was dried at 40°C under nitrogen. The residue was derivatized by adding hydroxylamine hydrochloride solution (300 µL; 1.5 mol/L) and sodium hydroxide solution (50 µL; 10 mol/L) and heated at 90°C for 1 hour. The resulting mixture was extracted with methyl-tert butyl ether (2 mL) and dried under nitrogen at 40°C. The residue was reconstituted with 1:1 methanol:water (100 µL) and injected (50 µL) onto a Cyclone-P TurboFlow® column (0.5 x 50 mm, Thermo Fisher Scientific, Waltham, MA) followed by a Kinetex® C18 analytical column (2.1 x 50 mm, 2.6 µm; Phenomenex, Torrance, CA) on a TLX2 system using a gradient elution with methanol and water. Steroids were detected in multiple reaction monitoring mode on a TSQ Vantage® mass spectrometer using a positive heated electrospray ionization source (Thermo Fisher Scientific).

Results: The lower limits of quantification (LLOQ) ranged from 0.024 to 1.6 ng/mL for all the steroids tested. The linear range was 0.15-122.3 ng/mL for pregnenolone, 0.24 -27.9 ng/mL for 17α-hydroxyprogesterone, 0.10-31.7 ng/mL for 11-deoxycortisol, 0.29-78.6 for progesterone, 0.024-31.4 for androstenedione, and 1.6-1490.5 ng/mL for cortisol. The relative recoveries from the linearity study ranged from 92.3-127.6%. Intra- and inter-assay CVs were 0.90-18.1% and 4.3-16.7%, respectively. All compounds were stable at 4°C for 48 hours in serum and for 7 days in the final extracts.

Conclusions: This sensitive LC-MS/MS method simultaneously measures six adrenal steroids important for diagnosis and therapeutic management of four most commonly encountered subtypes of CAH.

B-101

Faster Intraoperative PTH Testing Does Not Result in Shorter Surgery

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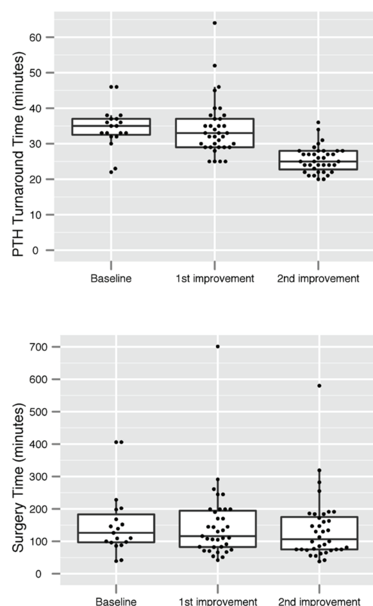
Background: Intraoperative parathyroid hormone (intraOP-PTH) monitoring permits rapid documentation of surgical correction of hyperparathyroidism. Another driving force for implementing this service is the perception by the surgical teams that rapid turnaround time (TAT) reduces overall surgical and anesthesia time. The objective of this study was to establish if a reduction in intraOP-PTH TATs translates into shorter surgeries and less time under anesthesia.

Methods: IntraOP-PTH TATs, surgery times, and anesthesia times were collected for parathyroid surgeries performed over a 3-year period (n=90) for which times were documented. Two laboratory process improvements were made over the 3-year period. The 1st introduced a paging system to facilitate communication between laboratory and surgery staff. The 2nd improvement moved testing from the Core Laboratory to the surgical unit. Data were thus split into 3 groups defined as Baseline (n=20), 1st improvement (n=35), and 2nd improvement (n=35). IntraOP-PTH TATs, surgery times, and anesthesia times were compared between groups using ANOVA and Tukey post-hoc tests; Krustal-Wallis and Mann-Whitney post-hoc tests were used for surgery and anesthesia times, which were not normally distributed. IntraOP-PTH TATs were tested for correlation against surgery and anesthesia time.

Results: Moving intraOP-PTH testing near the operating room improved TATs significantly (P<0.001). However, this does not appear to impact either surgery duration or anesthesia time. There was no correlation between TATs and either surgery duration (r2=0.002, P=0.66) or anesthesia times (r2=0.005, P=0.49).

Conclusions: IntraOP-PTH testing performed near the surgery suite significantly decreased TATs; however, neither surgical nor anesthesia times decreased in response. The perception that faster intraOP-PTH TAT directly leads to shorter surgery and anesthesia times needs to be re-evaluated.



B-103

Strategy for the development of a mass spectrometry assay for measuring sex hormone binding globulin (SHBG) in human serum

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Objective: Design a robust method for measuring SHBG in human serum. Relevance: SHBG measurements are clinically important to calculate the concentrations of the biologically active forms of testosterone and estrogen. An assay using peptide mass spectrometry (MS) could help standardize measurements. Multiple factors must be considered, such as target peptides, trypsin digestion conditions, and prior immuno-extraction to concentrate and select the appropriate circulating forms of SHBG.

Methods: SHBG peptides were selected using in-silico prediction models based on specificity, predicted MS signal strength and low probability of post-translational modifications which would alter the charge/mass signal. The existence and MS properties of the peptides were validated using trypsin digested SHBG measured on a ThermoFinnigan LTQ Orbitrap Hybrid mass spectrometer. Isotopically labeled internal standards were synthesized and dose response curves were established on API 5000 spectrometer. Mouse anti-SHBG antibodies (clone 1A5 from GenWay) were coupled to paramagnetic beads and used for extraction SHBG from 100uL serum samples. The trypsin digest was performed directly on beads. Efficiency of SHBG extraction was measured with Siemens Immulite immunoassay. Calibrators and controls were made by adding SHBG from CellSciensces to rabbit sera. Lyphochek controls were purchased from Biorad.

Results: Three target candidate peptides of SHBG were selected for analysis with minimum SHBG detection level from 5 nmoles to150 nmoles.

Peptide	AA sequence SHBG	Native peptide transition	Stable isotope-transition
IAL1	IALGGLLFPASNLR	721.92+/657.4	727.92+/663.3
ILA2		721.92+/804.4	727.92+/810.4
ILA3		721.92+/917.5	727.92+/923.4
QAE1	QAEISASAPTSLR	666.22+/644.4	672.42+/663.4
QAE2		666.22+/731.4	672.42+/744.4
QAE3		666.22+/889.5	672.42+/902.6
LDV1	LDVDQALNR	522.62+/716.4	528.52+/723.2
LDV2		522.62+/828.5	528.52+/815.4
LDV3		522.62+/943.4	528.52+/930.5

Efficiency of SHBG immuno-extraction was 98%. Five SHBG standards from 6 to 100 umol/L showed linear dose responses on MS for each of the 3 peptides. The MS peak areas ranged from 384,000 to 5,000,000 for IAL peptide; 3,100 to 383,000 for QAE peptide; and 7,400 to 97,400 for LDV peptide. Controls yielded values close to immunoassay targets. Conclusion: With this strategy the development of a MRM method for SHBG in serum looks feasible. The utility of this assay for standardizing sex-steroid activity measurements awaits further trials.

B-105

Development of Simultaneous Tandem Mass Spectrometric Method to Quantify Free Estrone and Free Estradiol

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Background: Measurement of free estrone and free estradiol will correlate with hormone activity better than measurement of total hormone concentrations. Both estrone and estradiol bind to sex hormone binding globulin (SHBG). The latter is polyglycated/polyglycosylated at multiple binding sites which could change the conformation of the binding site for both estrone and estradiol. Theoretically this may alter the Kd's by a factor of >10, thereby greatly increasing the concentration of free hormone. We propose to examine the free estrogen concentrations in patients with breast and ovarian cancers.

Methods: An AB SCIEX QTRAP 5500 tandem mass spectrometer equipped with TurboIonSpray source and Shimadzu HPLC system was employed to perform the analysis using isotope dilution with deuterium labeled internal standards, estrone-d_4 and estradiol-d_4 . 1 mL of human plasma/serum was filtered through a Centrifree YM-30 ultrafiltration device by centrifugation at 2800 rpm and 37°C for about 1 hour, and 20 µL of internal standard in methanol was then added to 500 µL of ultrafiltrate. This solution was treated twice with a simple liquid-liquid extraction using 2.5 mL of high purity hexane/ethyl acetate reagent. 70 uL of the reconstituted sample was injected onto a Phenomenex Gemini C6-Phenyl column where both estrone and estradiol undergo an on-line extraction, gradient chromatographic separation and elution. Quantitation by multiple reaction-monitoring (MRM) analysis was performed in the negative mode. The transitions selected were: mass-to-charge (m/z) 269.06/145.10 for estrone, 271.06/145.10 for estradiol, 273.06/147.00 for estrone-d_4 and 275.06/147.00 for estradiol-d_4. Nitrogen served as auxiliary, curtain, and collision gas. The main working parameters of the mass spectrometer were: collision gas medium, curtain gas 35, ion source gas 1 (GS1) 60, ion source gas 2 (GS2) 50, ionspray voltage -4500 V, entrance potential -10 V, probe temperature 650 °C, and dwell time 200 msec. For total estrone and estradiol, the iMethod from AB SCIEX was used.

Results and Conclusions: Our calibrators were prepared in 1,000 fold diluted (charcoal treated) serum giving protein concentrations similar to those in patient serum ultrafiltrates. 47 women were tested. The mean (range) for total, free, %free hormone concentrations were 53.4 (16.7-117.0) pg/mL, 1.5 (0.4-3.9) pg/mL, 2.9 (1.5-4.6) %for estrone and 90.1 (18.7-312.1) pg/mL, 0.9 (0.0-3.7) pg/mL, 1.0 (0.0-2.8)% for estradiol respectively. Our preliminary data reveal a significant difference in free hormone levels between patients. These studies will now be extended to determine the free hormone concentrations in newly diagnosed breast cancer patients and age matched controls.

B-106

Validation of a New and Improved Progesterone Assay on the IMMULITE/IMMULITE 1000 Immunoassay Analyzers

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Background: Progesterone is a steroid hormone essential in the preparation for and maintenance of pregnancy. During pregnancy, the placenta is the major source of the hormone. In nonpregnant females, it is secreted mainly by the corpus luteum, and small quantities are secreted by the adrenal cortex in males and females. Progesterone levels are low during the follicular phase and increase sharply during the luteal phase of the menstrual cycle in females. Measurement of serum progesterone may provide valuable information on the adequacy of the luteal phase of the menstrual cycle, on the effectiveness of ovulation induction, and in monitoring patients at risk for abortion during the early weeks of pregnancy.

Methods: The new and improved progesterone assay (LKPW), already available on the IMMULITE® 2000 analyzer (L2KPW), is now also available on the IMMULITE® and IMMULITE® 1000 systems. The assay time has been shortened to 30 minutes from 60 minutes, sample volume has been reduced from 50 µL to 25 µL, and the low-end accuracy has been improved.

The test design is based on a competitive immunoassay format. Progesterone in

patient sample competes with progesterone conjugated to bovine calf intestine alkaline phosphatase for binding to the progesterone antibodies coated onto the solid phase (bead). Unbound enzyme conjugate is then removed by a centrifugal wash. Finally, a chemiluminescent substrate is added to the bead and generates a signal which is inversely related to the amount of progesterone present in the patient sample. The assay range is 0.20-40 ng/mL. Two method comparisons were performed against the predicate device, the IMMULITE 2000 Progesterone Assay (L2KPW), using remnant samples from nonpregnant and pregnant females.

Results: For the method comparison performed at one site with three lots of the new IMMULITE Progesterone Assay (LKPW) on 168-172 samples, linear regression analysis yielded slopes of 1.08, 0.99, and 1.02 and correlation coefficients of 0.988, 0.991, and 0.986, respectively. For the method comparison performed at a second site using one IMMULITE kit lot and 400 remnant samples, the slope was 0.99 and the correlation coefficient 0.99. The three lots performed equivalently to the current IMMULITE 2000 Progesterone Assay.

Conclusions: The new progesterone assay for the IMMULITE and IMMULITE 1000 systems is comparable to the assay currently available on the IMMULITE 2000 system, with a faster turnaround time and overall improved performance over the former assay.

B-108

Distribution of ELF (Enhanced Liver Fibrosis) Test (and Component Assay) Results from Blood Donor Samples on the ADVIA Centaur Immunoassay System

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Background: The ADVIA Centaur® ELF™ Test is a composite score calculated from the results of three direct markers of liver fibrosis: HA, PIIINP, and TIMP-1.* Originally developed for the Immuno1™ Immunoassay System (Siemens), the ELF score, which correlates well with liver biopsy results (Rosenberg et al. Gastroenterology 2004: 1704-13), has been transferred to the ADVIA Centaur immunoassay systems (Dillon et al. Clin Chem 2011: A116). Previous studies have been done largely with samples from patients with known or suspected liver disease requiring biopsy. Knowing the distributions of results from individuals not requiring liver biopsy would be desirable.

Objective: Determine the distribution of HA, PIIINP, TIMP-1, and ELF results from blood donor samples on the ADVIA Centaur Immunoassay System.

Methods: The ADVIA Centaur ELF Test was conducted using a total of 594 samples from blood donors of known gender, ethnicity, and age. Cumulative distributions, 95th percentiles and, for ELF scores, percentages below each of the two standard cutoffs (7.7 and 9.8 score) were calculated.

Results: Observed ranges and 95th percentiles:

HA: 3.31-255.7, 95th percentile 54.39 ng/mL

PIIINP: 1.22-18.79, 95th percentile 7.85 ng/mL

TIMP-1: 16.02-419.3, 95th percentile 138.2 ng/mL

ELF: 5.15-10.75, 95th percentile 8.88 score, 47.8% below 7.7 and 99.2% below 9.8 score.

Gender (25.9% female) had little effect on the distributions. Ethnicity (47.8% Caucasian, 51.2% African-American, 1.0% Hispanic) had little effect on the HA and PIIINP distributions, but there was a subpopulation of African-Americans with TIMP-1 (and to a lesser extent ELF) less than expected on the basis of distributions in Caucasians, Hispanics and even the remaining African-Americans. Of the African-Americans, 20.4% had TIMP-1 results below 50 ng/mL; none of the Caucasians or Hispanics did. Similarly, 7.6% of the African-Americans had ELF scores below 6.5, compared to only 1.4% of the Caucasians and 16.7% (only one individual) of the Hispanics. It may be speculated that the low TIMP-1-African-American subpopulation may be related to keloid scarring, which is much more prevalent amongst African-Americans than Caucasians. The donors were from 18 to 66 years old. The results each had a modest, positive correlation with age: r = 0.37(HA), 0.40(PIIINP), 0.35(TIMP-1), 0.40(ELF). Comparisons, by biopsy stage, have been made with the Centaur-equivalent to the Immuno1 values from the 921-sample data set originally used to determine the ELF score calculation and cutoffs (Dillon et al. Clin Chem 2009: A169). The distributions of ELF scores from the blood donor samples and the Ishak biopsy score 0 are similar. The distributions with Ishak scores of 1 to 6 were progressively higher.

Conclusions: The distribution of HA, PIIINP, TIMP-1 and ELF were determined with 594 samples from blood donors. Gender and ethnicity had little effect (except for an African-American subpopulation with unusually low TIMP-1 values). The results had a modest, positive correlation with donor age.

* The ELF Test and the HA, PIIINP, and TIMP-1 assays are not available for sale in the U.S. This test and these assays are CE marked.

B-109

Establishment of reference intervals for thyroid function tests during pregnancy

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Background: Thyroid hormones are important for fetal development. Pregnancy involves physiological factors such as high beta-HCG and high thyroid binding proteins concentrations which affect thyroid physiology and thyroid function test results. This study aims to determine reference intervals for TSH, free T4 and total T3 in pregnant women.

Methods: Leftover serum samples from routine blood work during pregnancy were collected in our laboratory. Specimens were collected randomly . Gestational age was determined by using laboratory information system. Following this, specimen were made anonymous before analysis on Roche E70 Modular platform. Anti-TPO antibodies positive samples were excluded from the reference population. Percentiles for TSH, free T4 and Total T3 were computed for every trimester. ANOVA on logarithmically transformed data were performed to compare TSH, free T4 and Total T3 concentrations between trimesters of pregnancy.

Results: Overall , 546 samples were collected, of which 60 were positive for anti-TPO (11,0%), leaving 486 samples from our reference population analysis. Table 1 shows key results. TSH, free T4 and total T3 differed from the reference range in use in CHUQ for all adults as shown in table 1. With advancing gestational age, free T4 declined gradually and this was statistically significant. TSH upper reference limit in pregnant women 97.5th percentile was lower than 5 mIU/L used in CHUQ. Lower reference limit as defined by 2.5th percentile was higher than CHUQ usual lower reference limit. Total T3 was higher in pregnancy but did not vary significantly between different pregnancy ages.

Conclusions: We performed inhouse assessment of pregnancy-specific reference ranges for thyroid function tests directly in our population. Our data underlines the importance of using laboratory reference ranges tailored to specific populations (pregnant vs non pregnant in this case). Despite intrinsical design limitations, our study contributes to better define thyroid function tests reference intervals during pregnancy.

Table1 : Summary of trimester-based ANOVA performed on log transformed thyroid function tests results with corresponding trimester based test distribution and CHUQ reference ranges.

Parameter	TSH	Free T4	Total T3
Transformation	Ln(TSH) + 2	Ln (fT4)	Ln(tT3)
Comparison			
T1 vs T2	p < 0,001	p < 0,001	p = 0.0542
T1 vs T3	p = 0.0033	p < 0,001	p= 0.2339
T2 vs T3	p = 0.14	p < 0,001	p = 0.5039

Centile	TSH (mIU/L)			Free T4 (pmol/L)			T3T (nmol/L)		
	T1 (n=194)	T2 (n=142)	T3 (n=150)	T1 (n=194)	T2 (n=142)	T3 (n=150)	T1 (n=194)	T2 (n=142)	T3 (n=150)
1	0.22	0.23	0.29	9.60	8.36	8.0	1.66	1.70	1.66
2,5	0.45	0.51	0.47	10.36	9.54	8.64	1.81	1.80	1.80
5	0.54	0.73	0.67	11.00	9.85	9.20	1.97	1.94	1.89
25	0.94	1.23	1.38	12.69	11.34	10.22	2.24	2.20	2.28
50	1.34	1.58	1.88	13.55	12.42	11.18	2.51	2.59	2.56
75	1.96	2.39	2.51	14.55	13.68	12.07	2.80	2.89	3.00
95	3.39	3.29	3.94	16.57	15.52	14.14	3.27	3.52	3.69
97,5	4.35	4.54	4.78	17.81	16.32	15.14	3.60	3.73	3.92
99	4.75	6.06	5.27	20.67	16.86	16.29	3.74	4.07	4.01

CHUQ Adult reference limits	TSH (mIU/L)	T4L (pmol/L)	T3T (nmol/L)
	0.25 - 5	12 - 22	1.2 - 3.0

B-110

Development of a fully characterized picoPAPP-A chemiluminescence assay for healthy male and female serum evaluation*.

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Relevance: Pregnancy-associated plasma protein A (PAPP-A) is a large placenta-derived glycoprotein. During pregnancy it is produced in high concentrations by

the trophoblast and released into maternal circulation. In addition to trophoblasts, PAPP-A expression has been reported in various tissues, including endometrium, testis, atherosclerotic arteries, kidney, bone, colon, and other adult and fetal tissue. PAPP-A is potentially proatherosclerotic and has been proposed as a new marker of inflammation, as high serum PAPP-A levels are observed in patients with renal impairment, asthma, lung cancer, unstable angina, etc. Studies suggest that the PAPP-A form in non-pregnant females and males is dimeric and is not complexed with proMBP and proteolyse IGFBP-4 and IGFBP-5.

Methodology: We have developed a well characterized two-step sandwich-type enzymatic microplate glow based chemiluminiscence assay to measure PAPP-A levels in serum. The assay measures PAPP-A in 50 µL of serum sample against dimeric PAPP-A calibrators (0.1-25ng/mL). The antibody pair used in the assay measures dimeric PAPP-A and PAPP-A/proMBP complex and does not cross-react with proMBP, PAPP-A2 and MMP-9 at twice the physiological concentrations.

Validation: Total imprecision calculated on 3 samples over 12 runs, 4 replicates per run, using CLSI EP5-A guidelines was 3.01% at 0.979ng/mL, 1.41% at 1.45ng/mL and 2.86% at 3.02ng/mL. The limit of detection calculated using ten serum samples in the range of of 0.045-4.16ng/mL over 12 runs is 0.03ng/mL. The functional sensitivity of the assay at 10% CV was 0.05ng/mL. Dilution studies showed an average recovery of 104-110%. The median PAPP-A value on random male and female samples (n=12) was 0.82ng/mL.

Conclusions: A quantitative, robust and fully characterized microplate PAPP-A chemiluminiscence assay has been developed to measure PAPP-A in random male and female serum. The approximate median PAPP-A levels found in a random male and female serum can be measured with < 5 % CV using this assay. The performance of the assay is acceptable for investigation of clinical utility in inflammation related disorders.

* For Research Use Only. Not for use in diagnostic procedures.

B-111

Elevated progesterone concentrations in postmenopausal women: Evaluation of 6 immunoassays

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Background: Our clinical laboratory observed numerous samples with elevated progesterone concentrations from postmenopausal women. Immunoassays are known to be susceptible to interfering and cross-reacting substances and progesterone is no exception.

Methods: Specimens from postmenopausal women (≥55 years of age or ≥50 years of age with elevated follicle stimulating hormone or decreased anti-mullerian hormone, n=21) were matched with specimens from premenopausal women (≤34 years of age, n=21) according to progesterone results. A control group of healthy, self-reported postmenopausal women was also obtained (n=25). Progesterone testing was performed on the following immunoassay analyzers: Beckman Coulter Access, SIEMENS ADVIA Centaur and IMMULITE 2000, Abbott ARCHITECT *i*2000$_{SR,}$ Roche Modular E170, and Ortho ECi. Specimens were also tested for progesterone by liquid chromatography tandem mass spectrometry (LC-MS/MS) by a commercial lab. Organic solvent extractions were performed on both premenopausal and postmenopausal sample sets and subsequently tested by all methods.

Results: Immunoassay methods were compared to LC-MS/MS by absolute difference plots for each patient group before and after extraction, where applicable (Table). A statistically significant positive bias was observed for the postmenopausal group as compared with the premenopausal group for the Centaur, ARCHITECT, E170, and ECi. A statistically significant positive bias was also observed for the postmenopausal group compared to the postmenopausal control group for all methods. Additionally, the observed bias was statistically higher for the postmenopausal group before extraction compared to after extraction for all methods except the IMMULITE. After extraction, the premenopausal group was statistically lower than the postmenopausal group for all methods.

Conclusions: Solvent extractions may have removed the hydrophilic substance(s) causing the presumed false elevation of progesterone in postmenopausal women. The IMMULITE was least affected by the cross-reacting substance(s), while the Centaur was most affected. Caution should be taken when interpreting progesterone results in postmenopausal women.

Table. Absolute bias for progesterone methods as compared to LC-MS/MS.

	Bias (95% limits of agreement) (nmol/L)				
Method	Premenopausal	Premenopausal after extraction	Postmenopausal	Postmenopausal after extraction	Postmenopausal control group
Access 2	5.6 (-12.6, 23.7)	-20.3 (-53.3, 12.7)	15.8 (-16.0, 47.5)	-4.9 (-15.4, 5.7)	-0.6 (-1.4, 0.0)
ADVIA Centaur	-1.3 (-22.0, 19.5)	-20.7 (-54.5, 13.2)	36.4 (-40.0, 110.7)	-4.8 (-16.5, 6.9)	-0.8 (-1.5, -0.1)
ARCHITECT i2000SR	-3.0 (-12.6, 6.5)	-19.1 (-51.0, 12.8)	11.9 (-15.3, 39.1)	-4.4 (-14.1, 5.3)	-0.8 (-1.4, -0.2)
IMMULITE 2000	-8.6 (-25.8, 8.7)	-21.4 (-56.9, 14.2)	3.7 (-10.3, 17.7)	-5.9 (-17.4, 5.6)	-0.8 (-1.5, -0.1)
Modular E170	-1.8 (-14.3, 10.7)	-19.6 (-49.8, 10.5)	20.5 (-25.8, 66.8)	-4.4 (-15.7, 6.8)	-0.6 (-1.7, 0.5)
VITROS ECi	-2.3 (-23.3, 18.7)	-23.7 (-60.8, 13.5)	29.3 (-30.1, 88.6)	-5.9 (-18.2, 6.4)	-0.3 (-1.1, 0.6)

B-112

Comparative evaluation of automated immunoassays for the detection of 25-OH Vitamin D

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Introduction: With the increase in the number of manufacturers offering assays for the detection of 25 OH Vitamin D, it is important to compare the performance of each method in order to evaluate the accuracy of assay results.

Objective: A study was conducted in order to assess the performance of the following assays: DiaSorin LIAISON® XL 25 OH Vitamin D TOTAL Assay, Abbott ARCHITECT 25-OH Vitamin D, IDS iSYS 25-Hydroxy Vitamin D, Roche Elecsys Vitamin D total and Siemens ADVIA Centaur® Vitamin D Total (Vit D) assays against the DiaSorin RIA assay.

Materials: Sera collected for method comparison studies, which used a panel of 109 specimens, were obtained at an external European trial site as part of their routine analyses. The samples were stored at -20°C until analysis on each of the automated instruments and RIA. All methods were performed according to the instructions for use provided by each manufacturer. RIA assay results were used as the study reference method.

Results/Conclusions: The LIAISON® XL assay shows the closest agreement with the RIA reference assay (y=1.02x - 3.88, R=0.98) when compared with the 25-OHD assays of Architect (y=1.36 - 11.0, R=0.95), iSYS (y=1.29 - 4.70, R=0.97), Elecsys (y=1.05 - 2.81, R=0.94) and Centaur (y=1.12 - 5.23, R=0.96). A positive bias is seen (differing by up to 60 ng/mL from RIA) on the Architect, iSYS, and Centaur® assays at doses >60 ng/mL. Both the LIAISON® XL and Elecsys assays exhibit the closest agreement to the RIA method across the measuring range.

B-113

Change in Intraoperative PTH Assay Methodology Highlights Need for Standardization

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Background: Primary hyperparathyroidism is often characterized with multi glandular disease, requiring the exploration and identification of all glands on both sides during parathyroid surgery. Rapid intraoperative parathyroid hormone (RI-PTH) measurement is a cost-effective way of predicting the necessity of further neck dissection after the removal of an adenoma. As part of a laboratory reorganization, our intraoperative PTH assay using Advia Centaur (Siemens) platform needed to be transferred to the e411 immunochemistry system (Roche), using the rapid 9-minute STAT PTH assay.

Methods: Method verification consisted of precision, a calibration verification including AMR, and method comparison. To compare methods, intraoperative PTH results, both pre and post parathyroid excision, were initially obtained via our normal process on the Advia to ensure rapid resulting for clinical purposes, then the specimens were assayed on the e411. Because the clinical use of the PTH results depends on the percent drop in PTH concentration (pre vs. post), this parameter was used, in addition to comparison of actual concentrations, as an additional comparison element. Data was analyzed using EP evaluator software. In total, 59 specimens were compared, which corresponded to 27 actual patients/surgeries, because in several cases, multiple post excision specimens were received and assayed. Manufacturer control materials, as well as third party control materials were also compared on both instruments.

Results: The precision and AMR studies were suitable. In comparing the methods, though the correlation was excellent (R=.96), a substantial proportional bias was evident (slope= 0.6 by Deming regression), with substantially lower results from

the e411. When comparing the percent drop between pre and post specimens, the correlation was also excellent (R= .97, slope=.93), giving functionally identical results. Based on this data, a correction factor to align the results obtained from e411 with the Siemens-Advia Centaur results was used. The corrected results comparison data showed the slope of 1.001 [r= 0.96]. Comparison of quality control materials also showed the proportional bias. Examination of proficiency testing results from the past two years also demonstrated a bias of the same magnitude.

Conclusions: Lacking a suitable standardized reference material, we could not resolve the proportional bias observed in the comparison study. However based on this data, the rapid PTH on the e411 was placed into service with a correction factor to align the results with the Siemens-Advia Centaur results, previously used for both intraoperative and routine PTH testing. The substantial differences in absolute concentrations, demonstrated in patient samples and other (calibration, quality, and proficiency) materials, emphasizes the need for establishment of gold standard reference materials and standardization of PTH immunoassays.

B-114

False Elevations in the Beckman Coulter Hypersensitive TSH Assay in Patients with Suppressed Thyroid-Stimulating Hormone

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Background: Following thyroidectomy and/or radioiodine ablation, patients with differentiated thyroid carcinoma (DTC) are given high doses of levothyroxine to suppress Thyroid-stimulating hormone (TSH). Suppression below functional sensitivity of 3rd generation TSH assays (<0.015 mU/L) may be associated with improved outcome, however, guidelines suggest a therapeutic target for TSH of <0.1 mU/L and <0.5 mU/L for high and low risk patients, respectively. Very low TSH is also common in severe primary hyperthyroidism. We noticed several cases of unexpected elevations in TSH (Beckman Coulter DxI, hTSH assay) in hyperthyroid and DTC patients undergoing suppression therapy.

Objective: To investigate the performance of the Beckman Coulter 3rd generation hTSH assay in patients with low and suppressed TSH concentrations.

Methods: Results from physician ordered TSH performed on the Beckman Coulter DxI hTSH assay were evaluated for 13 weeks. Residual plasma specimens (BD Li-Heparin PST; first 10 weeks) or serum specimens (BD Serum; last 3 weeks) with TSH <0.5 mU/L were utilized (n=1102; 750 Plasma, 352 Serum). TSH was reanalyzed in the primary tubes on the Cobas e411 3rd generation TSH assay (Roche Diagnostics). If discrepant, specimens were aliquotted, re-centrifuged and re-analyzed on the DxI. Discrepancies were considered clinically significant if the TSH decreased by ≥50% or by > 25% and results went from normal to abnormal or to below 0.1mU/L. Matrix comparisons were performed in paired plasma and serum (n=20). An LIS query generated all TSH results <0.015 mU/L for two-2.5 month periods in which TSH was measured in plasma and serum, respectively. Results were compared using 1-way ANOVA and student T-test analyses.

Results: Validation studies showed agreement between the DxI and e411 TSH assays in Li-Heparin PST specimens (R=0.9827, slope 1.02). Matched plasma and serum specimens correlated well on the DxI TSH assay (R=0.9996, slope 1.03). TSH results were clinically and significantly different across the two platforms for 68 of 750 samples (9.1%), with average concentrations of 0.187mU/L (DxI) and 0.042mU/L (e411) (p<0.0001), and after re-centrifugation and repeat analysis on the DxI (average 0.079mU/L, p<0.0001). Among the discrepant specimens, results were below functional sensitivity in 40% of samples analyzed on the e411 compared to 3% on the DxI, p <0.0001. Only 3 of 352 (0.85%) TSH results measured in serum specimens were clinically and significantly different. In two-2.5 month periods, the rate of TSH suppression below functional sensitivity (<0.015 mU/L) was significantly different for plasma and serum specimens, 0.09% (18/19,175) and 0.49% (76/15,554), respectively (p <0.0001).

Conclusions: An interferent in BD Li-Heparin PST specimens produced ~10% false positive results on the Beckman Coulter DxI hTSH assay among specimens with original values <0.5mU/L. Re-centrifugation reduces the interference. Virtually no DxI TSH results were suppressed below functional sensitivity in plasma while utilization of serum decreased the false positive rate to <1% and increased the overall suppression rate to 0.5%. Results from BD Li-Heparin PST specimens measured on the Beckman DxI hTSH assay should be interpreted with caution especially in hyperthyroid and DTC patients with suppressed TSH.

B-115

Development of a well characterized PAPP-A2 chemiluminescence assay to measure PAPP-A2 in biological fluids.

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Relevance: Pregnancy-associated plasma protein-A2 (PAPP-A2) is a novel metalloproteinase identified as a homolog of PAPP-A in the metzincin superfamily of pappalysins. PAPP-A2 shares 46% sequence identity with PAPP-A. PAPP-A2 is a noncovalently linked dimer of two 220-kDa subunits. It exhibits proteolytic activity against IGFBP-5 and IGFBP-3. PAPP-A2 is expressed in a wide range of tissues and is abundant in placental syncytiotrophoblasts and the pregnant uterus. The physiological importance of PAPP-A2 is not known.

Methodology: We have developed a well characterized two-step sandwich-type enzymatic microplate CLIA to measure PAPP-A2 levels in the maternal serum and other biological fluids. The assay measures PAPP-A2 in 50 µL of sample (diluted 20 folds in sample diluent) against recombinant PAPP-A2 calibrators (0.25-25 ng/mL). The antibody pair used in the PAPP-A2 ELISA measures PAPP-A2 and does not detect proMBP, dimeric PAPP-A and PAPP-A-proMBP complex.

Validation Total imprecision calculated on 4 samples over 12 runs, 4 replicates per run, using CLSI EP5-A guidelines, was 4.46% at 1.01ng/mL, 7.38% at 1.83ng/mL, 2.94% at 2.87ng/mL and 3.88% at 7.48ng/mL . The limit of detection calculated using six serum samples in the range of 0.125-1.9ng/mL over 12 runs is 0.1ng/mL. The functional sensitivity of the assay at 20% CV was 0.18ng/mL. Dilution studies showed an average recovery of 98-110%. The median PAPP-A2 value on a second trimester samples (n=65) was 47.09ng/mL.

Conclusions: A quantitative, robust and fully characterized microplate PAPP-A2 CLIA has been developed to measure PAPP-A2 in maternal serum. The approximate median PAPP-A2 levels found in first and second trimester maternal serum can be measured with < 5 % CV using this assay. The performance of the assay is acceptable for investigation of clinical utility in a variety of maternity related disorders.

* For Research Use Only. Not for use in diagnostic procedures.

B-116

An LC-MS/MS Method for the Measurement of Aldosterone, Cortisol, 11-Deoxycorticosterone, Corticosterone, 18-Hydroxycorticosterone and 18-Hydroxy-11-Deoxycorticosterone with application to Adrenal Vein Sampling.

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Background: Primary Aldosteronism is a treatable and often curable form of hypertension. Causes include: bilateral adrenal hyperplasia, aldosterone producing adenoma (APA), glucocorticoid remediable hyperaldosteronism, and other rare forms. After diagnosis of PA, treatment approach (ie surgical vs medical) is directed by subtype classification which depends almost entirely on the biochemical analysis of adrenal venous sampling (AVS) procedures. One of the challenges in analysis of AVS samples is the biochemical proof of catheterization. Although it is commonly understood that cortisol is the optimal biochemical parameter for the identification of successful adrenal vein cannulation, it often fails to prove catheterization on biochemical grounds. In order to increase the demonstrable success of adrenal vein cannulation, ACTH stimulation is sometimes employed prior to sample collection. However, the downside of ACTH stimulation is factitious loss of lateralization. In order to address this problem we have developed a multiplex LC-MS/MS assay to investigate whether there is another steroid which might better serve to prove cannulation and to normalize left and right aldosterone results for the calculation of a "lateralization index"

Methods: The assay concomitantly measures aldosterone, cortisol, 11-deoxycorticosterone, corticosterone, 18-hydroxy-11-deoxycorticosterone, and 18-hydroxycorticosterone in a multiplex fashion using 250 µL of serum extracted by supported liquid extraction using SLE+ 400uL plates (Biotage, Charlotte, NC) with methyl-tert-butylether (Sigma, Oakville, ON). Calibrator standards were prepared in double charcoal treated human serum (Golden West Biologicals, Temecula, CA) from weighed-in steroid stock solutions. Stable isotope labeled steroids (d7-aldosterone, d4-cortisol, d8-corticosterone and d8-11-deoxycorticosterone) were used as internal standards. After SLE+ extraction, the samples were analyzed on an AB SCIEX API5000 triple quadrupole MS/MS in positive ESI mode (AB SCIEX, Foster City, CA) and a Shimadzu Prominence LC20AC (Shimadzu, Kyoto, Japan) on a Gemini-NX 100x2.1mm, 3.5m column (Phenomenex, Torrance, CA) maintained at 55 °C. The method was preliminarily applied to 8 adrenal vein collections, pre and post ACTH stimulation for 32 analyses in all.

Results: Application to adrenal vein samples has demonstrated that a number of the steroids may be equally good or superior markers for catheterization success based on adrenal vein to inferior vena cava gradients. However, in order to serve as a tool for localization calculation, the marker must not heavily correlate with aldosterone in order

that lateralization is not mathematically washed-out. Preliminarily we have found that 18-hydroxy-11-deoxycorticosterone may be a more sensitive marker of adrenal vein cannulation than cortisol based on venous to central concentration gradients. Additionally, compared to cortisol, 18-hydroxy-11-deoxycorticosterone shows similarly poor and statistically non-significant correlation to aldosterone in AVS (pre-ACTH: cortisol ρ=0.03, p=NS; 18-hydroxy-11-deoxycortisol ρ=0.03, p=NS; post-ACTH: cortisol ρ=0.07, p=NS; 18-hydroxy-11-deoxycortisol ρ=0.21, p=NS)

Conclusions: We present an LC-MS/MS method for 6 closely related adrenal steroids relevant to the investigation of mineralocorticoid excess. 18-hydroxy-11-deoxycorticosterone may serve as a better marker for cannulation and lateralization index calculation than cortisol.

B-117

Performance of ACTH Immunoassay in the setting of Hypothalamic-Pituitary-Adrenal Dynamic Testing

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Background: Adrenocorticotropic hormone (ACTH) is a polypeptide hormone secreted by the anterior pituitary gland. ACTH levels are often assessed during endocrine provocative tests, such as ACTH stimulation, corticotropin releasing hormone (CRH) stimulation and inferior petrosal sinus sampling (IPSS) with CRH stimulation, to evaluate hypothalamic-pituitary-adrenal (HPA) axis and establish etiology of glucocorticoid deficiency or excess. Such dynamic tests are complex and often involve highly invasive procedures. Thus, specimen collection, processing and analyses must be optimized and error-free. It is well known that ACTH is heat labile and susceptible to proteolysis.

Objectives: We performed stability studies to establish the specimen decay rate to minimize any error due to sample processing. Furthermore, during stimulation testing where serial ACTH levels are monitored, hormone levels exceeding the upper analyte measuring range of 1250 pg/mL are frequently observed. Since there are currently no clear instructions regarding high sample dilutions, we performed measuring range studies to validate the expansion of the clinical range of our assay. Finally, we were recently consulted on a case where the physician questioned the low-end precision of our assay. The patient in this case underwent CRH stimulation and their ACTH levels ranged from 10-30 pg/mL. These levels were too high for adrenal and too low for pituitary cause of patient's hypercorticolism, causing the clinician to question the sensitivity of our assay.

Method: ACTH was measured on Immulite® 2000 platform (Siemens Healthcare Diagnostics, Tarrytown, NY). ACTH stability was assessed using 18 de-identified patient samples split into 3 aliquot sets. Aliquot set #1 was stored at 4C and tested every day for 4 days. Aliquot sets #2 and 3 were both stored at -20C for 5 days and thawed at 4C (#2) and room temperature (#3) prior to analysis. For functional sensitivity study, 5 patient samples with ACTH levels ranging from <5 to 20 pg/mL were analyzed in five different runs. The limit of quantitation (LOQ) was arbitrarily defined as the lowest concentration with CV <15%. Finally, for a linearity study, high patient sample was diluted serially to cover the entire dynamic range of the assay. The recoveries were then measured and plotted against the expected values.

Results: ACTH samples were stable when stored at 4C for 48 hours and at least one freeze-thaw (F/T) cycle, with the analyte recovering within 20% of the initial (day 1) measurement. The LOQ of Immulite 2000 ACTH assay is 6.0 pg/mL with CV of 8.3%. Although ACTH diluent is provided by the manufacturer, we found it susceptible to matrix effects, resulting in under-recovery at higher dilutions. Serial dilutions of a high ACTH specimen obtained from a patient with Cushing disease diluted with a low-ACTH patient plasma up to 100-fold yielded an ACTH of 8500 pg/ml with ~85% recovery.

Conclusions: Immulite ACTH assay is a very sensitive assay that can be diluted with a low patient sample to extend the clinical reporting range. If plasma samples cannot be analyzed immediately, they may be stored up to 48 hours at 4C.

Tuesday PM, July 17, 2012

Poster Session: 2:00 PM - 4:30 PM

Technology/Design Development

B-118

Comparison of ARCHITECT I 2000 for determination of Cyclosporine with AxSYM

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Background: Cyclosporine (Sandimmune and Neoral) has been shown effective drug in suppressing acute rejection in recipients of allograft organ transplants. Adsorption of cyclosporine in the form of Sandimmune is highly variable varying from 5 % to 40 %. Whole - blood concentration correlates with the degree of immunosuppression and toxicity, but there is a poor relationship between dose and blood concentration.

Methods: The cyclosporine concentration of 96 blood EDTA samples was determined using CMIA (chemiluminesecent microparticle immnoassay) Architect i 2000 and FPIA (fluorescence polarization immunoassay) AxSYM Abbott diagnostic. All patients have transplantation of kidneys and were hospitalized at Department of Nephrology at the University Clinics Center of Sarajevo. The reference serum range of cyclosporine for kidney organ transplantation for maintenance lies between 50 and 150 ng/mL. The quality control, precision and accurancy of Architect i 2000 were assessed.

Results: The quality control was done using quality control serums for low (= 91 ng/mL), medium (= 328 ng/mL) and high (= 829 ng/mL). We have used commercial BIORAD controls and got CV 7.44 % to 12.14 % for Architect i 2000. It was established that the main difference between Architect i 2000 and AxSYM and it was statistically significant for $p < 0.05$ according to Student t-test. Correlation coefficient was r = 0.903 and regresion line had a slope 1.101 and a y axis intercept of 7.572. The AxSYM Cyclosporine Monoclonal Whole blood assay was performed od the same 96 human whole blood EDTA samples in the range 15.5 to 289.9 ng/mL. The average ng/mL difference bias exhibited by ARCHITECT Cyclosporine vs. AxSYM Cyclosporine Monoclonal Whole blood assay in this study was -25.75 ng/mL. The 95 % confidence interval of the ng/ml difference bias is -17.17 ng/mL to - 34.34 ng/mL.

Conclusions: The CMIA Architect assay has significant reduced cyclosporine metabolite interference relative to other immunoassay and is a convenient and sensitive automated method to measure cyclosporine in whole blood.

B-119

Verification of sample-to-sample carryover performance of TBA-120FR in HbA1c measurement

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Introduction: Currently, HbA1c testing is primarily performed by means of high-performance liquid chromatography (HPLC), which requires specialized equipment. However, some immunologic and enzymatic assays have been developed to measure HbA1c with a general-purpose biochemical analyzer. The inconvenience of these assays stems from the need for sample pretreatment that includes hemolysis. TBA-120FR (Toshiba Medical Systems Corporation, Japan) equipped with an HbA1c measurement unit allows direct sampling of red blood cells (RBCs) or whole blood, and can automatically process all steps from hemolysis to measurement of hemoglobin and HbA1c levels. One of the features of the instrument is that the sample-to-sample carryover performance is 0.1 ppm or less. We have verified the performance of the TBA-120FR in HbA1c measurement.

Methods: The following two protocols were used for evaluation of sample-to-sample carryover. (1) Orange G sample: A pooled serum contained washed RBCs and a high concentration of Orange G (150,000 ppm) was prepared as a hot sample. HbA1c in the hot sample was measured using the TBA-120FR. Next, twenty biochemical tests (sample volume of 2 µL to 15 µL) were performed consecutively in the TBA-120FR using physiological saline (zero sample). After biochemical testing, the absorbance of remaining physiological saline was measured at 476 nm and 572 nm, which were in the absorbance band of Orange G. Carryover was determined from the Orange G concentration in the zero sample. (2) Patient samples: HbA1c of a sample with high levels of HBs antigen and a sample with high levels of CA19-9 were measured using the TBA-120FR. Next, twenty biochemical tests (sample volume of 2 µL to 15 µL) were performed consecutively in the TBA-120FR using negative samples. Carryover was respectively determined from the amount of HBs antigen and CA19-9 included in the negative samples. Norudia®N HbA1c (Sekisui Medical Co., Ltd., Japan) was used to measure HbA1c. Measurement was performed in accordance with parameters specified by the manufacturer. Architect HBsAg QT and CA19-9 XR (Abbott Laboratories) were used to measure HBs antigen and CA19-9, respectively.

Results: (1) Orange G sample: The measurements were repeated 5 times. The sample-to-sample carryover of TBA-120FR was 0.1 ppm or less. (2) Patient samples: Two patient samples with HBs antigen level of 203,231 IU/ml and CA19-9 level of 3,110,519 U/ml, respectively, were used as the hot sample. The cut-off is 0.05 IU/ml for HBs antigen and 37 U/ml for CA19-9. After the carryover examinations, the amount of HBs antigen in the negative sample was 0.05 IU/ml or less. Similarly, the amount of CA19-9 in the negative sample was 2.5 U/ml or less. Neither sample gave a positive result for HBs antigen or CA19-9 due to sample-to-sample carryover.

Conclusions: The TBA-120FR can measure HbA1c in the clinical setting without being affected by sample-to-sample carry-over. Carryover performance of less than 0.1 ppm during HbA1c measurement is maintained.

B-121

Development of Microarray-Based Diagnostic Genotyping Assay for 5-Fluorouracil Toxicity in Cancer Patients

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Background: The fluoropyrimidine 5-fluorouracil (5-FU) is one of the most commonly used chemotherapeutic agents for the treatment of solid carcinomas. Since approximately 10-40% of patients develop severe to life-threatening, and in some cases even lethal, toxicity from 5-FU, predictive markers are of utmost clinical importance. Genetic variation in *DPYD* gene, encoding for dihydropyrimidine dehydrogenase, the key enzyme in the catabolism of 5-FU, has been shown to be a major predictor of 5-FU toxicity. In particular, by screening the currently known risk variants, up to 50% of the most severe (NCI CTCAE grade 4-5) toxicity cases can be identified prior to the initiation of therapy. For patients at an increased risk of toxicity, drug therapy can be adjusted by lowering the starting dose of 5-FU and further guided by therapeutic drug monitoring, or by applying alternative therapeutic regimen. The objective of this study was to develop a novel genotyping assay for the prediction of 5-FU toxicity utilizing an automated microarray platform optimized for clinical diagnostic use (INFINITI® AutoGenomics, Inc.).

Methods: Genomic regions covering seven major *DPYD* variants associated with 5-FU toxicity (c.234-123G>C, c.496A>G, c.775A>G, c.1129-5923C>G, c.1679T>G/A, c.1905+1G>A, c.2846A>T)[1] were amplified in a multiplex PCR reaction. Subsequent allele-specific primer extension with fluorescently labeled nucleotides, capturing via hybridization to the microarray, and scanning the arrays were automated within the INFINITI Analyzer. The assay was validated by analyzing 80 blood samples obtained from cancer patients previously re-sequenced for the *DPYD* gene.

Results: The results obtained with the two methods were 100% concordant. Since the genotyping results using the new assay can be obtained within one day (required time: 4 h for DNA extraction and multiplex PCR amplification, and 3.5 h + 0.5 h/sample for the INFINITI run), it is highly suitable for clinical diagnostic setting.

Conclusions: The developed assay provides a novel tool to improve the safety and efficacy of 5-FU-based cancer chemotherapy.

Reference:

1. Amstutz U, Froehlich TK, Largiadèr CR. Dihydropyrimidine dehydrogenase gene as a major predictor of severe 5-fluorouracil toxicity. *Pharmacogenomics* 2011; 12:1321-36.

B-122

AACC History Division Clinical Laboratory Analyzer Archive

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Since the 1950's, the advances and evolution of automated discrete and continuous flow clinical chemistry instrumentation has had a profound impact on today's laboratory and diagnostic medicine. In 2011, with the objective of documenting these clinical

instrumentation advances, the AACC History Division initiated the development of a digital Clinical Laboratory Analyzer Archive, available to all on the AACC History Division web site (www.aacc.org/members/divisions/history/pages/default.aspx#; Google: Clinical Laboratory Analyzer Archive). The scope of the Archive includes: clinical chemistry, coagulation, cytology, hematology, immunoassay, integrated systems, molecular, point-of-care, sample processing, and automation (track) systems from more than 60 companies. The archive currently contains over 230 examples of analyzers and in the future, the scope will be expanded to include: blood banking, histology, laboratory information systems, microbiology, virology, etc. The Archive can now be searched by company, category (chemistry, hematology, etc.), or by year. The search capability by Product Name is in development. When an instrument selection is made, the instrument can be highlighted, producing a specific instrument screen containing the Product Name, Year, Company, Category, Description, Source, Bibliography, and Documentation. Eventually, it is planned to also include other information on an analyzer such as advertisements and analyzer brochures and operation manuals. We believe that this Archive will provide an important clinical laboratory instrument reference and teaching source. AACC members and instrumentation companies are invited to contribute to the Archive by submitting missing instrumentation (introduction date, slides, photographs, brochures, advertisements, first bibliographic citation, and other relevant information) to Kricka@mail.med.upenn.edu.

B-124

Comparison between new UPLC and traditional HPLC methods for the determination of plasma iohexol

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Background: Iohexol clearance is a reference method for detection of the glomerular filtration rate, which is important in nephrology practice and research. High performance liquid chromatography (HPLC) method to determine plasma iohexol is well accepted. Ultrahigh performance liquid chromatography (UPLC) method is recently introduced as a new direction for the study. Both new UPLC and traditional HPLC methods for determination of plasma iohexol are compared for their efficiencies, and application in laboratories of different settings.

Methods: The HPLC (Waters Associates Inc, Milford, MA, USA) and UPLC (Agilent Technology, Wilmington, DE, USA) techniques were developed for iohexol determination in plasma. Spiked standard EDTA plasma was used to evaluate system suitability test and validate method. It was extracted by using 5% perchloric acid on ultrasonicator and filtered through 0.2 µm membrane. Iohexol in filtered solution was carried on Alltech alltima C18 column (Alltech Associates Inc, Deerfield, IL, USA) for HPLC and Zorbax Eclipse plus C18 (Agilent Technology, Wilmington, DE, USA) for UPLC. The system suitability test and method validation data were evaluated. The clinical samples (n = 54) were assessed by both methods and then their results were compared. We used Passing and Bablok regression, correlation coefficient, mean difference and paired-t-test for data analysis.

Results: HPLC and UPLC eluted iohexol out at 9.14 and 3.53 min, respectively. Both methods produced symmetry peak and clearly separated peak A and B of iohexol with the resolutions of 1.50 and 2.29, respectively. The RSD of retention time (t_R) and area were 0.07% and 1.80% for HPLC; 0.02% and 1.07% for UPLC. The average recoveries of HPLC and UPLC ranged from 99.8% ± 0.2% to 100.3% ± 2.3% and 97.2% ± 1.1% to 98.6% ± 0.9%, respectively. Intra-assay and inter-assay variants of HPLC and UPLC ranged from 0.94% to 1.88% and 0.94% to 2.44%, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.93 µg/mL and 3.10 µg/mL for HPLC and 0.64 µg/mL and 2.15 µg/mL for UPLC. The comparison equation was y= 0.9918x with a very high correlation (R^2= 0.9966, *p*-value <0.0001). Iohexol obtained by both methods was not statistically different, *p*-value >0.05 (mean difference = -1.00 µg/mL, 95% CI = -14.1 to 12.1).

Conclusions: HPLC and UPLC methods for determination of the plasma iohexol level yielded the same range of results without statistical difference. The traditional HPLC method was slightly easier by the equipment-maintenance process and overall cheaper machine, thus more suitable for a smaller laboratory. The UPLC method was faster that it should be considered for a larger and busier laboratory that timing is a significant constraint.

B-125

Using Lean Engineering Principles and Pre-Analytical Process Improvements to Decrease Specimen Processing Time

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Background: The majority of errors occur in the pre-analytical phase of clinical laboratory testing and it is imperative that pre-analytic processing of specimens is consistent and quality-focused. Once the specimen arrives in the laboratory, processing steps include specimen tracking in the laboratory information system, verifying that serum samples are clotted, and sorting to appropriate testing location. Pre-analytic automation has brought innovation and improvement to core laboratory specimen processing work flow. Beyond automating, additional efficiency may be gained by utilizing Lean engineering principles such as 5S (Sort, Set in order, Shine, Standardize, Sustain), Value Stream Mapping, Just in Time, and Pull Systems. **Objective:** To reduce pre-analytic specimen processing time in the central processing area of the core chemistry laboratory (CCL) at Mayo Clinic using Lean engineering principles.

Methods: The workflow in the central processing laboratory (CPL) from the time specimens are received in the laboratory to loading on the pre-analytic automation (Roche Modular Pre-Analytics, (MPA) Indianapolis, IN) was value stream mapped to identify potential waste in the system. Lab work stations were set up in a standard configuration and clearly labeled using 5S. Specimen processing time (time received in CPL to loading on MPA) data were collected before (8/1/2010 -1/31/2011) and after (2/1/2011 - 7/31/2011) process improvements. The percentage of serum specimens causing a “specimen clotted” error on the MPA was monitored during the same time periods. Daily specimen volume was also tracked and staff satisfaction after Lean improvements was surveyed.

Results: Specimen processing time (time received in CPL to loading on MPA) was reduced by 58% from 14.2±2.3 (mean±SD) minutes to 8.3±1.7 minutes after value stream mapping and 5S Lean principles were implemented. Specimen quality, measured as percentage of serum specimens causing a “specimen clotted” error on the MPA, was maintained at 2% before and after Lean improvements. After implementation of 5S Lean techniques, supplies are restocked when they are needed and sorted specimens are picked up when the operator is ready to load them on the MPA. Staff surveyed reported the perception of reduced workload; although the actual specimen volume increased by 24% from an average of 2595 specimens daily (1/27/10-2/2/2010) to 3235 specimens daily (1/23/2012-1/29/12).

Conclusions: The CPL was able to gain efficiency and reduce specimen processing time while maintaining specimen quality. Waste in the workflow was eliminated and laboratory practices were standardized. 5S is an integral part of maintaining the improvements in specimen processing time. Lean engineering affords an opportunity for process improvement by eliminating wasted effort, time, space, and supplies.

B-126

Total and Direct Bilirubin Measurements Using Eight Different Chemistry Analyzers

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Background: Total and direct bilirubin measurements have been shown to vary from laboratory to laboratory. When a patient is being transferred from a local hospital to a tertiary hospital, it is important to realize that the bilirubin measurements in the local hospital may not be identical to the tertiary hospital due to analyzer differences, although the patient is clinically stable.

Objective: We aimed to evaluate the comparability of total and direct bilirubin tested on eight different analyzers from the tertiary hospital, local hospitals, and outreach clinic.

Methods: Twenty pools of patient plasma with varying total and direct bilirubin results were made. Each pool was aliquoted into 8 vials and kept frozen at -80°C until testing was performed. The set of twenty samples was shipped on dry ice to the various local hospitals and outreach clinic for testing. Results from the various chemistry analyzers were compared to the Roche Modular in Dartmouth-Hitchcock Medical Center. Comparability was assessed using linear regression and Bland-Altman plots.

Results: Correlation results, biases and percent biases between analyzers are listed in the table below.

Conclusions: There was good correlation (r^2 >0.98) and acceptable bias (<10%) for total bilirubin measurements among all analyzers. For direct bilirubin measurements, there was poorer correlation (r^2 >0.89), and bias varied widely (0.2 - 53.2%) among the analyzers. This comparison data can serve as a useful tool for clinicians when interpreting bilirubin results from different sites.

	Total Bilirubin			Direct Bilirubin		
	Correlation	Bias	% Bias	Correlation	Bias	% Bias
Roche Modular vs Roche Cobas 6000	y= 1.0106x – R^2 =0.9987	0	-0.8	y= 0.9637x – R^2 =0.9985	-0.13	-8.1
Roche Modular vs Roche Cobas c111	y=0.9205x + R^2 = 0.9991	-0.32	-7.4	y =0.9314 x– R^2 = 0.9797	-0.21	-12.5
Roche Modular vs Vitros 5,1 FS	y =0.873x + R^2 =0.9946	-0.47	-9.7	y =1.0009 x– R^2 = 0.9859	-0.11	-8.1
Roche Modular vs Vitros 250	y=0.9196x + R^2 = 0.9923	-0.22	-1.2	y= 0.918x - R^2 =0.8969	-0.87	-53.2
Roche Modular vs Dimension ExL	y= 0.9886x – R^2 =0.9923	-0.13	-1.9	y =1.0465 x– R^2 = 0.9912	0.05	-2.3
Roche Modular vs Vista 1000T	y= 0.9477x – R^2 =0.9968	-0.23	-4.5	y=0.9925x+ R^2 =0.9903	-0.02	0.2
Roche Modular vs Architect c8000	y=0.9706x + R^2 = 0.9898	0.03	3.8	y=1.0396x+ R^2 =0.9852	0.31	22.2

B-128

Development and Validation of an Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2) Immunoassay Using Novel Electrochemiluminescent Technology

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Background: Serum Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2) levels may be used to help evaluate growth hormone deficiency. IGFBP-2 is also a highly sensitive marker of malignant progression in different tumors. We have developed a two-site immunoassay for the quantitative measurement of IGFBP-2 in serum using a novel plate based electrochemiluminescent detection technology (Meso Scale Discovery®, MSD).

Methods: The two-step reaction utilizes a monoclonal-polyclonal pair of IGFBP-2 antibodies. The monoclonal antibody is immobilized on the carbon surface of the MSD 96-well plate, acting as a capture antibody. The polyclonal antibody is conjugated with an electrochemiluminescent reporter molecule (MSD SULFO-TAG™) to generate a signal antibody. Serum samples containing IGFBP-2 are allowed to react in the capture antibody-coated plate well. Non-IGFBP-2 material is washed off and the IGFBP-2-bound plate is incubated with the signal antibody. IGFBP-2 present in each well is "sandwiched" between the plate-bound antibody and the signal antibody. After washing, bound IGFBP-2-complex emits light upon application of electrochemical stimulation initiated at the electrode surfaces of the microplate by the MSD instrument. The signal generated is directly proportional to IGFBP-2 concentration.

Results: The reportable range of the assay was from approximately 30 to 1700 ng/mL. The lower limit of detection was 0.5 ng/mL and the lower limit of quantitation was approximately 30 ng/mL. Precision (%CV) was validated using four levels of IGFBP-2 serum controls. Six replicates of each level were analyzed in four assay batches. The mean interassay CV at 95,179, 246 and 369 ng/mL was 5.5%, 4.5%, 3.9% and 4.9% respectively. Dilutional linearity of four IGFBP-2 samples diluted with the assay diluent produced mean recoveries of 113% - 123%. Method comparison with 27 serum samples previously tested with a commercial IGFBP-2 ELISA assay (Mediagnost®) yielded the following regression characteristics: slope = 1.08, intercept = -57.0 ng/mL and r = 0.95. Acceptable sample types were serum, serum with separator gel, EDTA plasma and heparin plasma. IGFBP-2 stability was evaluated using four freshly drawn serum samples and was demonstrated to be stable at ambient temperature for two days and refrigerated for up to three days. Stability was also confirmed after undergoing six freeze/thaw cycles.

Conclusions: The IGFBP-2 electrochemiluminescent assay combines novel immunoassay technology with high assay sensitivity and precision performance that is comparable to a reference commercial IGFBP-2 immunoassay.

B-130

Application of a PNA-clamping real-time PCR method to predict the response to tyrosine kinase inhibitors from pleural effusions of patients with non-small-cell lung cancer

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Background: Mutations in epidermal growth factor receptors (*EGFR*) is an important marker to predict the response to tyrosine kinase inhibitors (TKI) in non-small cell lung cancer (NSCLC) patients. Currently direct sequencing of the *EGFR* genes from lung biopsy samples remains the standard method to detect such mutations. However, for patients whose tumor biopsies are difficult to obtain, detection of *EGFR* mutations would not be easy. Pleural effusions are often observed in NSCLC patients and thus may serve as a potential substitute to detect the *EGFR* mutations. To evaluate whether pleural effusions are appropriate samples for the detection of *EGFR* mutations and the reliability of a newly designed PNA-clamping real-time PCR method.

Methods: A clamping PCR with specific peptide nucleic acid (PNA) probes targeting at the in-frame deletion at exon 19, a T790M mutation at exon 20, and a L858R point mutation at exon 21 of the *EGFR* genes was used to detect such mutations among the samples. In the preliminary experiments, paired samples of pleural effusions and lung biopsies were available from 13 NSCLC patients and were used to compare the PNA-clamping real-time PCR method with the conventional PCR and direct sequencing performed on biopsy samples. Clinical application of the PNA-clamping real-time PCR method was subsequently performed on 44 pleural effusion samples collected from patients diagnosed as NSCLC in Chang Gung Memorial Hospital, Linkou, Taiwan, during March 2010 and October 2011. The results were correlated to the TKI response evaluated by the Response Evaluation Criteria in Solid Tumor (RECIST).

Results: A total of 14 mutations were identified among the 13 biopsy samples and 12 of them were correctively identified by the PNA-clamping real-time PCR method. An exon 20 insertion and a G719A mutation were not identified by the PNA-clamping real-time PCR method because they are not located in the region covered by the PNA probes. The sensitivity and specificity of the PNA-clamping real-time PCR method were therefore estimated as 83.3% and 100%, respectively. By using the PNA-clamping real-time PCR method, *EGFR* mutations were further identified in 26 (59.1%) of the 44 pleural effusions. Majority of the mutations were in-frame deletions at exon 19 (14 samples, 53.8 %) and a L858R mutation at exon 21 (10 samples, 38.5%). A T790M drug-resistant mutation was further identified in 6 of these 26 samples with mutations. For patients with either the exon 19 deletion or the L858R mutation but without the T790M mutation, the rate of TKI response was 75.0%. Eighteen of the 44 samples did not have any *EGFR* mutations, but 3 (16.7%) of the patients had a good TKI response. All the 6 patients with the T790M drug-resistant mutation had an adverse progressive disease.

Conclusions: Pleural effusions appeared to be a good substitute for tissue biopsy in the detection of *EGFR* mutations among NSCLC patients. Compared to lung biopsies, pleural effusions are easier to be collected, making it useful in the prediction of treatment responses, monitor of disease progression, and early detection of treatment failures associated with secondary, drug-resistant mutations.

B-131

Development of sensitive assays for detecting EGFR and KRAS mutations using peptide nucleic acid probes

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Background: Mutations in epidermal growth factor receptor (EGFR) and KRAS genes determine the efficacy of targeted therapy with gefitinib and erlotinib in non-small cell lung cancers (NSCLC). Detection of these mutations is thus important before applying the therapy. However, if a clinical sample consists of <20% tumor tissue, the mutations would be difficult to detect using conventional PCR and sequencing. A more sensitive and specific method is required for detecting mutations in this kind of samples. The aim of this research is to develop sensitive diagnostic assays for EGFR and KRAS mutation detection, taking advantage of our newly established technology using peptide nucleic acid (PNA) probe as both PCR clamp and sensor probe.

Methods: We designed PNA probes and paired anchor probes covering 3 mutational hotspots of EGFR gene (L858R, exon 19 deletions, and T790M) and one mutational hotspot of KRAS gene (including different types of mutations in codons 12 and 13). The PNA probes served as PCR clamp for inhibiting the amplification of wild-type template but allow the amplification of mutant templates. The PCR components and reaction conditions were optimized for detecting rare mutant in the wild-type

background. Sensitivity and detection limit of the assays were determined using DNA extracted from mixed cell-lines, pleural effusions, and peripheral blood. Finally, the optimized components were assembled into ready-to-use reagents.

Results: Three ready-to-use reagents were established for detecting 21 EGFR mutations, including 19 small deletions in exon 19 and two point mutations at amino acid 858 and 790. One reagent was set up for detecting 12 KRAS mutations in codons 12 and 13. Using mixed cell-line DNA, all the assays could detect less than 0.1% mutant in the wild-type background. The loaded genomic DNA ranged from 200 ng to 50 pg can be successfully amplified. Each assay can be completed within 45 min in a single tube. Using DNA from pleural effusions and plasma, the assays detected around 90% of mutants discovered in original tumor tissues. In addition, the ready-to-use reagents are stable for 6 months when stored in the dark at -20°C.

Conclusions: We had developed sensitive and fast assays for detecting rare EGFR and KRAS mutations in the wild-type background. The assays are suitable for analyzing DNA from fresh tissue, pleural effusions, bronchial alveolar lavage, and blood.

B-132

Filtration Device for Centrifuge-Free Isolation of Liquid Plasma from Clinical Samples of Whole Blood

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Objectives. Demonstrate the effectiveness of a novel filtration device for the rapid isolation of liquid plasma from 0.8-3.0 mL of whole blood without the use of a centrifuge. Illustrate the application of the device for plasma sample preparation in terms of volume of collected plasma, hemolysis and target analyte recovery.

Materials and Methods: The filtration device comprises a plasma separation membrane, and a housing having an inlet, a downstream chamber, and an outlet. The plasma separation membrane is positioned in the housing across the fluid flow path such that, when a blood sample is loaded on the upstream surface of the membrane and absorbed by it's matrix, liquid plasma is collected from the downstream surface through the outlet. The device was tested using human blood collected from healthy donors into blood collection tubes containing EDTA anticoagulant. Whole blood samples of 0.8-3.0 mL were loaded onto the device. Plasma was collected, measured and analyzed for the presence of residual blood cells, free hemoglobin, concentration of total protein, total cholesterol and HPLC profiling of plasma proteins. Control plasma was prepared from the same blood by a common centrifugation method.

Results: It was demonstrated that 100-800 uL of liquid, cell-free plasma can be collected in 2-5 min from 0.8-3.0 ml of blood by a simple procedure avoiding centrifugation steps. The process of plasma separation on the tested filter device does not cause additional RBC hemolysis - hemoglobin concentrations measured using the device plasma and control plasma samples are comparable. The recovery of total protein and total cholesterol is greater than 90%. The HPLC protein profile of the collected plasma in the minor fraction is also very similar to the control plasma.

Conclusions: The study results demonstrate the efficient performance of the filtration device in the rapid centrifuge-free production of plasma. Samples of 100-800 uL cell-free plasma can be collected from 800-3.0 mL of whole blood in 2-5 minutes. The quality of plasma collected using the filter device is comparable to plasma prepared by centrifugation with respect to the degree of RBC hemolysis, total protein, total cholesterol and the HPLC protein profile.

B-133

A Novel Multiplex Analysis of Filaggrin Polymorphisms. An Universally Applicable Method for Genotyping

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Background: Filaggrin is a protein located in the epidermis with a repetitive filaggrin gene located in the epidermal differentiation complex on chromosome 1q21 and the protein is important for skin barrier function. Deficiency due to functional null-polymorphisms affects 8-10% of the population in Northern Europe and cause ichthyosis vulgaris, and is a strong risk factor for atopic dermatitis, asthma, rhinitis, and food allergies. We describe a method for genotyping of three common variations in the filaggrin gene. The method is versatile and universally applicable for multiplexed genotyping.

Methods: Five known techniques are combined i) allele-specific PCR, ii) PCR with tagged primers, iii) asymmetric PCR, iv) multiplexed PCR, and v) hybridization of single-stranded PCR products to spectrally coded microbeads carrying tag-sequences as individual capture-probes. Asymmetric PCR is accomplished as the tagged and allele-specific forward primers of the three individual PCR's are present in limiting concentrations relative to the corresponding reverse primers. Asymmetry ensures that the later PCR cycles generate only single-stranded reverse strand products. This leads to highly improved assay sensitivity and allows for easy assay optimization

Results: Specificity of the tags was verified by single PCR with wildtype and homozygote samples. Only PCR-products with the appropriate anti-tag hybridized to the corresponding beads, indicating a specific signal. The hybridization signal strongly dependent upon single-stranded PCR products resulting from the depletion of forward primers. After 40-48 PCR cycles double-stranded PCR products are clearly present, but only the single-stranded PCR products generated in later cycles hybridize and elicit the strong signals that allow for unambiguous genotyping.

Conclusions: We have tested > 17,000 samples for three filaggrin polymorphisms using this method with a call-rate exceeding 99.0% and at reagent cost of only 0.75 US $ per sample. The method is universally applicable for multiplexed genotyping, and may be adapted for genotyping of for instance hereditary hemochromatosis, lactose intolerance, or cystic fibrosis.

B-136

Multiplex Autoantibody Detection Using MagArray GMR Biosensors

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Background: We are developing multiplex autoantibody assays based on magnetic sensors of the MagArray platform. The serum autoantibodies were selected for their demonstrated potential in the diagnosis and prediagnosis of lung cancer. The Magarray platform utilizes magnetic nanotags for signal generation and detection. By monitoring multiple sensors on a single chip, multiplexed detection of autoantibodies is made possible. Also, since magnetic signals rather than optical signals are generated and detected from the tags, interferences with optical output by biological matrices in a conventional optical-based detection system have no effect in the current platform.

Objective: Feasibility study of multiplex detection of autoantibodies in serum samples that are related to lung cancer, which are anti-14-3-3theta, anti-LAMR1, and anti-ANXA1 autoantibodies.

Methodology: We have screened and selected recombinant human proteins of 14-3-3theta, ANXA1, and LAMR1 as targets of interested autoantibodies. The antigens were spotted onto magnetic sensors in replicates on individual MagArray chips. Individual sera collected from 5 patients were hybridized to individual microarrays. The electronic readout of the magnetic signals from the assays were recorded and compared with corresponding fluorescence-based microarray data.

Results: Detection of autoantibodies on series dilutions of serum samples is demonstrated and the results compared with regular fluorescence microarray data. Most of the data points obtained from MagArray chips qualitatively agreed with fluorescent results with the exception of 2 out of 20 data points that don't agree. Intra-assay CVs are below 10% for the range of autoantibody concentrations studied, and inter-assay CVs are below 15% which are defined as chip to chip signal variations when measuring the same sample.

Conclusions: MagArray platform shows excellent performance in the multiplex detection of autoantibodies related to the diagnosis of lung cancer. The instrument is novel, multiplex, easy to use, and free of matrix effect.

B-137

Evaluation of EGFR and HE4 on MagArray High Sensitivity Immunoassay Platform

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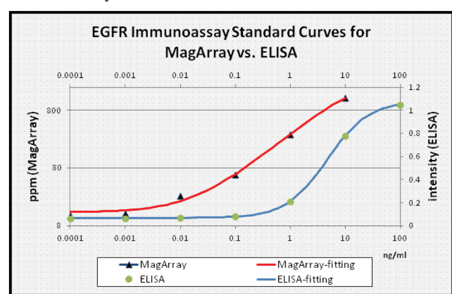
Background: HE4 and EGFR are important clinically relevant biomarkers. We report here that the MagArray platform is particularly suitable for the detection of HE4 and EGFR with ease of use and high sensitivity. Rather than detecting optical labels, the MagArray platform is based on the detection of magnetic labels that are free of optical interference in biological matrices. We report here that MagArray's magnetic biosensors is a sensitive and simple platform for detecting HE4 and EGFR in human sera.

Methods: Using MagArray biochips, antibodies for EGFR and HE4 (Human epididymis protein4) were spotted on individual sensors. Serum samples were hybridized on the microarrays followed with detection antibody incubations adopting a standard ELISA configuration. Instead of enzymatic labeling and signaling mechanism, magnetic tags were labeled and detected. The accuracy and sensitivity of

results on magnetic sensors were compared with standard ELISA assays.

Results: The detection limits of EGFR is 100 times better than that of ELISA as shown in the Graph, and HE4 20 times better. The inter assay CV was less than 20% for both biomarkers between 5 runs in the concentration range studies.

Conclusions: MagArray platform is demonstrated to be a sensitive and easy-to-use platform for immunoassays.



B-138

Development of a rapid real-time model system for isothermal amplification

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Despite the widespread applications of thermal-cycling polymerase chain reaction (PCR) technique in molecular diagnosis, there remain needs of a miniaturized amplification system which can be operated in both well-equipped and resource-limited areas. Recently, more and more novel approaches based on isothermal amplification are proposed and validated. The objective of this study is to evaluate and develop isothermal amplification systems for applications in a point-of-care or resourced-limited setting. With specifically designed primers, a modified loop-mediated isothermal amplification (LAMP) method can be realized under a single incubation temperature thereby reducing the cost and complexity of the instrument. In this presentation we report the design and development of a model system for the modified LAMP method. The model system is able to amplify the target sequence with high selectivity, since it recognizes the target by six distinct sequences initially and then by four distinct sequences. The detection method for amplified product is based on measuring the turbidity caused by increasing quantity of Magnesium pyrophosphate in solution. The miniaturized optical system includes laser diode array, photodetector array, and well-aligned aperture to increase signal to noise ratio. Evaluation of optical simulations by ZEMAX has been done to optimize the detection efficiency. The thermal controller can achieve the operating temperature in 3 minutes from room temperature, and the stability of ±0.1 degree. The amplification run time is less than 30 mins. Performance comparison of the model system and a commercial machine are demonstrated with Lamda-DNA. In conclusion, a novel rapid detection system which achieves real-time and quantitative detection for LAMP reagents has been developed. It integrates both efficient reagents and detection platforms in one system. The benefits of simplified system and shortened reaction time have the potential of point-of-case applications.

B-139

Automation of Assays Based on Single Molecule Arrays: Development of a Flow-Cell Based Procedure on Microfabricated Polymer Array Assemblies

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Objective: The aim of this work was to develop the next generation immunoassay analyzer using a method that isolates individual paramagnetic beads in arrays of femtoliter-sized wells and detects single enzyme-labeled proteins on these beads using sequential fluid flows in microfabricated polymer array assemblies for ultra-sensitive medical diagnosis. This approach, which is developed based on previously reported single-molecule arrays (SIMOA) technology, allows for automated, low-cost and high-throughput precise measurements of clinically relevant biomarkers at unprecedentedly low concentrations over a broad dynamic range.

Methods: Detection of single molecules using SIMOA has been reported previously. In brief, proteins are captured on antibody-coated beads (2.7-mm diameter) and labeled with single enzymes, followed by partitioning single beads into arrays of 50,000 femtoliter-sized wells and sealing the arrays in the presence of a fluorogenic substrate. We developed enclosed polymeric assemblies that perform the functions of loading and sealing of paramagnetic beads associated with single enzyme molecules in arrays of femtoliter-sized wells using only fluidic flow. We employed a micro-replication technique (injection molding) using a well-characterized, low fluorescence polymeric material, namely cyclic olefin polymer (COP), to fabricate the femtoliter-sized well arrays and the fluidic structures to allow the delivery of fluids to the arrays. Using the microfabricated COP arrays, we developed a non-mechanical method to load, seal, and image individual paramagnetic beads in arrays of femtoliter-sized wells. Sealing was accomplished using fluorocarbon oil that is immiscible with the aqueous assay medium. We compared the performance of this device to our current method based on optical fiber bundle arrays and achieved comparable, and, in some cases, superior results. By integrating bead loading, sealing, and imaging into a single device that is operated with minimal manual intervention, we could enable the development of a fully automated, low-cost instrument for performing single-molecule immunoassays.

Results: Using this approach for PSA detection by digital immunoassay, we demonstrated a limit of detection of 0.016 pg/mL, over 1000-fold more sensitivity than conventional PSA immunoassays. Over 4 logs of dynamic range were demonstrated with high precision. Ultra-sensitive PSA detection has proven to have important clinical implications such as post radical prostatectomy monitoring and recurrence prediction.

Conclusions: We have developed an approach for performing single-molecule isolation and detection in large arrays of microwells in a low-cost polymeric device using fluidic flow. This approach leverages established high-volume polymer manufacturing to realize low-cost, disposable single molecule arrays. These devices could enable the development of fully automated instrumentation for performing high-throughput testing of samples in single-molecule arrays for applications in life science research and *in vitro* diagnostics.

B-140

Introduction of the Vantera® Clinical Analyzer for NMR-Based Testing

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Background and Objective: For over four decades, nuclear magnetic resonance (NMR) spectroscopy has served as a powerful analytical tool used mostly for chemical structure determination and basic biomedical research. Although the NMR signal intensity is quantitative in nature, the complexity of the technology, lack of automation, and absence of a “killer app” have kept NMR spectroscopy from being employed in clinical laboratory medicine. We have developed the first NMR Clinical Analyzer, named *Vantera*®, to enable reliable, fully automated NMR-based quantification of lipoproteins and small molecule metabolites by laboratory personnel with no NMR expertise.

Features: *Vantera*® consists of an automatic sample handler, 400MHz (^{1}H) superconducting magnet with a flow probe, and an Agilent 400MR console, all of which is packaged in an enclosure to give the touch and feel of a regular clinical analyzer. Proprietary software integrates the entire system from automatic calibration and magnetic field shimming, reading the tube barcode, sample preparation and delivery, NMR acquisition and processing, and reporting patient results directly or through commercial lab interfaces. The touch screen graphical user interface makes operation of the clinical analyzer by personnel lacking any formal NMR training easy and intuitive.

Applications: *Vantera*® was developed as a multi-assay platform for easy expansion of menu. The initial application, the *NMR LipoProfile* test (lipoprotein particle test), uniquely provides quantification of the particle concentrations of several different-size VLDL, LDL, and HDL lipoprotein subclasses. Despite previously being offered only as a laboratory developed test (LDT), clinicians have ordered the test over 7 million times to help assess and manage the risk of atherosclerotic cardiovascular disease and guide treatment with LDL-lowering drugs. The particle test takes only 101 sec. to run on the *Vantera*® platform, and provides good precision (4 - 8 % CV) for the different classes of lipoproteins measured. The key assay characteristics are presented separately. *Vantera*® also provides triglyceride and HDL-cholesterol results that correlate well with the traditional chemical methods of analysis. Potential future applications include analysis of bio-fluids for high throughput metabolomic studies and disease prediction including multivariate analysis.

Conclusions: A fully automated NMR clinical analyzer platform has been developed to enable routine high-throughput NMR analysis of bio-fluids for medical diagnostics with widespread potential in areas including atherosclerosis, diabetes and oncology.

Tuesday PM, July 17, 2012

Poster Session: 2:00 PM - 4:30 PM
Electrolytes/Blood Gas/Metabolites

B-141

Laboratory Approach in a patient with Hereditary Renal Hypouricemia presenting with Rhabdomyolysis

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Renal Hypouricemia is a rare disorder in which there is isolated defect of UA transport in the renal tubules leading to profound hypouricemia along with increased Urinary UA excretion. This condition is very rare and most cases till date have been seen in Japanese population with a prevalence rate of 0.12 %. Nephrolithiasis and exercise induced ARF (EIARF) have been found to be associated with patients with Renal Hypouricemia. Patients with renal hypouricemia have a 200-fold greater predisposition to exercise-induced ARF than those without renal hypouricemia. Hereditary hypouricemia complicated by nephrolithiasis and EIARF has mainly been reported in Japanese patients with loss-of-function URAT1 gene mutations.

We present a 19 year old patient who presented with Rhabdomyolysis along with Acute renal Failure after an episode of unaccustomed, severe exercise. On admission he had a Creatinine of 8.3 mg/dl, Urea 100 mg/dl and CK of 10,127 U/L.He was treated with few sessions of hemodialysis which led to recovery in a few days with serum Creatinine of 1.5 mg/dl at discharge. 2 months later during follow up, he was found to have a severe hypouricemia (Uric Acid 0.3 mg/dl).His Fractional Excretion of Uric Acid and 24 hour Urinary UA was estimated in order to evaluate the cause of hypouricemia.The FE UA was 150% with 24 hour Urine UA of 437 mg/dl.This high FE UA in the presence of marked hypouricemia led to the diagnosis of Rhabdomyolysis induced by Renal Hypouricemia.We screened the patients brother for the same parameters.His Serum UA was 0.2 mg/dl along with a FE UA of more than 150%.Thus we confirmed this as a case Hereditary Renal Hypouricemia.

UA is the most abundant aqueous antioxidant and helps in preservation of endothelial dilation in the time of oxidative stress. Uric acid is a powerful antioxidant, and is a scavenger of oxy¬gen free radicals which play a vital role is maintenance of this redox balance. Hence the lack of UA in plasma of patients with Hereditary Renal hypouricemia predisposes them to ischemic acute renal failure when exercise leads to renal vasoconstriction and excessive production of free radicals in renal tubules. This case also highlights the fact that exercise induced free radicals might be responsible for myocyte injury because of the impaired antioxidant balance which can lead to Rhabdomyolysis.

This is the first known case of Renal Hypouricemia presenting with Rhabdomyolyis. Previous literature reports mainly cases of this disorder presenting with EIARF. But this case of ours shows that hypouricemia might not affect just the renal tubules but also the muscle leading to oxidative stress induced myocyte injury. Simple and easily available laboratory tests can be of vital importance in diagnosis of this uncommon but potentially dangerous disorder.

B-142

Design and prototype of an inter-laboratory comparison program for urine bicarbonate

R. L. Benjamin, P. Berman, J. King. *University of Cape Town, Cape Town, South Africa*

Objective: Construct an inter-laboratory comparison (ILC) program for proficiency testing (PT) of urine bicarbonate.

Relevance: The classification and diagnosis of renal tubular acidosis (RTA) requires the calculation of the fractional excretion of bicarbonate (Fe(HCO_3)). In South Africa, Fe(HCO_3) is not waived, RTA prevalence is unknown and the presentation of failure to thrive (FTT) is common. Accredited laboratories cannot offer urine bicarbonate analysis without PT. Thus screening for RTA in the FTT population requires urine bicarbonate PT. This ILC model can be generalised for the PT of other unstable analytes such as pyruvate.

Methodology: The Shapiro-Wilk test and QQ-plots excluded normality. The 95% confidence interval (CI) of the median was established using the Wilcoxon signed rank test. Urine bicarbonate analysis was validated on the Beckman-Coulter® DxC using the recovery of spiked analytical grade $NaHCO_3$ (80-103% for central 90%) and NH_4HCO_3 (74-91% for central 90%) at three levels (13.5 mmol/L, 27 mmol/L and 54 mmol/L). Spiked urine stored in sealed microcentrifuge tubes was stable for at least twenty four hours at room temperature and at 4°C. The functional sensitivity was 5 mmol/L with an intra-laboratory coefficient of variation (CV) of 18% (at 10 mmol/L). The manufacturer's analytical range is 5-50 mmol/L. The recoveries at the three levels were 82% (CI 77-89% at 13.5 mmol/L), 85% (CI 78-92% at 27 mmol/L) and 80% (CI 74-86% at 54 mmol/L), thus establishing linearity. The ILC program distributes three microcentrifuge tubes, containing fixed masses of $NaHCO_3$, to each of three laboratories. Each laboratory adds a predefined volume of random urine (one urine specimen per laboratory) to each of the microcentrifuge tubes such that three $NaHCO_3$ concentrations are obtained. The differences between the two highest concentrations and the lowest concentration are plotted on a Youden plot. The differences are also plotted as a standard deviation index (SDI). The limits of acceptability were based on the CV of the original validation results and on the CV of the group.

Results: Both $NaHCO_3$ and NH_4HCO_3 were suitable as substances for the ILC program. $NaHCO_3$ was selected as it is cheaper, more soluble and less irritant. One of the three laboratories performed poorly when graphed on either a Youden plot, SDI or recovery provided the original validation criteria were used. The marginal cost of implementing the program in the Western Cape of South Africa is US$0.50 per quality control cycle (three levels in duplicate) per laboratory. The marginal cost to the evaluated laboratory is US$2.12 per quality control cycle.

Conclusions: It has been possible to construct a cost-effective ILC program based on the difference in recovery of an unstable analyte (bicarbonate). The matrix-appropriate analyte concentrations were reconstituted by adding random urine from the evaluated laboratory - each laboratory could only use one urine specimen. The urine specimens differed among laboratories. The approach was sensitive enough to identify a non-conformance in one of the laboratories tested. This finding proves both that the approach can be used for unstable analytes and that a need for PT of urine bicarbonate exists.

B-143

Ionized Calcium Reference Ranges Determined from Archived Patient Results

P. R. Bach. *Primary Children's Medical Center, Salt Lake City, UT*

Background: Ionized calcium is frequently measured for diagnostic and acute care purposes. When clinicians suggested that our reference ranges were too high, we evaluated them using archived patient results.

Methods: Archived patient results obtained between Sept 2010 and Aug 2011 were retrieved. Some were from whole blood collected in blood gas syringes and measured by Radiometer ABL800 FLEX analyzers, Some were from whole blood collected by various means and measured by *i-STAT* (Abbott Point-of-Care), and some were from serum collected by venipuncture and measured by AVL 9180 Electrolyte Analyzers. ICD9 codes were used to exclude patients with diagnoses or procedures known to affect ionized calcium. Results for each specimen type/test method combination were plotted versus patient age, and age brackets were determined visually from these plots. Reference ranges (central 95% of normal distribution) were determined by the method of Hoffman (*Am J Clin Pathol* 2010;133:180-6), a statistical tool for identifying normal distributions within data sets containing numerous outliers.

Results: All ranges are in mmol/L. After excluding diagnoses and procedures known to affect ionized calcium, enough results remained to determine whole blood ranges for all age groups and serum ranges for adults. Except for serum in older adults, the same age brackets were used for all specimen types and instruments. Whole blood (ABL) ranges we obtained were: 0-2d, 1.04-1.39, n=828; 3d-1m, 1.19-1.50, n=732; 2-11m, 1.19-1.45, n=225; 1-9y, 1.12-1.39, n=617; 10-17y, 1.09-1.33, n=398; >17y, 1.03-1.25, n=4722. Except for ages 0-2d, i-STAT and ABL ranges were very similar. We obtained separate serum ranges for adults: 18-49y, 1.03-1.36, n=514; >49y, 1.03-1.43, n=1064.

Discussion: The "ideal" reference range study uses specimens from known healthy subjects, collected and analyzed by a standardized protocol. We chose to use patient data because it represents clinical practice. Ranges we previously used for both serum and whole blood were 1.15-1.48 (pediatric) and 1.16-1.32 (adult). The new pediatric ranges are similar to the old, but the new adult ranges are lower. Hospitalized patients often have lower ionized calcium. Increasing altitude stimulates hyperventilation, increasing pH and lowering ionized calcium at our altitude of 1400m by about 0.05 mmol/L, not enough to explain the difference in adult ranges. Instrumentation has changed since the previous ranges were determined 20 years ago. But hospitalization, altitude, and instrumentation would be expected to affect children and adults similarly. The difference

in adult ranges remains unexplained. Agreement between ABL and i-STAT was better than expected considering the differences in specimen type (arterial in a closed syringe versus capillary or venous exposed to air) and testing methodology. The new serum ranges are about 0.10 mmol/L higher than whole blood at the upper end. The serum data included more outpatients. We could not identify or exclude patients taking calcium or vitamin D supplements and this may explain the higher serum values.

Conclusions: This study illustrates the need to confirm published reference ranges before use, and the possibilities and limitations of determining reference ranges from patient data.

B-144

Utility of the Factored Jaffe IDMS Creatinine on Siemens Dimension Systems: Alignment with IDMS Traceable Methods

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Objectives: To compare the utility of the Siemens Dimension® systems Factored Jaffe IDMS creatinine (Fact-IDMS) method to IDMS traceable methods.

Methods: Forty patient plasma samples were tested on Dimension® Xpand®, Dimension® EXL™ and two Dimension Vistas® using the non-IDMS Jaffe creatinine method. A Siemens validated factor was used to convert the results to IDMS-like **Results:** Fact-IDMS = Jaffe - 14.9 umol/L (0.17 mg/dL). The samples were also tested using IDMS traceable methods - enzymatic creatinine (Vista, Xpand, EXL, Abbott i-STAT, Ortho Vitros) and Jaffe creatinine (Roche Integra).

To assess the impact of any differences in creatinine results for the different methods/analyzers on estimated Glomerular Filtration Rate (eGFR), the mean creatinine result for each method/analyzer was used to calculate eGFR (MDRD and CKD-EPI) for both genders at three different ages (30, 50 and 70 years).

Results: The non-IDMS Jaffe creatinine concentration range from Vista was 46 - 397 umol/L (0.52 - 4.49 mg/dL). Data analysis included only results for the 33 samples with non-IDMS Jaffe creatinine within the concentration range (27 - 221 umol/L, 0.30 - 2.50 mg/dL) used by Siemens to derive the factor. Results are shown in the table.

Analyzer	Siemens Dimension				Abbott	Ortho	Roche
	Vista 1	Vista 2	Xpand	EXL	i-STAT	Vitros	Integra
Mean Creatinine umol/L							
Jaffe	105.8	110.4	111.6	110.1			
Fact-IDMS	90.8	95.6	96.6	95.2			
IDMS	98.4	94.0	86.5	91.7	96.4	95.4	93.4
Mean Creatinine mg/dL							
Jaffe	1.20	1.25	1.26	1.25			
Fact-IDMS	1.03	1.08	1.09	1.08			
IDMS	1.11	1.06	0.98	1.04	1.09	1.08	1.06

As expected, the non-IDMS Jaffe creatinine results showed positive bias compared to Fact-IDMS results and results from IDMS traceable methods, including the Roche IDMS traceable Jaffe method. Comparison of the means of the Fact-IDMS results showed acceptable agreement between Vista, Xpand and EXL. These results also showed acceptable agreement to the means for the IDMS traceable creatinine results on Vista, Xpand, EXL, i-STAT, Vitros and Integra. Consequently, there was good agreement of the eGFR's (MDRD and CKD-EPI) for the Fact-IDMS and the IDMS traceable creatinine methods on each analyzer.

Conclusions: Fact-IDMS and corresponding eGFR (MDRD and CKD-EPI) results were appropriately aligned with creatinine and corresponding eGFR results for IDMS traceable creatinine methods.

B-145

Plasma Ammonia Reference Range Establishment

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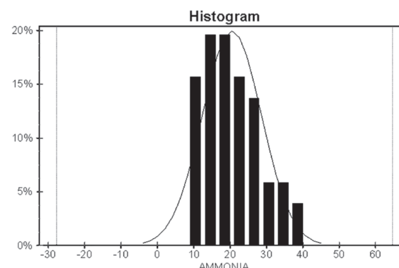
Background: Measurement of plasma ammonia is important in diagnosing inherited disorders of urea metabolism cycle and Reye's syndrome and in the differential diagnosis of encephalopathy. Appropriate reference ranges allow for effective utilization of an assay. The aim of the current work was to establish ammonia reference ranges in a local healthy population.

Methods: Collection of blood samples for reference range determination was approved by the Institutional Review Board. In brief, EDTA whole blood samples (n = 51) were collected from healthy adults (12 males), aged 19-64 y (38.8 ± 12.6), after a minimum of 8 hour fasting. The exclusion criteria were: pregnancy; body mass index (BMI) below 15 or above 30; having a cold, flu, virus or other infection in the past two weeks; a diagnosis of diabetes, malabsorption syndrome or Crohn's disease; gastric or intestinal surgery, or frequent diarrhea; chemotherapy in the past year, current immunosuppressant therapy. Blood samples were stored and transported on ice, spun and assayed on a P modular system (Roche Diagnostics, Indianapolis, IN) within 2 hours of collection. Statistics were performed using EP Evaluator Release 9 (Data Innovations, South Burlington, VT).

Results: Ammonia results displayed a slight positive skew in the distribution for this reference population (Figure 1). The reference interval for ammonia using a transformed parametric method provided a overall range of 9.0 to 41.0 µmol/L, while the range for females (n=39) was 8.8 to 35.6 µmol/L and for males (n=12) was 12.5 to 51.0 µmol/L.

Conclusions: The reference range determined in this healthy adult population was gender dependent with higher levels for males.



B-146

Determination of ABL800, ABL90, GEM 3000 and iSTAT whole blood ionized calcium reference intervals in a healthy population: Evaluation of instrumentation and sampling biases.

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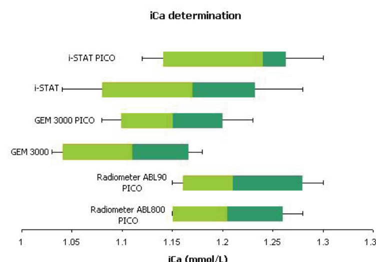
Background: Ionized calcium (iCa), the biologically active fraction of calcium, is primarily regulated by the parathyroid. There is a significant variation in the published reference intervals of healthy individuals. In our study, we wished to determine the reference intervals for iCa using either blood collection tubes containing lithium heparin or commercially available syringes containing electrolyte balanced heparin

Methods: Venous blood samples were drawn from 70 healthy subjects after 10 minutes of sitting into 1.5 mL PICO syringes containing balanced heparin (Radiometer, Westlake, OH) and 2 mL Becton Dickinson lithium heparin blood collection tubes (Franklin Lakes, NJ). Syringe samples were analyzed by Radiometer ABL800 and ABL90 as well as the GEM 3000 (Instrumentation Laboratory, Bedford, MA) and i-STAT (Abbott Diagnostics, Abbott Park, IL). Samples from blood collection tubes were analyzed by GEM 3000 and i-STAT. We used the inner 95 percentile limits to define the reference interval.

Results: The figure summarizes the results. The external vertical lines (whiskers) represent the 2.5-97.5 percentile range. With the balanced heparin syringe, the iCa reference intervals are similar for the ABL800, ABL90 and i-STAT. When the lithium heparin blood collection tubes are used with the i-STAT and GEM 3000, the reference interval is shifted to the left. The use of balanced heparin syringes with the GEM 3000 did not completely shift the mean to that of the Radiometers' iCa mean.

Conclusions: Good agreement exists between the Radiometer ABL800 and ABL90. We recommend the use of the electrolyte balanced heparin syringes for the measurement of iCa by i-STAT to obtain results comparable to the Radiometers. The GEM 3000 iCa reference intervals are very different from the Radiometers, regardless of whether the balanced heparin syringe is used or not. A similar discrepancy is noted in i-STAT when the samples are analyzed from the lithium heparin blood collection tubes.



B-148

Are the acid-base profiles of elite and amateur runners at rest different?

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Background: Elite long-distance runners (ER) show better performance when compared to amateur long-distance runners (AR). However, the electrolyte and acid-base profile in these two groups has not been well described yet. The aim of this study was to compare the blood-gas, electrolyte and acid base parameters between ER and AR at rest.

Methods: Participated this study twenty five AR (male=23 and female=2; $VO_2max = 38.3\pm6.3$ $ml \cdot kg^{-1} \cdot min^{-1}$) and twenty ER (male=14 and female=6; $VO_2max = 54.0\pm9.0$ $ml \cdot kg^{-1} \cdot min^{-1}$), age = 30.6 ± 6.0 years. Blood samples were collected at rest state through digital puncture (Clinitubes®, Radiometer, Copenhagen®) and analyzed immediately in the blood gas analyzer Stat Profile® pHOx®Plus L (Nova Biomedical®,USA) and included: bicarbonate concentration (HCO_3^-), pH, pCO_2, pO_2, base excess, O_2 saturation (SaO_2), anion gap, hematocrit, hemoglobin, sodium, potassium, chloride and lactate concentrations. External control Stat Profile was run in parallel with the analysis. The coefficients of analytical variation (CV_A) are showed in Table 1. Unpaired t test was applied to verify differences between ER and AR blood parameters.

Results: The Table 1 shows the main results. Significant higher values were found in ER compared to AR for HCO_3^- and chloride concentrations, base excess and pCO_2. Anion gap and sodium concentration were lower in ER in comparison to AR. SaO_2, pH, hemoglobin, potassium and lactate concentrations were not different between ER and AR.

Table 1. Mean values for acid-base and blood gas profile in AR and ER.

Analysis	Amateur Runner	Elite Runner	p	CVA (%)
HCO-3 (mmol/L)	25.7 ± 1.65	28.3 ± 2.0	<0.001	-
pH	7.48 ± 0.02	7.49 ± 0.03	0.470	0.9
pCO2 (mmHg)	33.9 ± 2.9	36.9 ± 3.7	0.004	6.2
pO2 (mmHg)	76.9 ± 10.6	79.9 ± 6.7	0.306	7.2
Base Excess (mmol/L)	3.21 ± 1.43	5.6 ± 1.6	<0.001	-
SaO2(%)	95.9 ± 1.8	93.4 ± 1.1	0.356	0.5
Anion gap (mmol/L)	26.6 ± 6.1	16.9 ± 5.7	0.002	-
Hemoglobin (g/dL)	15.2 ± 0.9	14.8 ± 0.8	0.129	1.4
Hematocrit (%)	45.7 ± 2.9	44.4 ± 2.7	0.137	3.2
Sodium (mEq/L)	143.5 ± 3.3	140.0 ± 4.1	0.026	0.7
Potassium (mEq/L)	5.27 ± 0.72	5.18 ± 1.00	0.720	0.4
Chloride (mmol/l)	100.1 ± 3.9	104.4 ± 3.8	0.005	0.2
Lactate (mmol/L)	2.86 ± 0.86	2.6 ± 0.9	0.357	4.9

Conclusions: The blood parameters that showed significant differences at rest are in some way related to buffering capacity that may justify different performance levels in AR and ER. Analyzing acid-base and blood gas profile of athletes can be an useful tool to differentiate performance levels.

B-150

Multicenter Evaluation of a New Homocysteine Assay on Roche Clinical Chemistry Analyzers

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Background: The present abstract describes the analytical performance of a new homocysteine assay (HCYS, Roche Diagnostics GmbH, Mannheim, Germany). The evaluation was performed on Roche/Hitachi MODULAR *ANALYTICS* <P> analyzer, COBAS INTEGRA® 800 instrument and on the cobas® 6000 analyzer series (cobas c 501 analyzer). The Homocysteine Enzymatic Assay is intended for the quantitative determination of total L-homocysteine in human serum and plasma. The assay can assist in the diagnosis of patients suspected of having hyperhomocysteinemia or homocystinuria.

Methods: Total homocysteine (tHcy) is measured by an enzymatic assay originally developed by Diazyme which was applied on Roche systems. In this assay, oxidized homocysteine is first reduced to free homocysteine which then reacts with a co-substrate, S-adenosylmethionine (SAM), catalyzed by a homocysteine S-methyltransferase. The co-substrate conversion product is amplified by coupled enzymatic cycling reactions. The tHcy level in the sample is indirectly proportional to the amount of NADH conversion to NAD^+. This method has been standardized against NIST SRM 1955 reference material. The assessment of the analytical performance of the assay under routine conditions includes analysis of repeatability, precision, recovery in controls and Ring Trial samples and a method comparison. 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 GmbH.

Results: Within-run precision with n=21 replicates (repeatability) demonstrated CV's below 2 % using control materials and below 3 % using human sample pools covering the measuring range (3 - 50 µmol/l). Recovery in controls and Ring Trial samples (10 samples, 3 runs, n = 3/sample) was found within a range of 100 ± 10 % homocysteine. CV's calculated from daily routine simulation experiments (n = 21 days) using control material and human sample pools (n = 4 replicates) gave results for repeatability (CV ≤ 4%) and intermediate precision (CV ≤ 4.5 %) on all systems over the whole measuring range. Statistical Passing/Bablok analysis of method comparison against Abbott homocysteine FPIA assay (AxSYM), Diazyme HCY assay (cobas c 501 analyzer), Siemens homocysteine FPIA (Centaur), HPLC and LC/MS/MS yielded correlation coefficients > 0.96, slopes between 0.97 and 1.00 except for HPLC (slope of 1.25) and intercepts from -1.76 - 0.511 µmol/l homocysteine using ≥ 101 serum or plasma samples. The slope of about 1.25 in the method comparison against HPLC seems to be related to different standardizations of the methods (NIST material vs. lab internal standards).

Conclusions: In this study, the new homocysteine assay demonstrated reliable and precise analytical performance, good correlation with existing routine homocysteine methods and convenient reagent handling during routine use on COBAS INTEGRA and Roche/Hitachi instruments.

Disclaimer: The Roche Homocysteine assay is not yet cleared for use in the U.S. COBAS, COBAS C, COBAS INTEGRA and MODULAR are trademarks of Roche.

B-151

Evaluation of Radiometer ABL800, Radiometer ABL90 FLEX, and IL GEM 4000 Premier for Blood Gas, Electrolyte, and Metabolite Testing

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Background: We evaluated the Radiometer ABL825, Radiometer ABL90 FLEX (Radiometer Medical ApS, Brønshøj, Denmark) and IL GEM 4000 Premier (Instrumentation Laboratory Company, Bedford, Massachusetts) blood gas analyzers, compared to our internal reference method Radiometer ABL725. For analytes that showed significant biases between methods (glucose and hemoglobin), additional comparisons were done using the Roche Cobas Integra 400 Plus (Roche Diagnostics, Indianapolis, Indiana) and Sysmex XT-2000*i* (Sysmex America Inc., Mundelein, Illinois) as secondary reference methods.

Methods: Precision was determined using commercially available quality control material. Method comparisons (N=160) using waste heparinized blood gas syringes submitted for clinical testing were analyzed on each test platform. Heparinized whole blood from normal donors (N=20) was used to compare glucose results on the blood gas analyzers to heparinized plasma on the Integra 400; while heparinized whole blood from these same donors was compared to EDTA whole blood hemoglobin results obtained from the Sysmex XT-2000*i*.

Results: Between-run precision studies conducted over 20 days using three levels (low, normal, high) of quality control material yielded coefficients of variation (CVs) of ≤1.9% on all parameters on the ABL825 except normal COHb (CV=5.3%). All parameters on the ABL90 were ≤4.8% CV except normal COHb (CV=7.5%). GEM 4000 yielded ≤4.5% CV except all three levels of pO2 (CVs=5.0-7.9%) and low-range pCO2 (CV=5.7%). Compared to our internal reference (Radiometer ABL725), mean biases (SD) observed for blood gas and electrolyte parameters were as follows:

		ABL825		GEM 4000		ABL90	
	Units	Mean Bias	SD	Mean Bias	SD	Mean Bias	SD
pH	pH units	-0.01	0.02	-0.02	0.03	0	0.02
pCO2	mmHg	0	6	2	7	-1	6
pO2	mmHg	2	6	7	11	1	8
TotalHb	g/dL	-0.1	0.2	0.7	0.3	0.1	0.2
FO2Hb	%	-0.5	1.9	-1.2	3	-0.6	2
COHb	%	0.1	0.2	1	0.6	0.2	0.4
MetHb	%	0	0.3	1.2	0.5	0.4	0.3
Na	mmol/L	-1	2	0	2	1	1
K	mmol/L	0	0.1	0	0.2	0	0.1
iCa	mg/dL	-0.45	0.2	-1	0.2	-0.12	0.18
Glucose	mg/dL	0	14	-3	7	2	5

Glucose biases compared to the Integra were 0±2 for ABL825; -5±3 for GEM 4000; and 0±3 mg/dL for ABL90. Compared to Sysmex XT-2000i, hemoglobin biases were 0.2±0.2 for ABL825; 1.2±0.2 for GEM 4000; and 0.4±0.1 g/dL for ABL90.

Conclusion: The IL GEM 4000 Premier showed significant bias for glucose and hemoglobin when compared to both an internal blood gas reference method and secondary reference methods.

B-154

Comparison of Four Immunoassay Analyzers for Quantitative Determination of Total 25-Hydroxy Vitamin D Level in the Blood

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Objectives: Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol) are the most abundant forms of Vitamin D in the body. Vitamin D3 is synthesized in the skin from 7-dehydrocholesterol in response to sunlight. The measurement of 25-OH vitamin D concentration in the serum or plasma is the best indicator of vitamin D nutritional status. The total 25-hydroxyvitamin D (25-OH Vit D) level (the sum of 25-OH vitamin D2 and 25-OH vitamin D3) is the appropriate indicator of vitamin D body stores. In this study we compared four immunoassay analyzers with chromatography assay (HPLC) for quantitative measurement of total 25-hydroxy vitamin D in the blood.

Methods and Patients: This was a multi-medical centres evaluation study during year 2011. Blood samples were collected from 60 apparently healthy subjects, however, only 33 samples were compared with the chromatography method. Samples were left to be clotted for 30 minutes and then centrifuged at 3000 RPM for 10 minutes. Serum were separated, aliquoted, and shipped to each centre. Samples were analyzed immediately or otherwise stored in freezer with temperature of -70 C until further analysis. The total 25-hydroxy Vitamin D level was performed on four immunoassay analyzers: Liasion chemiluminescent immunoassay (CLIA) from Diasorin (Stillwater, MN, USA); Architect i2000 chemiluminescent microparticle immunoassay (CMIA) Abbott (IL, USA); E170 and Cobas 8000 both using electrochemiluminescent immunoassay (ECLIA) from Roche (Basel, SWZ). All four analyzers were compared to the chromatography method HPLC alliance Water (Austria). The regression and statistical analysis were performed using the Microsoft excel sheet version 2007. The significant of P value were set at <0.05 using 2-tailed t-test. Haemolysed samples were avoided and 2 or 3 levels of quality control were used.

Results: There were 18 male and 42 female with age range from 1 up 82 years. There was a direct linear relationship between HPLC and the four immunoassay analyzers with Pearson correlation coefficient (r) for Liasions (0.8143); E170 (0.9111); Cobas (0.9156) and Architect (0.8603) with p-values of 0.005, 0.0001, 0.170, and 0.440 respectively. The mean and standard deviation (Mean ±SD) values were found to be 63.3±34.6; 81.4±33.2; 73.9±33.9; and 73.3±36.5 respectively. The mean and SD for chromatography analyzer HPLC was found to be 71.4±35.0.

Conclusions: In light of all the results obtained to compare the correlation between the four ummunoassays with the gold standard method HPLC for measuring total 25-hydroxy vitamin D, we found a different degree of correlation compared to HPLC where the hightest correlation was with Roche Cobas and the lowest was for Diasorin Liasion.

B-155

Assessment of Whole Blood Lactate on the Siemens RAPIDPoint 500 Blood Gas Analyzer

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Background: Lactic acid is the endpoint product of the anaerobic metabolism of glucose. Lactate levels can be elevated by strenuous exercise, carbon monoxide poisoning, respiratory failure, alcoholic or diabetic ketoacidosis, and numerous other causes. Determining the blood lactate level is helpful in assessing the supply of oxygen at the tissue level. Increased oxygen deprivation causes the normal oxidation of pyruvic acid to lactate and can cause severe acidosis (Levinsky NG. Acidosis and alkalosis. In: Isselbacher KJ et al. Harrison's principles of internal medicine. 13th ed. New York: McGraw-Hill; 1994. p. 253-62). Patients with lactate levels greater than 4 mmol/L have a significant increase in mortality rate (Trzeciak S, et al. Intensive Care Med. 2007;33:970-7). The RAPIDPoint® 500 point-of-care system provides a stable lactate sensor* with a 28-day use life, enclosed in a maintenance-free cartridge. An internal validation of whole blood lactate* measured on the Siemens RAPIDPoint 500 Blood Gas Analyzer is presented.

Methods: Test methods were adapted from Clinical Laboratory Standards Institute (CLSI) EP09-A2-IR. A total of 102 paired lactate whole blood samples (prepared and unaltered) that spanned the analytical range of interest (0.2-30 mmol/L) were evaluated in a method comparison on RAPIDPoint 500 systems and RAPIDLab® 1265 systems.

Results: Deming regression analysis yielded a slope of 1.04 and an intercept of 0.01, with R^2=0.978 across the lactate range. No outliers or discordant results were observed.

Conclusions: Method comparison testing based on CLSI EP09-A2-IR showed that the RAPIDPoint 500 whole blood lactate method provides analytical results comparable to those of the RAPIDLab 1265 system.

* Under FDA review. Not available for sale in the U.S.

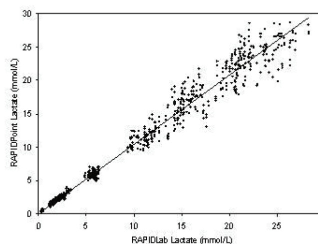

Figure 1. Lactate on the RAPIDPoint 500 system vs. the RAPIDLab 1265 system.

B-156

Blood Lactate - Comparison between Central Laboratry Reference Method on the Cobas Integra 400 and Siemens RAPIDPoint 500 POCT system.

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Introduction: The prognostic value of raised blood lactate levels has been well established in septic shock patients, particularly if the high levels persist. Blood lactate levels have been shown to have greater prognostic value than oxygen-derived variables. Obtaining a lactate level is essential to identifying tissue hypoperfusion in patients who are not yet hypotensive but who are at risk for septic shock. Consequently, blood lactate levels are an important conduit within the Emergency department to effectively triage severity of illness of patient and ensure physicians adhere to institutional care pathways.

This pilot study investigated whether lactate levels obtained on critically ill patients on the RAPIDPoint 500 corresponded to those obtained in the central laboratory to ensure the adequacy of the POCT system.

Methodology: 60 heparinised arterial whole blood samples were obtained from critically ill patients. Samples were analysed on the Central laboratory reference method, the Roche COBAS® Integra 400, and at the Point of Care on the Siemens RAPIDPoint® 500 blood gas system. Statistical analysis: Spearman Rank Correlation and Linear Regression, Bland Altman analysis, Student Paired T test.

Results: Lactate patient results were obtained, ranging from 0.6 mmol/l - 19.1 mmol/l with COBAS Integra and 0.5 mmol/l - 18.6 mmol/l with RAPIDPoint 500. Correlation coefficient r^2=0.998 with slope 0.97 and intercept 0.06. Student Paired T test: p= 0.25

no significant difference between methods. Bland Altman analysis: Mean bias = -0.04 ± 0.24 with 95 % confidence intervals -0.67 - 0.61 mmol/l.

Conclusion. This pilot study showed excellent correlation between Siemens RAPIDPoint 500 and Central Laboratory reference method. No significant statistical difference was demonstrated between groups. Bland Altman analysis demonstrated minimal bias across the measuring range demonstrating that the RAPIDPoint 500 Blood lactate parameter could be used at the Point of Care to effectively triage and treat the critically ill patient.

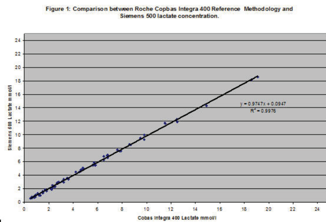

Figure 1: Comparison between Roche Copbas Integra 400 Reference Methodology and Siemens 500 lactate concentration.

B-157

Diagnostic accuracy of icteric index for the differentiation between pathological bilirubin values

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Background: Total bilirubin tests are individually inexpensive but can result in huge costs to the health care systems worldwide, since they are very commonly requested. The main objective of this study is to evaluate the diagnostic accuracy of icteric index, a test with zero reagent cost, as a preliminary biomarker to decide when total bilirubin measurement is necessary.

Methods: The laboratory is located at the University Hospital of San Juan (Alicante, Spain), a 370-bed suburban community hospital that serves a population of 234551 inhabitants. We retrospectively reviewed all the requested total bilirubin values and their corresponding icteric index results in a 2 years period. Receiver operating characteristic (ROC) curve analysis was used to find out the optimal icteric index cut-off value to discriminate between normal and abnormal bilirubin values (>1.2 mg/dL). The study included 100207 patients' total bilirubin and icteric index results.

Results: The ROC curve analysis suggested 2 mg/dL as the optimal icteric index threshold to identify abnormal bilirubin values. The area under the curve was 0.981. Regardless of the patient population studied (primary care, hospital and different individual hospital departments), sensitivity was superior to 94% and specificity to 98%. Likelihood ratios values

provided strong evidence that total bilirubin is <1.2 mg/dL when the icteric index is < 2 mg/dL. In the whole population the number of false positive was 1241 (1.2 %), and 164 (0.16 %) false negative results that showed a mean total bilirubin of 1.27 mg/dL (95%CI: 1.26-1.28 mg/dL).

Conclusions: The present study supports the use of the icteric index to identify patients with abnormal total bilirubin values. Considerable economic savings can be achieved if this strategy is applied in a clinical daily basis. Its implementation and report should be discussed and planned by laboratory professionals together with requesting physicians.

Prediction of abnormal total bilirubin using a 2 mg/dL icteric index threshold in the whole populati				
	Whole population	Inpatients	Outpatients	Primary Care Patients
Sensitivity (%)	96,48	94,96	96,31	98,46
Specificity (%)	98,70	99,28	98,82	98,42
Positive predictive value (%)	78,38	94,25	77,08	67,19
Negative predictive value (%)	99,83	99,37	99,85	99,95
Likelihood ratio positive **(95%CI)**	74,28 (70,27-78,52)	131,01 (106,96-160,47)	81,49 (74,47-89,16)	62,23 (57,72-67,09)
Likelihood ratio negative**(95%CI)**	0,04 (0,03-0,04)	0,05 (0,04-0,06)	0,04 (0,03-0,05)	0,02 (0,01-0,02)

B-159

Evaluation of the Next Generation Uric Acid Assay (3P39) on the Abbott ARCHITECT Chemistry System

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Objective: To evaluate the analytical performance of the Abbott ARCHITECT Uric Acid Assay (List #3P39) for measurement in human serum/plasma and urine.

Methods: Precision, linearity, method comparison, and potential interferences were assessed as per CLSI protocols. Precision (20 days) and linearity were evaluated using Bio-Rad Chemistry Controls and Maine Standards calibration verifiers, respectively. Results were compared to those obtained with the current Abbott ARCHITECT Uric Acid Assay (List #7D76). Impact of common interferences was assessed at low and high concentrations of patient pools (232 and 406 µmol/L, respectively). Reference interval verification was performed using healthy subjects.

Results: Total imprecision was 0.6 %CV and 1.1 %CV at 282 and 547 µmol/L uric acid for serum/plasma, and 1.3 %CV and 1.4 %CV at 788 and 1425 µmol/L for urine, respectively (single reagent lot and calibration). Linearity was verified to 2142 µmol/L for plasma and 5071 µmol/L for urine. Comparison of plasma results (x) versus the current assay (y) gave x = 0.978y + 26.4 (r^2 = 0.987) while urine gave y = 0.960x + 18.0 (r^2 = 0.970). The assay was not affected by unconjugated bilirubin (1026 µmol/L), hemoglobin (3 g/L), lipemia (20 g/L Intralipid), and ascorbic acid 680 µmol/L) with <10% error. The manufacturer's reference interval was verified (males ≤ 450 µmol/L; females ≤ 415 µmol/L).

Conclusions: The Next Generation ARCHITECT Uric Acid assay demonstrated acceptable precision and agreement with the current method. Linearity was verified over a broad range. In comparison to the current assay, the new assay showed minimal interference from hemolysis, lipemia, unconjugated bilirubin, and ascorbic acid.

B-160

Reference interval analyses of cerebrospinal fluid lactate and pyruvate concentrations and of the CSF lactate:pyruvate ratio

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Background: Determinations of cerebrospinal fluid (CSF) lactate and pyruvate concentrations and of CSF lactate:pyruvate ratios (L/P) are important in the diagnostic evaluation of inherited defects of mitochondrial energy homeostasis, as well as some acquired disorders affecting the central nervous system. Existing data regarding reference intervals are limited, especially for CSF pyruvate and CSF L/P. The objective of this study was to establish reference intervals using a large number of stringently selected subjects.

Methods: We evaluated data from 360 patients who had concurrent determinations of CSF lactate and pyruvate from 2001-2011 at the Cleveland Clinic. Inclusion criteria for normal reference intervals were normal CSF cell counts, glucose and protein; exclusion criteria were chronic brain disorder, epilepsy, seizure within 24 hours, liver or renal dysfunction, or acute changes or basal ganglia abnormality on brain MRI. CSF cytology, IgG synthesis/index, oligoclonal banding and CSF alanine were also normal, if done. CSF lactate and pyruvate were assayed spectrophotometrically with lactate oxidase and lactate dehydrogenase, respectively.

Results: There were 69 patients that fulfilled all inclusion/exclusion criteria, including 34 females (median age 16.2 years, range 0.8-60.2) and 35 males (median age of 8.8 years, range 0.3-48.5 years, p = 0.117). There are no significant differences of CSF lactate or pyruvate or of CSF L/P between males and females (Table 1). There are weak positive correlations between concentration of lactate (p = 0.015; r = 0.295) or pyruvate (p = 0.035; r = 0.256) and age by Spearman's correlation.

Conclusions: Using stringent criteria regarding both CSF characteristics and inclusion/exclusion criteria regarding medical conditions, the normal reference population selected here excludes most known conditions that may affect the levels of CSF lactate and pyruvate. This work, therefore, provides a large normal dataset for CSF lactate and pyruvate levels and of CSF L/P.

Table 1. Normal reference intervals for CSF lactate and pyruvate and CSF L/P ratio							
(mM)	N	Mean	SD	Median	5%*	95%*	p-value#
Male&female							
Lactate	69	1.43	0.28	1.40	1.10	2.01	
Pyruvate	69	0.10	0.03	0.10	0.04	0.15	
L/P	69	16.1	7.10	14.0	9.96	28.1	
Male							
Lactate	34	1.43	0.28	1.50	0.94	1.88	
Pyruvate	34	0.10	0.04	1.10	0.03	0.16	
L/P	34	16.6	8.10	14.2	10.0	40.2	
Female							
Lactate	35	1.43	0.29	1.30	1.10	2.15	0.43
Pyruvate	35	0.10	0.03	0.10	0.04	0.15	0.89
L/P	35	15.7	5.99	14.1	9.50	27.1	0.94
*Percentile							
# Male vs Female							

B-161

Abbott ARCHITECT ICT (ISE) Module Evaluation of Interferences

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Objective: Characterize the Abbott ARCHITECT Integrated Chip Technology (ICT), solid-state ISE module for suspected interferents.

Relevance: ARCHITECT ICT provides STAT results for Sodium, Potassium and Chloride using 15 µL of serum, plasma or urine that may contain interferents that could elevate or depress results.

Methodology: CLSI EP7-A2 Appendices B, C and industry data sheets were used as guidelines for evaluating interferents, concentrations and number of replicates.

Validation: ICT modules were calibrated and the Calibration Slopes, Serum and Urine Controls and the responses to the baseline serum or urine were used to verify correct operation of the ICT Modules.

Results: ICT responses were evaluated against 25 potential interferents in Serum and Urine. The cutoff value was determined following EP7-A2. The substances below, at stated concentrations, did not demonstrate interference (change from serum baseline less than the Total Allowable Error (TEa) from RiliBÄK):

Interferent Cutoff (Highest concentration tested), mmol/L or %				
Serum	Ammonium Nitrate	Bicarbonate	Sodium	Ascorbic Acid
	1	35	200	0.342
	Ethylene Glycol	Triton X100	Iodide	Acetaminophen
	1%	0.1%	4.1	1.32
	β-hydroxybutyrate	Naproxen	Sulfate	Hydroxyurea
	6	2.17	2	0.92
	Benzalkonium Chloride	Salicylate	Bromide	Acetoacetate
	0.008%	4.34	4.1	2
	Lithium Lactate	Ibuprofen	Fluoride	Sodium Azide
	6.6	2.42	0.105	36.7 (0.24%)
	Monophosphate	Ampicillin	Thiocyanate	Acetylcysteine
	9	0.15	6.9	8.4
Urine	Ammonium Nitrate	Potassium	Sodium	Acetylcysteine
	80	300	400	8.1

Sodium and potassium were affected by Benzalkonium Chloride (>0.008%), a cationic surfactant and preservative in IV lines. The Chloride sensor, Ag/AgCl based, while free from bicarbonate and phenolic drug metabolite interference, was affected by Bromide and Iodide > 4.1 mmol/L, Azide > 36.7 mmol/L (or 0.24% NaN3), and Acetylcysteine > 8.4 mmol/L. We found Benzalkonium Chloride, Bromide and Iodide gave error codes associated with sensor recovery on ARCHITECT cSystems.

Conclusion: This Interference Study demonstrates the ICT system is robust, stable and accurate and free from typical drug metabolite interferences. Many interferents produced an error warning message, preventing an unacceptable result from being reported. All Sodium and Potassium electrodes require flushing catheter lines free of preservative to prevent interference. The Abbott ICT Module is well suited for use in the Clinical Chemistry Laboratory providing Na/K/Cl results within 3 minutes from 15µL of Serum, Plasma or Urine and warranted for 20,000 samples (60,000 tests) or three months use.

B-162

Evaluation of Instrumentation Laboratories' Gem Premier 4000 Blood Gas Analyzers

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Background: Our objective was to evaluate the Gem Premier 4000 blood gas analyzers from Instrumentation Laboratories (IL) as the Hamilton Regional Laboratory Medicine Program (HRLMP) Core Laboratory blood gas analyzers.

Methods: Nine instruments were introduced across four sites. Their performance was compared to the Radiometer ABL800 Flex blood gas analyzers currently used in the laboratories. Analytes offered on the Gem instruments that were not available on the ABL analyzers were compared to the Roche P Modular. The method evaluation was performed according to HRLMP Standard Operating Procedure. Agreement between methods was assessed by running twenty arterial and twenty venous patient samples on each Gem instrument and its comparators and graphing the results with respect to total allowable error for each analyte. Precision of the Gem machines was assessed by running Gem System Evaluator quality control material at three different analyte levels in duplicate twice a day for five days. Precision was compared to the precision goals of Ontario's Quality Management Program - Laboratory Services (QMP-LS), where available. Manufacturer's precision claims were used for analytes without QMP-LS precision goals.

Results: QMP-LS precision goals were not met for PCO_2, PO_2, and glucose. The manufacturer's precision claims were not met for lactate, carboxyhemoglobin, and methemoglobin. Correlations with ABL and Roche results were acceptable and reference range transference indicated no reference range changes are requried.

Conclusions: Despite the imprecision for PCO_2, PO_2, glucose, lactate, carboxyhemoglobin, and methemoglobin, the Gem Premier 4000 instruments will be the new blood gas analyzers used by the Core Laboratories at HRLMP.

B-164

Validating Normal Reference Intervals for Glucose in Cerebrospinal Fluid Specimens.

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Introduction: It is impractical to verify the manufacturer's recommended normal reference interval for the measurement of glucose in cerebrospinal fluid (CSF), given the invasive nature of CSF specimen collection. Additionally, it is not our routine practice to interpret CSF glucose (CSFG) results along with a concurrent plasma or serum glucose (SG), however the relationship has been reported in the literature (CSFG=0.6•SG).

Objective: Verify the manufacturer's reference intervals for adults (CSFG=40-70 mg/dL) and those published (Tietz) for infants/children (CSFG=60-80 mg/dL). Establish the relationship between CSFG results and paired SG measurements.

Methodology: Retrospective CSFG results from Mayo Clinic patients over the last ten years were compiled along with ICD-9 diagnostic codes associated with each patient visit within ±30 days of the CSFG result. Plasma or SG results reported <4 hours prior to CSF collection were included, when available. Two separate populations were constructed based on exclusion criteria and the application of both exclusion and inclusion (combined) criteria. Exclusion criteria included diagnostic codes for infection, central nervous system abnormalities, diabetes, as well as an increased cell count (>5/µL total cells). The inclusion criteria included patients with headache and migraine. The central 95% reference intervals, using quantile regression (SAS V9, Cary, NC), along with the 95% confidence intervals (reported in parenthesis) were calculated for each population and compared to the manufacturer's recommendations. Observed CSFG values were compared to those expected, based on the CSFG=0.6•SG relationship, using a paired t-test along with a 95% confidence interval (in parentheses) for the average difference.

Results: An initial n=25,758 CSFG results were compiled, n=1,608 CSFG results comprised the exclusion population, and the combined population contained n=143 results. The exclusion population demonstrated children <3 years (n=304) have CSFG=38 mg/dL (95% CI: 36-40) to 81 mg/dL (63-99) and 3-17 years (n=179) have CSFG= 42 mg/dL (41-43) to 79 mg/dL (74-84). Adults >18 years (n=1125) all have a lower-limit of CSFG=47 mg/dL (46-48), however the upper reference interval increases with age; 18-39 years (n=359) have CSFG=83 mg/dL (78-87), 40-59 years

(n=471) CSFG=86 mg/dL (81-91), >60 years (n=295) CSFG=89 mg/dL (82-96). The combined population have CSFG=47 mg/dL (44-50) to 80 mg/dL (62-98). On average, the observed CSFG was significantly less than the expected (0.6•SG). The average difference was -3.8 mg/dL (-4.5 to -2.8, p<0.0001) in the entire population and -4.6 mg/dL (-8.2 to -0.9, p=0.01) for the exclusion population (n=138).

Conclusions: The combined population is a reasonable approximation to normal. The exclusion and combined adult reference intervals agree and display a positive bias compared to the manufacturer recommended range for our assay. The increased upper-limit of normal for the exclusion group may reflect inclusion of undiagnosed diabetics, a limitation of the study design. The pediatric reference range, based on the exclusion population is 22 mg/dL lower than the lower-limit cited in Tietz. The relationship of CSFG and SG is slightly lower than expected, but within the precision of the assay (CV<5%). This suggests that the interpretation of CSFG is most accurate with a concurrent SG measurement.

Wednesday AM, July 18, 2012

Poster Session: 10:00 AM - 12:30 PM

Cancer/Tumor Markers

C-01

Plasma cell myeloma mimicking lung cancer

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Background: Plasma cell myeloma (PCM) is a malignant hemaotologic disease characterized by proliferation of neoplastic plasma cells and produce excessive amounts of immunoglobulin (Ig) or light chain (LC). We are presenting a extremely rare case of PCM with an extremely rare presentation of pleural effusion and plasmacytoma mimicking lung cancer, and the case is characterized by presence of M components in serum and pleural fluid electrophoresis.

Methods: A 59 year Korean female complaining anorexia and weight loss for 6 weeks was transferred to our hospital to evaluate an incidental finding of lung mass at routine check-up from a local clinic. Chest CT presented lung mass with lobulating contour in left lower lobe with pleural effusions, which were suggested lung cancer with pleural metastasis, or PCM for the second possibility. PET/CT and bone scintigraphy also suggested lung cancer with multiple bone metastases as a primary impression.

Results: In the FLC assay, however, serum lambda FLC increased upto 183 mg/L (reference range: 5.71-26.3 mg/L), and the kappa/lambda FLC ratio (rFLC) was markedly reversed to 0.048 (reference range: 0.26-1.65). Capillary electrophoresis with serum and urine samples showed a discrete peak with a definite immunosubtraction in lambda LC, suggesting a monoclonal gammopathy. In pleural fluid, gel electrophoresis revealed monoclonal band in lambda antisera and lambda FLC was quantificated to 14000.0 mg/L. In bone marrow examination, plasma cells with eccentric nucleus and basophilic cytoplasms are counted upto 18.6% and biopsy sections showed packed marrow. The day after BM examination, a surgical operation was done for pleural mass excision. The immunohistochemical stains in biopsy specimens from the left pleural mass were compatible with plasmacytoma as follows: CK(-), CD5(-), CD45 (+), CD138 (+), Kappa (-) and Lambda (+).

Conclusions: Therefore, PCM with extramedullary dissemination into the lung was made on the basis of these results. The patient was referred to the hematology department for chemotherapy, thereafter autologous stem cell transplantation was performed. Extramedullary existence of plasmacytoma is not common and the incidence of thoracic cases is low. Moreover, it is extremely rare that PCM simultaneously presents with pulmonary plasmacytoma and myelomatous pleural effusion (MPE) to simulate a pleural mesothelioma or lung cancer. When MPE and pleural involvement were concomitantly observed like this case, the precise diagnosis of PCM could be difficult only with clinical and imaging studies. Because a delay in differential diagnosis might affect on the clinical outcome and entail medicolegal consequences, it might be important that the clinician and laboratory physician were aware of these conditions like our case. Thorough examinations should be performed to exclude systemic disease and laboratory approaches to confirm the presence of monoclonal components could be very helpful to differentiate extramedullary PCM from other malignancies.

C-03

Diagnostic usefulness of tumor antigen CA 15-3, CEA and ferritin in malign and benign disease

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Background: The CA 15-3 concentrations increase was observed in various malignant tumors, but this is a useful marker for breast cancer metastasis and is determined in monitoring disease progression and success of therapy. It is not used as screening test or as a test for primary diagnosis because it has low diagnostic sensitivity. We have make determination of CA 15-3, CEA and ferritin at patents with breast or lung cancer and patients with diagnosis of mastitis.

Material and Methods: The concentrations of CA 15-3, CEA and ferritin in 500 serum samples were determined using CMIA (chemiluminesecent microparticle immnoassay) Architect i 2000 Abbott diagnostic. All of 300 patients were hospitalized at Department of Gynecologic Oncology and Department for Oncology at the University Clinics Center of Sarajevo and 200 healthy subjects. The normal serum range of CA 15-3 between 0,0 - 31,3 U/ml, CEA 0-5,00 ng/mL and ferritin 4,63 - 204,00 ng/ml. Collected data were statistically analyzed using programs SPSS version 11.0 and Microsoft Office Excel 2003.

Results: We had 100 patients with breast cancer a mean value of CA 15-3 was 116.38 U / ml, while the mean value for CEA was 165.61 ng / ml for ferritin and 188.03 ng / ml. The mean values of the control group were in reference range. The patients group with the diagnosis of mastitis (100) have mean value of CEA 4.11 ng/ml, CA 15-3 was 92.37 U/ml, and ferritin 164.58 ng / ml. The patients with mastitis have CA 15-3 is high and ferritin in reference value. The patients with diagnoses of lung cancer (100) have the mean value of CA 15-3 was 37.52 U / ml, CEA was 63.67 ng / ml, and ferritin was 540.10 ng / ml. We found good correlation between CA 15-3 and CEA correlation coefficient was r = 0.688. The correlation between CEA and ferritin was very low r = 0.024. Our studie have show the correlation between CA 15-3 and ferritin with correlation coefficient r = 0.210.

Conclusions: The CA 15-3 and CEA are useful markers in patients with confirmed diagnosis of beast and lung cancers. The ferritin concentration has not increased in patients with breast cancer but it increased in lung patients. It is reactant of acute phase and it could be used eventuality be in patients with lung cancer. The concentration of CA 15-3 can increase in mastitis patients, but CEA and ferritin were in reference value. Therefore the CA 15-3 is not high specific as tumor marker in breast cancer and diagnosis should be proved with using other laboratory tests.

C-05

Detection of tumor cells in cerebrospinal fluid of children with acute leukemias by flow cytometry

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Background: Central nervous system (CNS) involvement is one of the important risk factors in childhood acute leukemia (AL). Tumor cells detection in cerebrospinal fluid (CSF) is one of the main signs of CNS lesion. Traditionally blasts presence in CSF is assessed by conventional cytomorphology (CM) of cytospin slides. However, sensitivity of this method is relatively low. Flow cytometry (FC) having a higher sensitivity could provide better diagnostic applicability for CSF blasts detection. Aim. To compare results of tumor cells detection in CSF of children with AL by flow FC and CM.

Methods: 183 samples from 52 boys and 31 girls aged from 5 months to 15 years with different types of acute lymphoblastic leukemia (ALL) (77 patients), acute myeloid leukemia (AML) (5 patients) and acute biphenotypic leukemia (1 patient) were investigated. 17 positive samples obtained by traumatic lumbar puncture were excluded from analysis because tumor blasts were also detected in peripheral blood. Comparison between FC and CM data was performed in 166 samples. Among these samples 61 was taken at the time of initial diagnostics, 34 - during AL follow-up, 17 - at relapse and 54 - during relapse monitoring. Monoclonal antibodies panels were constructed according to immunophenotype of tumor cells in bone marrow.

Results: In 24 out of 166 samples (14.5%) tumor cells were detected by CM. In all these cases blasts were also found by FC, while FC allowed finding blasts in other 35 samples. Thus the total number of FC-positive samples was 59 out of 166 (35.5%). This frequency was significantly higher than rate of CM-positive cases ([[Unsupported Character - р]] < 0.0001). Among initial diagnostics samples there were 20 FC-positive and only 10 CM-positive patients (32.8% vs. 16.1%, p=0.0585). At relapse 9 (52.9%) patients were FC-positive, while 6 (35.3%) were CM-positive (p=0.4897). In both B-lineage and T-lineage ALL, analyzed separately, FC detected blasts in CSF frequently than CM (p=0.0098 and p=0.0002 respectively). Absolute blast count in 1 ml in CSF samples, positive by both methods was significantly higher than in samples, positive only by FC (median = 418, range 8-158171 and median = 34, range 5-2762 respectively, [[Unsupported Character - р]] = 0.0002). Thus FC allows detecting tumor cells in CSF much more frequently than conventional CM, which could be explained mainly by higher FC sensitivity. Moreover FC is applicable also for qualitative and quantitative monitoring of CNS lesion. Nevertheless prognostic impact of FC CSF investigation is questionable. Among 13 patients in whom discordant results were obtained in initial diagnostics samples and at relapse, only for one patient risk stratification could have been changed. For all other patients there were other risk factors, that decreased significance of FC leukemic blast detection in CSF.

Conclusions: Flow cytometry allows more frequent detection of tumor blasts in CSF of children with AL, while prognostic significance of these findings is still unclear and needs to be confirmed in large prospective trials.

C-08

A Novel Method for Prediction of Risk of Malignant Transformation of Oral Epithelial Dysplasia (OED) Using p16 Methylation Biomarker in A Clinical Cohort Study

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Background: Since 17-25% of the leukoplakia lesions contain OED lesion, approximately 8% of oral leukoplakia will progress to squamous cell carcinoma. OED diagnosis is primarily based on morphologic criteria. OED progression to cancer cannot be determined based on histopathologic grounds alone. *P16* (CDKN2A) methylation is a potential biomarker for malignant transformation of OED. Previously, a 115-bp novel MethyLight assay for clinical detection of *p16* methylation was reported. It was evaluated using the published data in a prospective cohort study (Zhou et al. BMC Med Genet 2011, 12:67; Cao et al. Clin Cancer Res 2009, 15:1578-83). This study was to develop a diagnostic kit for malignant potential of OED determination with a purpose to undertake multi-central follow-up clinical trial.

Methods: Genomic DNA (~500ng) were extracted from paraffin-embedded oral mucosa biopsies from enrolled OED patients (*n*=176; 78 from Center-A, 67 from Center-B, 30 from Center-C) and modified with sodium bisulfite to convert the unmethylated cytosines to uridines. The methylation status of *p16* gene was determined by the optimized 115-bp *p16* Methylation MethyLight Kit (BioSino Bio-technology & Science Inc. Beijing, China). Briefly, methylation-specific primers (5'-CgCgg tCgtg gttag ttagt-3' and 5'-tacGc tcGac Gacta Cgaaa-3') and TaqMan probe (5'-6FAM-gttgt ttttC gtCgt Cggtt-TAMRA-3') were used to detect the 115-bp methylated fragment of the sense-strand of *p16* exon-1, which completely overlapped to the 150-bp MSP amplicon within the antisense-strand. The 150-bp methylation-specific PCR (MSP) and sequencing were also used. All patients in the trial were regularly follow-up every three months since Dec 2010. The difference of oral cancer incidence would be compared between *p16*-methylation positive and negative patients by 2013.

Results: Total 176 OED patients (i.e. 80 males and 96 females; average age 56 years old, ranged from 25-78) were enrolled into the trial. *P16* Methylation was detected in 57 OED samples (32.4%) by MSP and 39 OED samples (22.2%) by the kit, respectively. The result of the optimized 115-bp MethyLight correlated with 150-bp MSP significantly (P<0.0000001): 36 of 39 methylation-positive samples by the kit were also MSP-positive and 116 samples were *p16* methylation-negative in both assays. The results were confirmed with clone sequencing. Using MSP as a golden standard, the sensitivity, specificity, and accuracy for the 115-bp MethyLight was 63.2%, 97.5% and 60.2%, respectively. This was consistent with previously reported data (sensitivity 70.5%, specificity 84.5%, and accuracy 55.0%). In the first follow-up year (the compliance of 100%), no patient was lost, 6 patients developed oral cancer, and 3/4 cancers were *p16* methylation-positive.

Conclusions: *P16* Methylation could be detected via optimized 115-bp MethyLight consistently. It is a biomarker for malignant potential of OED in multi-central prospective clinical trials.

C-09

Next-Gen Deep Sequencing Improves FGFR3 Mutation Detection in the Urine of Bladder Cancer Patients

J. M. Millholland, S. Li, C. A. Fernandez, A. P. Shuber. *Predictive Biosciences, Lexington, MA*

Background: FGFR3 mutations have been identified in ~60-70% of low-stage, non-invasive tumors. Our group and others have developed assays to detect FGFR3 mutations in the urine of bladder cancer patients. However, urine-based assays have been limited by the technical ability to detect rare events in a dilute medium where there is a high background of normal DNA. In these assays, FGFR3 mutations are generally found in ~30% of the urine samples, which is < 50% concordance with the expected detection in tissue. We have now developed an ultra-deep amplicon sequencing technique that increases FGFR3 mutation detection in urine to ~67%, close to the expected detection frequency if every mutation found in tissue could be detected in urine.

Methods: Amplicons were designed against FGFR3 exons 7, 10, and 15 using PCR primers containing the adapter sequences for unidirectional sequencing. Primary amplification was performed from DNA isolated from 4 ml of urine. The resulting PCR products were used as template for emulsion PCR and these were then sequenced using the Roche 454 GS Junior. Samples were analyzed for total DNA reads per sample and number of mutant sequencing reads to determine percent mutation.

Results: Urine samples from 43 patients with bladder cancer were analyzed by both our previously described qPCR method and the new ultra-deep sequencing approach. Using ultra-deep amplicon sequencing, 24 out of 43 (55.8%) were positive for FGFR3 mutations, while only 5 out of 43 (11.6%) were positive for mutations by qPCR. The urine samples from the 15 newly identified mutations using deep sequencing contained FGFR3 mutations as low as 0.05% mutant DNA. The sensitivity achieved using deep sequencing was 91% concordant with the FGFR3 mutations observed in tissue.

Conclusions: We have developed a highly sensitive non-invasive urine based assay that can detect FGFR3 mutant DNA when present at < 1% of the sample and is > 90% concordance with the mutations found in tumor tissues. To our knowledge, this is the first practical application of next generation sequencing technology for diagnostic use.

C-10

Anticancer Activities of Lesser Galangal (Alpinia officinarum Jam1), Turmeric (Curcuma longa) and Ginger (Zingiber officinale) against Acute Monocytic Leukemia: A Preliminary Study

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Background: Acute Monocytic Leukemia (AML) is one of several types of leukemia that have posed a challenge for a cure. The use of chemotherapy for management is known to be harsh due to the harm done to normal cells by the chemotherapeutic drugs in the vicinity of the target leukemia cells. Besides, relapse may sometimes be experienced by patients following some promising expressions of remission after bone marrow transplantations.

Methods: In this study, leaf and rhizome organic solvents extracts of three plants, lesser galangal (*Alpinia officinarum*), turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) were examined for their anticancer activities against THP-1 AML cells *in vitro*. Extracts (1%) were introduced into THP-1 cultures and incubated for 24 hours at 37 °C with 5% CO_2 against positive and negative controls. Data obtained were subjected to statistical error analysis to ascertain reproducibility.

Results: Lesser galangal leaf extracts in organic solvents of methanol, chloroform and dichloromethane maintained distinctive anticancer activities even over a 96 hr. period. Dilution of the extracts in these solvents to 0.05% still proved potent within a 24 hr. period, which suggests that cell death was by apoptosis rather than necrosis. This was confirmed by MTS viability test. In contrast, 1% lesser galangal rhizome extract in chloroform, dichloromethane and acetone showed strong anticancer activities within a 24 hr. period. The 1% turmeric leaf and rhizome extracts and 1% ginger rhizome extract in methanol showed the most distinctive anticancer activities among the range of extracting solvents employed. The 1% methanol extract of lesser galangal leaf was separated into 13 fractions and a subsequent 18 fractions using reversed phase-high performance liquid chromatography (RP-HPLC). Separation was achieved with gradient elution in acetonitrile: water mixture (15:85 - 95:5 percentage ratio, v/v) through a C18 RP column, at a flow rate of 1 ml/min, for 30 min. Fraction 9 and fraction 16 respectively showed the greatest anticancer activity.

Conclusions: These results from this preliminary study indicate that the use of plant extract might be a safer approach to finding a lasting treatment for AML. Further investigations are being carried out to identify the compounds in these extracts that possess the anticancer activities.

C-11

Serum free light chains in the diagnosis and monitoring of monoclonal gammopathies

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Introduction: The detection of monoclonal inmunoglobulin free light chains (FLCs) is very important for the diagnosis and monitoring of patients with multiple

myeloma (MM) and others monoclonal gammopathies such primary amyloidosis (AL), nonsecretory multiple myeloma (NSMM) or light chain deposition disease (LCDD). When the serum FLCs are present in low concentrations, they are difficult for the detection by conventional methods as serum protein electrophoresis (SPE) and immunofixation (IF). The detection for serum FLCs by quantitative turbidimetric assays are more sensitive for FLCs in serum than conventional electrophoretic techniques. We report here four patients for whom FLCs were either undetectable or barely detectable using the conventional qualitative assays.

Methods: sera of the four patients were sent to the protein laboratory for the study of the monoclonal gammopathy. SPE were peformed on CAPILLARYS 2™ (Sebia), the monoclonal component were identified by IF on HYDRASYS™ (Sebia), serum inmunoglobulins (IgA, IgG and IgM) were measured by nephelometry on BNII nephelometer (Dade Behring) and the FLCs were measured by FREELITE™ (The Binding Site) turbidimetric assay.

Results: in the table below we show the results obtained.

Conclusions: the turbidimetric assay of FLCs (FREELITE™) allows us an accurate quantification of serum FLCs in the diagnosis and monitoring of patients with monoclonal gammopathies. This is due to the high specificity of free light chains and the high sensitivity of this assay that enables an early identification of FLCs that couldn´t have been detected by conventional methods (SPE and IF).

FLCs, SPE and IF in the the diagnosis and monitoring of monoclonal gammopathies

Case	Patient age	Patient sex	SPE	IF	Kappa (mg/L)	Lambda (mg/L)	Kappa/ Lambda ratio	Diagnosis
1	66	Male	Normal	Normal	1,42	524	0,002	Relapse of Lambda Multiple Myeloma
2	74	Female	Normal	Normal	17,7	1800	0,009	Primary Amyloidosis
3	57	Male	Weak peak	Kappa	289,9	6,74	43	Light Chain Deposition Disease
4	64	Male	Weak peak	Weak Lambda	6,36	752	0,008	Relapse of Nonsecretory Multiple Myeloma

C-12

Estrogen Receptor Alpha Gene Polymorphisms And The Risk Of Breast Cancer In A Group Of Egyptian Women

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Background: Estrogen receptors (ER) are members of the steroid nuclear receptor superfamily of ligand-dependent transcription factors. The effects of estrogens are mediated primarily through ER in breast tissue, and polymorphisms in the ER genes may alter the functions of these receptors. Polymorphisms in ER alpha (ESR1) have been reportedly associated with breast cancer risk; however, the results are not fully consistent. The aim of the present study was to examine the possible association between two ESR1 polymorphisms, one located in exon 8: rs2228480 (G/A) and the other in the 3′-UTR: rs3798577(C/T), and the susceptibility to breast cancer in a group of Egyptian women.

Methods: Seventy unrelated Egyptian breast cancer patients and fifty age-matched controls were enrolled in this study. The two ESR1 polymorphisms were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: The frequency of T allele of rs3798577 was significantly higher in patients (67.1%) than controls (52%) ($p = 0.02$), and T homozygote genotype was associated with an increased risk of breast cancer [odds

ratio, 6.8; 95% confidence interval, 2.4-19.1; $p < 0.0001$). Although the frequency of rs2228480 risk allele A was higher (5.7%) in breast cancer patients than in controls (2%) it did not reach statistical significance ($p > 0.05$). Also, there was no difference in the genotype distribution of this polymorphism between cancer patients and controls.

Conclusions: This study provides evidence that ESR1 polymorphism rs3798577 is associated with breast cancer risk among Egyptian women.

C-13

Performance Characteristics of the Abbott Architect ci8200 Beta-2-macroglobulin assay: Comparisons with Roche Modular P, Siemens Immulite 2000 and Beckman Immage methods

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Background: Beta -2- macroglobulin (β2M) is a protein of 18 KDa present in most nucleated cell membranes has multiple clinical uses that include diagnosis, prognosis of several diseases and monitoring of cancer patients following treatments. Enhanced test volumes in clinical laboratories have prompted to load this assay in routine automated chemistry analyzers from traditional nephelometric assay platforms that use reagents from various manufacturers.

Objective and Methods: We sought to consolidate tests into an assay platform from multiple instruments for better efficiency of laboratory operation. We evaluated performance characteristics of the Abbott Architect ci 8200 method and compared its accuracy with Roche Modular P, Beckman Immage and Siemens Immulite 2000 methods.

Results: The limit of detection of this immunoturbidometric method is 0.013 mg/L and the analytical measurement range the assay is 0.48-17.6 mg/L. Total imprecision (%CV) was determined to be 0.9% and 7.3% at concentrations of 0.9 mg/L and 3.9 mg/L respectively. β2M concentration in specimens collected from various patients was compared in pairs separately between ci8200, Modular P, Immulite 2000 or Immage methods. In addition, we have calculated mean biases derived from Bland-Altman plots of these comparisons. These results are given below.

Conclusions: We conclude that the Abbott Architect ci8200 immunoturbidometric β2M assay is sensitive, precise and a rapid method. However, its accuracy and mean bias varies when compared between any pair of given methods.

Statistical Analysis of Patient Specimen Comparisons: n= 60

Deming Regression Analysis Method Pair (y vs x)	r	Slope (CI)	Intercept (CI)	Mean Bias* y-x mg/L
Architect ci 8200 vs Modular P	0.93	1.09 (0.98 to 1.19)	0.29 (-0.84 to 1.42)	1.11
Architect ci 8200 vs Immulite 2000	0.81	1.63 (1.38 to 1.89)	0.23 (-1.62 to 2.07)	4.22
Architect ci 8200 vs Immage	0.98	0.80 (0.76 to 0.85)	1.24 (0.66 to 1.83)	-1.02
Immage vs Modular P	0.89	1.39 (1.22 to 1.56)	-1.51 (-3.3 to 0.29)	2.14
Immage vs Immulite 2000	0.78	2.14 (1.79 to 2.49)	-1.95 (-4.49 to 0.59)	5.24
Immulite vs. Modular P	0.89	0.70 (0.61 to 0.78)	-0.23 (-1.17 to 0.70)	-3.10

*Bland Altman Analysis, n= Number of Specimens, r= Spearman Correlation Coefficient, CI= Confidence Interval

C-14

Development of a multiplex test for detection of non small cell lung cancer (NSCLC)

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Background: Lung cancer is the leading cause of cancer mortality in the US. Support for low-dose helical computed tomography (CT) screening for lung cancer has recently emerged from the National Lung Screening Trial (NLST). CT screening was shown to confer a 20.3% reduction in lung cancer mortality in a high risk population, relative to screening with chest x-ray. However, concerns remain regarding the low specificity of CT scanning and the resulting cost and morbidity associated with biopsy or resection of benign pulmonary nodules. In the CT arm of the NLST, solitary nodules that were determined not to be cancerous were detected in about 25% of the subjects. Non-invasive lung cancer biomarkers may serve as a useful complement to imaging, providing a simple, cost-effective means to further clarify the diagnosis of patients with suspicious pulmonary nodules identified by radiologic imaging. We previously reported the identification of a protein biomarker panel, configured using traditional ELISA technology, which accurately classified non-malignant nodules identified through low-dose helical CT scanning. Here we describe the characterization of a multiplex version of the assay.

Methods: A 9 marker (CEA, CYFRA, MDK, MMP2, OPN, SCC, SLPI, TFPI, TIMP1) multiplex assay was developed on the Luminex platform using similar reagents to those previously employed in traditional ELISA analyses. The Luminex analyzer is a dual laser, flow-based platform that uses differentially-labeled microspheres to enable simultaneous quantitation of multiple analytes. Conditions were established that enabled robust dose response while minimizing cross-reactivity between analytes. Optimization of detection-antibody conjugated-bead concentration and titration of detection antibodies was employed to enable accurate measurement of

analytes across the expected serum range using a 1:5 matrix dilution. The specificity of the multiplex assay was confirmed by testing each analyte in turn in the presence of conjugated beads and biotinylated antibodies for all analytes. A cohort of lung cancer serum samples (40 NSCLC, 40 smoker controls) was tested to compare the accuracy of the multiplex assay with the ELISA tests. NSCLC cases included major histological cell types and all stages of disease.

Results: Each analyte represented in the multiplex assay exhibited a linear dose response of 2-3 logs. Minimal cross-reactivity was observed between analytes (0-40 ng/mL). Analysis of the NSCLC serum samples revealed similar accuracy of individual markers in classifying disease using the multiplex and ELISA tests. Moreover, the multi-marker panel demonstrated comparable overall accuracy in detecting lung cancer specimens (AUC = 0.898; 77.5% sensitivity at 92.5% specificity) relative to the ELISA assays (AUC = 0.925; 77.5% sensitivity at 90% specificity). The multiplex assay utilized a significantly lower serum volume (50 uL) relative to the ELISA assays (450 uL).

Conclusions: The described 9 marker multiplex assay offers considerable advantages over the previously employed ELISA-based format requiring a significantly lower serum volume so that archival samples can more readily be tested, higher throughput capability to facilitate targeted screening with demonstration of clinical utility, and comparable performance. Additional studies are necessary to confirm the performance of the multiplex assay in subjects with indeterminate pulmonary nodules.

C-15

Insulin Resistance In Malignancy

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Background: Malignancy is a chronic disease scenario affecting people from all aspects of life. Malignancy is more prevalent among diabetics and Acute Ischemic Heart Disease(AIHD) is 3-4 folds common in diabetics and 2-3 folds common in cancer sufferers. However the pathological mediator for these diseases remains obscure. Since lack of insulin or resistance to insulin play the key role in diabetics which faces exponential increase in AIHD, a study on insulin resistance in malignancy resulted the positive outcome in terms of its available markers.

The common markers of insulin resistance are hyperinsulinaemia, low HDL, elevated HbA1c%,various cytokines and endothelial dysfunction expressed as deranged Nitric Oxide(NO)synthesis.The adipocytokines are biologically active polypeptides that are produced either exclusively or substantially by the adipocytes, and act by endocrine, paracrine, and autocrine mechanisms. Adiponectin, an adipocytokine is also an endogenous insulin sensitizer. Circulating concentrations of adiponectin are determined primarily by genetic factors, nutrition, exercise, and abdominal adiposity. Adiponectin knockout mice manifest glucose intolerance, insulin resistance, and hyperlipidaemia and tend to develop malignancies especially when on high-fat diets. Circulating concentrations of adiponectin are lower in patients with diabetes, cardiovascular disease, and several malignancies.

Methods: We measured HDL, C-Peptide, Insulin, HbA1c%, Nitric Oxide, Resistin and Adiponectin levels in the blood of cancer patients and control subjects (healthy sex and age matched individuals). Spectrophotometric and Enzyme Linked Immunometric assays were used to quantify the above mentioned analytes from the serum, whole blood and blood cells

Results: HDL and NO levels were significantly low (P<0.05),HbA1c% was raised, C-Peptide and Insulin levels were significantly higher (p<0.05) and not complimentary to plasma glucose levels than those observed in healthy controls. Low Adiponectin and high resistin levels reciprocated with HDL, NO, C-peptide and Insulin levels in the blood of cancer sufferers than that in normal healthy volunteers

Conclusions: Insulin Resistance is a common phenomenon in malignancy. It could be either a causative factor in the pathogenesis of malignancy or a sequel to malignancy. There is greater need of further research in this area which could bring a dramatic change or modulation in the diagnosis as well as management of cancer.

C-16

validation of a multiplex immunoassay for serum angiogenic factors as biomarkers for aggressive prostate cancer

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Assays used for discovery of biomarkers should be robust and high-throughput, capable of analyzing a sufficiently large number of samples over a sufficiently long period of time with good precision. In this study, we evaluated the analytical performance of the Bio-Plex Pro Human Cancer Biomarker Panel 1, a 16-plex multiplex immunoassay, in serum for composite profiling of angiogenic factors. Because prostate cancer progression and metastasis are pathological events closely linked to angiogenesis, serum angiogenic factors are ideal candidates as prognostic biomarkers. Our 5-day evaluation indicated that all 16 assays in the panel had good reproducibility (total precisions over 5 independent plates in 5 days of less than 20%), adequate sensitivity (LOQs of majority of the assays less than 100 pg/mL), and wide dynamic ranges (linearity of majority of the assays spanning across 3 logs in concentrations). Applying the panel to sera from prostate cancer patients with Gleason score of 6, 7, 8-10, tumor stages that correlated with clinical outcome, we identified the levels of sTIE-2, a soluble form of the transmembrane tyrosine kinase receptor for angiopoietins, were elevated in patients with Gleason score of 8-10. Future studies are necessary to determine whether sTIE-2 could be used as a prognostic biomarker for identifying aggressive prostate cancer.

C-19

Validation of a comprehensive NGS-based cancer genomic assay for clinical use

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Background: Molecular diagnostics are increasing in importance to clinical oncology, as the number of therapies targeting specific molecular alterations and pathways in cancer grows. This trend has led to a proliferation of single-target biomarker assays, which are constrained by scarce tissue material and restricted in the breadth of genomic alterations assessed. To overcome these limitations, we developed a CLIA certified, pan solid tumor, next-generation sequencing (NGS) based test that enables comprehensive identification of clinically actionable genomic alterations present in routine FFPE specimens. The test uses minimal (≥50ng) DNA to achieve >500X average unique sequence coverage across 3,240 exons and 37 intronic intervals in 189 cancer genes, permitting identification of single-base substitutions, small insertions and deletions (indels), copy number alterations, and selected gene fusions, even when present in a minor fraction of input cells. To support clinical adoption, we conducted a series of experiments to validate test performance for substitution and indel mutations.

Methods: To characterize mutation detection performance across the entire targeted genomic region (~1Mb) and allele frequency (AF) range of the test, we pooled cell line genomic DNAs to model somatic mutations at AFs expected in clinical samples. For single-base substitutions, two pools of 10 cell lines each were generated, with germ-line SNPs in individual cell lines modeling 766 substitutions across the targeted region, with AFs from 5%-100%. A similar design was used for short (1-40bp) indel mutations; DNA from 28 tumor cell lines containing known indels was used to make 40 pools of 2-10 cell lines, modeling 315 indels with AFs from 10-100%. Genomic alterations identified in regions covered ≥100X in the pooled samples were compared to the alterations known to be present in the constituent cell lines. To assess applicability of these model findings to clinical samples, we contrasted sequence coverage from the pooled model samples with sequence coverage from all clinical samples processed since laboratory certification in October 2011 (n=129), representing the typical range of laboratory operating conditions.

Results: Together, these experiments demonstrated highly sensitive and specific mutation detection performance. An average coverage of 772X was obtained in the pooled samples. 100% of single-base substitutions at ≥10% AF (477/477) and 96% of single-base substitutions at 5% AF (278/289) were correctly identified, with a positive predictive value (PPV) of >99% (755/758, zero false positives at >5% AF). 93% of indels present at ≥20% AF (140/150) and 85% of indels present at 10-20% AF (141/165) were correctly identified, with a PPV of 99% (453/458, zero false positives at ≥20% AF). Average coverage of 1,057X has been achieved in clinical samples tested to date, with ≥99% of exons covered at ≥100X in 96% of samples (124/129),

indicating that sufficient coverage depth for mutation calling can be obtained in clinical samples.

Conclusions: This study presents a validation design that demonstrates the high performance of a comprehensive, NGS-based, cancer gene test for clinical samples. The accuracy of mutation detection observed, coupled with the ability to interrogate most potentially actionable alterations, suggests that this type of testing can now become a routine component of cancer patient care.

C-20

The effect of CC chemokine receptor (CCR5) 59029 gene polymorphism on the occurence and clinicopathological status of prostate cancer

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Background: Chronic inflammation seems to play a key role in prostate cancer. Chemokine receptors are believed to be important risk factors on tumor occurence and progression. The aim of this study was to investigate the effect of CC chemokine receptor (CCR5) 59029 gene polymorphism on the risk and clinicopathological characteristics of prostate cancer.

Methods: Consecutive patients with histologically confirmed prostate cancer (n= 152) and healthy controls with normal serum total PSA (< 4 ng/ml) and DRE (n= 156) were prospectively enrolled in this study between 2008 and 2011. CCR5 59029 gene polymorphism was determined using polymerase chain reaction (PCR). The association between genotypes and degree of differentiation (high Gleason score versus low Gleason score), clinical T stage (low stage versus high stage) was calculated by Pearson's χ^2 test as well. Age, BMI and smoking status adjusted odds ratios (OR) and 95 % confidence intervals (95%CI) were calculated using logistic regression model .

Results: There were no significant differences in terms of the age and BMI between prostate cancer patients and controls. There was found no statistical significance between CCR5 59029 gene polymorphism and risk of prostate cancer among controls and prostate cancer patients (p>0.05). In the mean time, no association was found regarding Gleason score and the stage of prostate cancer risk after adjustment of age, BMI and cigarette smoking.

Conclusions: We suggest that the CCR5 59029 gene polymorphism is not a risk factor for both the occurence and progression of prostate cancer in Turkish men.

C-21

Measurement of Chromogranin-A serum levels with the new Kryptor® assay.

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Background: 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. The aim of this study was to assess the reliability of the Kryptor® assay for CgA measurement.

Methods: Imprecision of the Kryptor® CgA assay was determined with two levels of CgA concentration. Reference values for the Kryptor® CgA (B.R.A.H.M.S GmbH, Thermo Scientific, Germany) assay were obtained from eighty five healthy subjects. Method comparison was performed with our routine CgA assay (Dako, Glostrup, Denmark) with eighty five patients' samples.

Results: Between run imprecision (n = 12) performed with quality controls materials for the CgA assay were 3.5% and 1.9% for mean concentrations of 98 ng/mL and 481 ng/mL, respectively. The median CgA of the healthy population was 33.6 ng/mL (range: 6.2 to 120.5 ng/mL). CgA levels measured with the Kryptor® were significantly correlated with CgA levels obtained with our routine assay (r =0.96, p <0.0001).The agreement between the two methods was very good (weighted kappa coefficient: 0.84). However, seven cases were discrepant between the two methods. For low concentrations (below 23 UI/L with the routine assay), Passing and Bablok regression analysis showed a slope of 5.36 and an intercept of 37.38 (n=40). Bland and Altman plot evidenced a positive bias (mean difference of 22.1) with higher values for Kryptor® assay. For high concentrations (above 23 UI/L with the routine assay), Passing and Bablok regression analysis showed a slope of 3.49 and an intercept of 10.76 (n=36). Bland and Altman plot evidenced a positive bias (mean difference of 526.4) with higher values for Kryptor® assay.

Conclusions: Our study showed that the Kryptor® CgA assay have satisfactory analytical performances and is strongly correlated to our routin CgA assay. However, CgA assays are not commutable and laboratories must inform the physicians of the characteristics of potential new routine assay.

C-22

Performance Evaluation of the VITROS® Total PSA II Assay on the VITROS® ECi/ECiQ and VITROS® 3600 Immunodiagnostic Systems and the VITROS® 5600 Integrated System

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We have evaluated the performance of VITROS® Total PSA II (tPSA) assay (in development), which consists of VITROS Total PSA II Reagent Pack and the VITROS Total PSA II Calibrators on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System using Intellicheck® Technology. The assay utilizes the reaction of total prostate specific antigen (PSA) present in the sample with a biotinylated mouse monoclonal anti-PSA and a horseradish peroxidase-labeled anti-PSA antibody conjugate to form antigen-antibody complexes that are captured by streptavidin coated wells. Unbound materials are removed by a wash step and the bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the instrument system. The amount of HRP conjugate bound is directly proportional to the concentration of total PSA present. The assay is calibrated against the World Health Organization reference standards for PSA (90:10) (NIBSC Code 96/670) and recognizes free PSA and PSA-alpha-1-antichymotrypsin complex on an equimolar basis. Linear regression analysis showed linearity across the range of 0.004 to 113 ng/mL, supporting a claimed measuring range of 0.010 to 100 ng/mL. Precision study over 22 days showed excellent precision with samples at mean total PSA concentrations of 0.0195 ng/mL, 0.197 ng/mL, 3.37 ng/mL, 4.51 ng/mL, 45.2 ng/mL, and 78.9 ng/mL resulting in within-laboratory percent coefficient of variation (%CV) of 10.3%, 3.0%, 2.4%, 2.7%, 3.3% and 3.8%, respectively. The accuracy of the VITROS tPSA assay was evaluated with 188 patient specimens (range: 0.071 to 97.3 ng/mL) against a commercial method. The following regression statistics using the VITROS ECi/ECiQ Immunodiagnostic Systems were obtained: VITROS tPSA = 1.06*Comparative Method -0.32; Correlation Coefficient (r)=0.98. No significant interference or cross-reactivity were observed with bilirubin (20 mg/dL), hemoglobin (1000 mg/dL), cholesterol (250 mg/dL), triolein (3000 mg/dL), human kallikrein (100 μg/mL), prostatic acid phosphatase (1000 ng/mL), common prostate cancer drugs, over-the-counter and commonly prescribed medications. The VITROS tPSA assay has excellent sensitivity useful in monitoring patients following prostatectomy with a limit of detection of 0.008 ng/mL. In summary, the VITROS tPSA assay demonstrates reliable and acceptable performance on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System.

C-23

Analytical performance of two Thyroglobulin assays in the low range: impact on clinical decisions.

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Background: Thyroglobulin (Tg) determination is used for follow-up and monitoring of patients with differentiated cancer of the thyroid. In these patients, Tg levels drop to undetectable (or very low) levels after total or near-total thyroidectomy and successful treatment with radio-labelled iodine. If a rise in Tg levels is observed during the follow-up, it can indicate a recurrence of the cancer. Thus, analytical performance of the different available Tg assays in the lower range of measurement is of importance. Unfortunately, this has been evaluated in very few studies.

Methods: We used two Tg automated methods (Beckman-Coulter Access,Brea,CA and Roche Modular,Mannheim,Germany), supposed to be calibrated against the same material (CRM457). Roche claims a limit of detection (LOD) <0.1 ng/mL and a functional sensitivity (FS) <1 ng/mL. Beckman also claims a LOD of 0.1 ng/mL but is less clear on the FS, even if they claim a CV<10% for concentrations >1 ng/mL. We thus defined the LOD (value significantly different from zero with a probability of 99.7%) and the FS (lowest value who gave a CV of 20% when low values samples

were assayed in triplicates on 5 different days) of the two assays. Then we defined the beta-expectations tolerance limits for 5 samples free of anti-Tg antibodies ranging from about 0.2 to 2 ng/mL by running them on triplicate during 5 different days. Finally, we focused on 36 patients presenting low Tg levels (with no anti-Tg antibodies) to compare the results obtained with the two assays.

Results: LOD of 0.10 and 0.14 ng/mL and FS of 0.31 and 0.26 ng/mL for Modular and Access, respectively. For Access, the mean(upper and lower beta-expectations tolerance limits) on the low values samples were, in ng/mL: 0.22(0.19-0.26), 0.30(0.25-0.35), 0.47(0.42-0.51), 0.74(0.69-0.78), 0.89(0.80-0.98) and the risk to fall out of the calculated beta-expectations were <5%. For Modular, the same samples gave a mean(upper and lower beta-expectations tolerance limits) of 0.22(0.08-0.36), 0.40(0.24-0.57), 0.86(0.70-1.02), 1.26(1.04-1.48) and 1.71(1.57-1.86). The risk to fall out the calculated beta-expectation was >5% for samples 1 and 2. In other words, a patient presenting a Tg value of 0.30 with the Access could either range fro 0.25 to 0.35 with less than 5% of probability to fall out of this range. Run on Modular, this patient would have a value that could range from 0.24 to 0.57 with a chance greater than 5% to fall out of this range.

Passing-Bablock regression gave the following equation: Access= 0.65XModular -0.04. The Bland-Altman plot showed that the difference between the 2 methods increased with increasing values.

Conclusions: The performance of the 2 instruments is acceptable in the lower range of Tg assays, but Access presents more robust results. There is also a calibration problem with the Roche assay, even if claimed to be calibrated against CRM457. The first of the two calibrators used to fit to the Master curve of the instrument is probably too high (around 4 ng/mL) whereas Access uses four calibrators, the first around 1 ng/mL. Results obtained with the 2 methods are not transposable.

C-24

Evaluation of Accuracy and Comparability of 4 Analyzers for Total PSA

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Background: Prostate-specific antigen (PSA) testing is used for early detection and monitoring of prostate cancers. There are several assay methods commercially available for routine PSA tests. Different assays for PSA produced different results depending on their assay principles and calibrators. However, the cut-off value of total PSA has been applied to detect prostate cancers regardless of assay methods. In this study, the purpose of this test is to investigate the accuracy and comparability of 4 commercial analyzers being commonly used in Korea.

Methods:4 analyzers - Abbott Architect i200(Abbott Diagnostics, Abbott Park, IL, USA), Siemens ADVIA Centaur (Siemens Diagnostics, Tarrytown, NY, USA), Roche Modular Analytics E170 (Roche Diagnostics, Hitachinaka, Ibaraki, Japan), Beckman-Coulter Access (Beckman-Coulter, Chaska, MN, USA) were evaluated. We measured total PSA levels in WHO 96/670 reference preparation to evaluate the accuracy. In addition, the comparison of 4 analyzers was performed with CAP survey specimens (LN23-A) and 20 patient samples. Statistical tests for the results of each analyzer were performed by linear regression.

Results: Coefficients of variance (CV) of Architect, Centaur, E170, Access were 3.4%, 1.8%, 1.5%, 5.8% respectively. Regression slopes and intercepts of 4 analyzers were Architect(1.0919, 0.1178), Centaur(0.9895, 0.0793), E170(1.0176, 0.2890) and Access(1.3593, -0.0213) in the test with WHO 96/670 reference preparation diluted based on 5 concentrations (2.50, 6.88, 11.25, 15.63, and 20.00). Regression slopes and intercepts plotted against Architect were Centaur(0.7939, 0.7047), E170(0.9524, 0.1190) and Access(1.1117, -0.0968) in the comparison with patient samples, while regression slopes and intercepts plotted against Architect were Centaur(1.0953, -0.3523), E170(1.1770, -0.1250) and Access(1.2766, -0.5318) in the comparison with CAP survey specimens (LN23-A). Access showed higher regression slope in the test with patient sample and WHO material compared to Architect, while other analyzers show lower.

Conclusions: Even in the test based on WHO reference preparation, there were significant differences among each analyzers. However, PSA values in patient samples showed a bit smaller differences than those in WHO reference preparation. In addition, the difference-trends among 4 analyzers were similar to each other in the test with patient samples and WHO materials both, but the test with CAP survey specimens showed a different tendency from other 2 tests. And more study is required to figure out the reason cause these differences. Under this result, further efforts are still needed to standardize PSA assays.

C-25

Kallikrein-related peptidase 4 (KLK4) mRNA expression: a novel molecular tissue biomarker for the prediction of short-term relapse in colorectal adenocarcinoma.

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Background: Kallikrein-related peptidase 4 (KLK4) is a trypsin-like serine protease belonging to the KLK family, members of which are aberrantly expressed in various malignancies. *KLK4* mRNA expression is an unfavorable prognostic predictor in breast and ovarian cancer. KLK4 represents a novel endogenous activator of protease-activated receptor 1 (PAR1) in HT-29 colorectal adenocarcinoma cells, inducing PAR1 signaling and subsequent ERK1/2 activation. The aim of this study was to analyze *KLK4* mRNA expression in colorectal adenocarcinoma specimens and to examine its prognostic value and potential clinical application as a novel molecular tissue biomarker in colorectal adenocarcinoma.

Methods: Total RNA was isolated from primary tumors of 81 colorectal adenocarcinoma patients. After testing the RNA quality, cDNA was prepared by reverse transcription. A highly sensitive real-time PCR methodology, based on SYBR® Green chemistry, was developed for *KLK4* mRNA quantification (detection limit: 10 mRNA copies), and *KLK4* expression analysis was performed. Calculations were made with the comparative C_T ($2^{-\Delta\Delta CT}$) method, using *B2M* as an endogenous control gene and the HT-29 cell line as a calibrator. In the biostatistical analysis, the X-tile algorithm generated for *KLK4* expression an optimal cut-off point of 3.55 mRNA copies/10^6 *B2M* mRNA copies (c/Mc).

Results: *KLK4* mRNA expression in colorectal adenocarcinoma tissues ranged from 0.059 to 2565.0 c/Mc, with a mean±S.E.M. of 111.2±38.8. Dukes stage and histological grade were significantly associated with *KLK4* mRNA status, as colorectal adenocarcinomas of advanced stage or high grade were more frequently *KLK4* mRNA-positive in contrast with early-stage or low grade tumors (P=0.049 and P=0.028, respectively). *KLK4* mRNA status was also strongly associated with tumor size (P=0.004). In Cox univariate survival analysis, the risk of recurrence was significantly related to *KLK4* mRNA expression (hazard ratio [HR]=1.37, 95% confidence interval [95% CI]=1.04-1.81, P=0.024), considered as a continuous variable. Furthermore, a significantly increased risk of relapse was shown to be associated with *KLK4* mRNA-positive values using the cut-off of 3.55 c/Mc. Therefore, in addition to Dukes stage and histological grade that were confirmed as significant predictors of DFS (P=0.003 and P=0.004, respectively), *KLK4* mRNA expression was shown to predict poor DFS in colorectal adenocarcinoma, since patients with *KLK4* mRNA-positive tumors were at higher risk of recurrence (HR=2.68, 95% CI=1.06-6.77, P=0.036). Kaplan-Meier survival analysis, also, demonstrated that *KLK4* mRNA expression constitutes an unfavorable prognostic biomarker in colorectal adenocarcinoma (P=0.029), in terms of disease-free survival (DFS), especially for lymph node-negative patients (P<0.001) or those suffering from early-stage disease (P<0.001). We also developed a multivariate Cox regression model, adjusted for nodal status and tumor size, in which *KLK4* mRNA expression remained a statistically significant predictor of DFS in colorectal adenocarcinoma, as patients with *KLK4* mRNA-positive tumors were more prone to relapse (HR=2.73, 95% CI=1.04-7.13, P=0.040). Regarding overall survival, *KLK4* mRNA expression did not show any prognostic impact.

Conclusions: Our findings suggest that *KLK4* mRNA expression can be regarded as a novel potential tissue biomarker predicting short-term relapse of colorectal adenocarcinoma patients.

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C-26

Evaluation of Prostate Volume and [-2]proPSA for Prostate Cancer Detection, Using the Beckman Coulter Access 2 Immunoassay System, in a Multi-Center Prospective Clinical Trial

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Introduction And Objectives: Previous studies have reported that the receiver operating characteristics (ROC) of PSA testing are more favorable in men with a small prostate volume. We have found that [-2]proPSA (p2PSA), a free PSA (fPSA) isoform, provides superior discrimination of cancer from non-cancer compared to total PSA or %fPSA in men with a total PSA of 2-10 ng/ml and findings not suspicious for cancer on digital rectal examination (DRE). In this study, we evaluated the impact of prostate volume on the ROC of p2PSA in a multi-center, prospective clinical trial.

Methods: 738 subjects (341 PCa and 397 benign by biopsy) with trans-rectal ultrasonographically-determined prostate volume and a PSA ranging from 2-10 ng/mL were enrolled from 7 clinical centers in this study. Subjects were ≥ 50 years of age with non-suspicious DRE. 99% of the subjects had ≥ 8 and 98% had ≥ 10 core biopsies. PSA, fPSA and p2PSA were analyzed on the Beckman Coulter Access 2 Immunoassay System[1,2]. We compared the area under the curve (AUC) for PSA, %fPSA, %p2PSA and a formula combining PSA, fPSA, and p2PSA (called Beckman Coulter Prostate Health Index or *phi*[3]) to assess risk of PCA in men with different prostate volumes.

Results: Comparing patients with prostate volumes above and below the median for the study population, the AUCs of %p2PSA and *phi* were superior and less affected by prostate volume than were those of PSA and %fPSA (Table 1). However, among patients with a volume in the upper tertile (≥ 54 cc), %p2PSA and *phi* provided more discrimination than PSA or %fPSA.

AUC As A Function of Prostate Volume

Prostate Volume (cc)	Total PSA	%fPSA	%p2PSA	phi	%fPSA vs phi p-Value
≤ 44	0.60	0.62	0.65	0.68	0.056
> 44	0.53	0.56	0.68	0.68	0.008
≥ 54	0.52	0.56	0.65	0.64	0.067

Conclusions The AUCs of %p2PSA and phi are superior to those of total and %fPSA and are less influenced by prostate volume.

[1]Not intended as off-label promotion of any BCI product; [2]All trademarks are the property of their respective owners; [3]Not available in the US

C-27

miR-145 And Its Target, L-Dopa Decarboxylase (DDC), Are Promising Prognostic Biomarkers For The Disease-Free Survival of Prostate Cancer Patients

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Background: MicroRNA-145-5p (miR-145-5p) is a well-characterized tumor suppressor miRNA, the expression of which is decreased in prostate cancer (PCa) and many human malignancies. miR-145-5p targets many cancer-related genes, including those encoding IGF-IR, c-MYC, FLI1, MUC1 and p21. Furthermore, has been predicted to be a post-transcriptional regulator of L-Dopa decarboxylase (DDC) expression. DDC was recently found to interact with the androgen receptor (AR), promoting an increased ligand-dependent AR transcriptional activity, which is a hallmark of PCa development and signifies disease progression to an androgen-independent state. The clinical relevance of DDC for PCa has already been highlighted by the ability of *DDC* expression levels to discriminate PCa patients from those suffering from benign hyperplasia. The measurement of post-treatment serum PSA is the method of choice for the treatment monitoring of PCa patients. However, the significant variability in patients' outcomes strengthens the need for novel biomarkers able to provide an early indication of patients' prognosis.

Recognizing the emerging role of the DDC/AR axis for PCa pathobiology, the objective of our study was the evaluation of the clinical significance of *DDC* and miR-145-5p for the prognosis of radical prostatectomy-treated patients.

Methods: Total RNA was isolated from 70 prostatic tissue specimens obtained from PCa patients who underwent radical prostatectomy. The eligibility criteria for the participating patients were the absence of positive surgical margins and the absence of prior hormonal- or radio-therapy. *DDC* mRNA and miR-145-5p levels were determined by two gene-specific SYBR Green-based quantitative PCR assays, following the polyadenylation of 1ug total RNA and the subsequent oligo(dT)-mediated first-strand cDNA synthesis by reverse transcription. The $2^{-\Delta\Delta CT}$ relative quantification method was applied for the expression analysis of the target genes, using the LNCaP prostate cancer cell line as our assays' calibrator, and the *GAPDH* and RNU48 reference genes for the normalization of the *DDC* and miR-145-5p levels, respectively. Quality control of the two assays was completed prior to the screening of the tissues. The adopted cut-off values for both *DDC* and miR-145-5p expression levels, as indicated by the X-tile algorithm, were equal to the median values of the target genes' expression levels.

Results: Cox proportional regression analysis pointed out a 3-fold higher risk of biochemical relapse for the *DDC*-positive patients (HR=2.72; 95%CI=1.16-6.36) compared to *DDC*-negative ones (p=0.021). The pour outcome of the *DDC*-positive patients was also supported by their significantly reduced disease-free survival (DFS), which was highlighted by Kaplan-Meier survival analysis (p=0.015). Moreover, *DDC* expression status were associated with advanced disease stages (p=0.003) and a higher Gleason score (p=0.039). As expected, the downregulation of miR-145-5p was correlated with high Gleason score (p=0.004) and late-stage tumors (p=0.027). The unfavorable prognosis of the miR-145-5p-negative patients was strongly designated by their shorter DFS periods, compared to the positive ones, indicated by Kaplan-Meier survival analysis (p=0.019).

Conclusions: Our data clearly demonstrate the significant clinical potential of the assessment of *DDC* and miR-145-5p expression levels for the prediction of PCa patients' outcome.

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C-28

Macrophages Show Differential Expression of Nucleoside Phosphorylase 1 and Synuclein-alpha in Pancreatic Cancer Compared to Chronic Pancreatitis

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Background: Current literature supports the paradigm that benign components of the tissue microenvironment secrete cytokines that support the growth of cancer. We hypothesize that the protein expression of leukocytes infiltrating pancreatic ductal adenocarcinomas (PDACs) are likewise expressed in circulating leukocytes. If our hypothesis is correct, circulating leukocytes may express molecular biomarkers signaling the presence of PDAC. Chronic pancreatitis (CP) is a risk factor for the development of PDAC and can be found adjacent to PDAC in pancreatic tissues. Molecular expression analyses suggest that CP and PDAC represent a biological continuum of progression from benign to malignant disease. We previously analyzed proteins by mass spectrometry from the pancreatic juice of PDAC and CP patients. Purine nucleoside phosphorylase 1 (Np1) and Synuclein-alpha (Snca) were upregulated in PDAC pancreatic juice samples compared to CP samples. The molecular role of Snca in PDAC or CP is unknown, however, Np1 is known to be a mediator of inflammatory signaling, particularly in the extracellular milieu of tissues. Intense inflammation is frequently present in PDAC and CP.

The objective of this study was to investigate whether Np1 or Snca was expressed by leukocytes present in PDAC and CP tissues and, if so, to measure significance, sensitivity and specificity for PDAC.

Methods: Archival formalin-fixed paraffin-embedded tissues were examined for the presence of Np1 and Snca by immunohistochemistry. Seventy-nine PDAC cases and 31 cases of CP were analyzed. A case was scored positive if more than 5 cells of each cell type per case stained positive by light microscopy.

Results: Differential staining of endothelial cells, neutrophils and lymphocytes did not correlate with the presence of PDAC. Macrophages, however, were significantly positive for both Np1 ($p<0.0001$) by Chi-Square test and Snca ($p<0.0001$) by Fisher's exact test. Np1 sensitivity for PDAC was 63% and specificity was 87%. For Snca, sensitivity was 96%, specificity 45%.

Conclusions: Our data shows that macrophage protein expression differs significantly in PDACs compared to CPs. Our future experiments will explore the expression of these proteins in circulating monocytes of patients with PDAC or CP.

C-29

Evaluation of the CYFRA 21-1 assay for the ARCHITECT system

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Background: CYFRA 21-1 levels are elevated in non-small cell lung cancer, particularly in squamous cell tumors. The CYFRA 21-1 level can be used as an assistive tool to diagnose lung cancer, monitor tumor responses to chemotherapy, and predict survival during lung cancer therapy. In this study, the ARCHITECT CYFRA 21-1 assay (Abbott Laboratories, USA) was evaluated for its analytical performance.

Methods: The ARCHITECT CYFRA 21-1 assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of soluble fragments of human cytokeratin 19 in human serum and plasma. Three levels of controls were used for evaluating the precision and linearity of the CYFRA 21-1 assay.

Results: In the precision study, the within-run, between-day, and total coefficient of variation (CV) for the low controls (5 ng/mL) were 3.5%, 4.7%, and 5.9%, respectively; the corresponding values for medium controls (15 ng/mL) were 2.7%, 3.0%, and 4.3%, respectively, and those for high controls (35 ng/mL) were 1.6%, 1.8%, and 2.6%, respectively. In the dilution experiments, linearity of the CYFRA 21-1 results was observed in the studied range of 35 ng/mL through a correlation coefficient of 0.9993.

Conclusions: The ARCHITECT CYFRA 21-1 assay showed good precision and linearity for the 3 levels of controls.

C-30

Diagnostic and prognostic value of urinary PCA3 for prostate cancer: Comparison with, total and free PSA, and with PSA velocity

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Background: Prostate cancer is the most prevalent cancer in man and the second cause of cancer leading death. The widespread use of serum prostate specific antigen (PSA) for diagnostic screening has led to dramatic increase in the incidence rate for prostate cancer. Serum PSA has a low specificity and frequently results in negative biopsy outcome. Another problem with PSA based screening is over diagnosis due to increased detection of latent and clinically insignificant cancers. Recently a new prostate cancer marker the prostate cancer antigen (PCA3) has become available (Gen-Probe). PCA3 mRNA is over expressed in prostate cancer cells. The ratio of PCA3 to PSA mRNA reflects the proportion of cancer cells in the urine sample. We evaluated the clinical performance of PCA-3 and compared it to total PSA, % free PSA and PSA velocity in predicting biopsy outcome.

Methods: We retrospectively retrieved and reviewed 200 patients with urinary PCA3 measurements (Avero diagnostics Irving, TX) during the period of May 2010 to November 2011. Among these, 72 had benign prostate diseases, 45 had prostatic intraepithelial neoplasia (PIN) and 83 had prostate cancer based on multiple-core biopsy cytology/pathology results. The diagnostic performance of PCA3 was compared with free and total PSA and PSA velocity.

Results: PCA3 score was significantly higher in patients with prostate cancer and PIN than in those with benign prostate diseases ($p < 0.001$). Among prostate cancer patients, Gleason score 7 or more had significantly higher PCA3 Score than those with PIN and Gleason score of 6 or less ($p < 0.001$). The patients with chronic or acute prostatitis had increased PSA concentrations ($P < 0.05$) but similar PCA3 scores ($p > 0.05$) compared to patients with negative prostate biopsy. At sensitivity level of 95 to 80%, the specificity was 20 to 44% for PCA3; compare to 14 to 30% for total PSA, 24 to 36% for free PSA, and 20 to 40% for PSA velocity. To compare the diagnostic performance of PCA3 with total PSA, free PSA and PSA velocity, ROC analysis was performed. The area under the curve for PCA3 (0.69) was significantly higher (p=0.03) than total PSA (0.57) but was not significantly different from free PSA or PSA velocity.

Conclusions: Urinary PCA3 test provide slightly better diagnostic accuracy and potentially increases the detection of clinically significant prostate cancer as compared to total PSA. However, its diagnostic performance was not significantly better than of free PSA and PSA velocity. Our results suggest the need of future studies to explore if the use of multivariate analysis including free PSA and/or PSA velocity would further improve the diagnostic value of PCA3.

C-31

An EIA screening array assay for the determination of IgG subclass heavy light chain kappa lambda ratios in normal blood donors and specific detection in IgG monoclonal gammopathies

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Background: Serum protein electrophoresis (SPEP) is routinely used to identify and monitor patients with monoclonal gammopathy. Whilst this is adequate for gross quantities of protein it is inadequate for the identification serum free light chain (FLC) gammopathies and low levels of intact immunoglobulin monoclonal gammopathies. Immunofixation (IFE) can improve sensitivity but it is a qualitative measurement and therefore of limited use as a tool for monitoring response. Recently, a solid phase array has been developed which can quantify IgG subclass heavy/light chain concentrations in serum (i.e. IgG1κ/IgG1λ, IgG2κ/IgG2λ etc.). In this study we establish normal range data for each assay and show the ability of the assay to identify previously confirmed monoclonal gammopathy samples, finally we compare the sensitivity of the assay with SPEP and IFE.

Methods: The assays have been developed using the Dynex bead array system. Briefly, individualbeads were coated with specific anti-IgG subclass antibodies, subclass antibodies in diluted test samples were captured by the bound antibody and probed using either sheep anti-total human κ or λ HRP labelled antibodies. In this study, forty six normal human sera (NHS) together with 52 IgGκ (29 IgG_1, 16 IgG_2, 6 IgG_3, 1 IgG_4) and 40 IgGλ (23 IgG_1, 8 IgG_2, 7 IgG_3 and 2 IgG_4) sera from patientswith monoclonal gammopathy were assayed using the subclass array. To compare sensitivity of the array to SPEP, six kappa and eight lambda myeloma samples (representing all subclasses) were diluted in triplicate in normal human serum of known IgG subclass concentration (1:3, 1:9, 1:27 and 1:81) and assayed using the array.

Results: The following normal ranges (mg/mL) were found: $IgG_1κ$=1.49-7.1, $IgG_2κ$=0.87-3.92, $IgG_3κ$=0.13-1.35, $IgG_4κ$=0.019-0.99, $IgG_1λ$=1.2-2.82, $IgG_2λ$=0.60-4.87, $IgG_3λ$=0.097-0.52 and $IgG_4λ$=0.008-0.317. The κ:λ ratio ranges were: IgG_1=0.98-3.39, IgG_2=0.63-2.23, IgG_3=0.74-3.58 and IgG_4=1.37-7.52. The summated κ and λ subclass values were correlated with the subclass values obtained using IgG subclass nephelometric assays, Pearson correlation values (R^2) were: IgG_1 0.83, IgG_2 0.84, IgG_3 0.78 and IgG_4 0.95. The subclass concordance of the 52 IgGκ and 40 IgGλ samples was confirmed in all cases by abnormally high (κ) or low (λ) ratios compared to the subclass ratio normal ranges. Sensitivity between the array and SPE using the serially diluted samples was comparable for 7/8 of the IgG_1 and IgG_2 diluted myeloma samples, the one SPE negative IgG_1 kappa sample gave an abnormal ratio on the array at 1:81 dilution. All IgG3 kappa and lambda samples were negative by SPEP at the 1:81 dilutions but gave abnormal ratios on the array. The IgG4 kappa and lambda samples were also undetectable by SPEP at the lowest dilution, again the array reported abnormal ratios.

Conclusions: Based on these data and the incorporation of previously presented assays based on this platform for κ and λ free light chain and IgA and IgM heavy light chain detection, this EIA array assay combination offers the potential for a sensitive high throughput assay for screening for monoclonal gammopathies.

C-32

Increase in positive biopsies and the safe use of tumor markers.

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Background: The WHO Patient Safety strategies refer to carrying out procedures in a correct manner. The European Group on Tumor Markers (EGTM) has stated that the use of these should be limited to follow-up of the disease and monitoring of treatments. However, incorrect usage of these is frequent. The use of these tests for screening puposes results in other investigations that are invasive for the patient and affect his safety. Objective. To measure the impact of the application of a protocol for the determination of tumor markers on the biopsies carried out. Relevance. The suspicion of cancer causes much anxiety in the patient and an inappropriate use of tumor markers can lead to unnecessary and injurious tests being carried out on the patient, at the same time as overloading the rest of the hospital services and increasing the cost.

Methods: Period of study: 2010 and 2011. Requests for all the most frequent tumor markers were analyzed: CEA, CA 15.3, CA 19.9 and CA 125, from 2010 and 2011. The Oncology Service was excluded given that it treats confirmed tumors, but biopsies that were requested later were studied. In 2011, clinical services were informed of the application of a new protocol for determining tumor markers according to EGTM instructions. All requests for tumor markers that did not provide the necessary

information requested in the protocol were rejected. To check the impact of such a measure the biopsies carried out on patients according to the protocol were analyzed, as well as the biopsies requested from those patients in which the request for tumor markers had been rejected. Validation. During the two years of the study 7080 requests were made for the analysis of tumor markers. Those from the Oncology Service were discarded. The positive results and the biopsies generated were studied as follows

Results: In the year 2010, 5316 tumor markers were requested. 42% of the requests were diagnostic. Pathological results were only found in 14.3% of cases. In the biopsies generated only 21% gave a result of neoplasia. The introduction of the protocol in 2011 caused a decrease of 58% in the number of requests for tumor markers. The percentage of requests for diagnostic tests remained at 42%. Only 7.45% of the samples analyzed reached a pathological level. 35% of the biopsies carried out later were positive for neoplasia. The rejected requests rose to 40%. The clinician only decided to request a biopsy in 10% of cases. 63% of the biopsies carried out on these patients were positive for neoplasia.

Conclusions: The safe use of tumor markers, according to EGTM, means a decrease in the number of requests. It reduces the number of negative biopsies carried out at the same time as increasing their usefulness. It is confirmed that tumor markers should not be used as screening tests. This translates into an improvement in patient safety, as it avoids invasive tests which are not properly justified.

C-33

Differential regulation of N-myc dowstream-regulated gene 1 (NDRG1) splice variants in MCF-7 and HepG2 cells by desferoxamine and phenanthroline

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Background: N-myc downstream-regulated gene 1 (NDRG1) is a cellular protein that is up-regulated under a multitude of stress and growth-regulatory conditions. Previous reports have demonstrated that NDRG1 is strongly up-regulated by chemical iron chelators and hypoxia. Studies using iron chelators such as desferoxamine (DFO) have shown that Fe deprivation results in G_1/S arrest and apoptosis. NDRG1 transcription is under genomic control of a wide variety of transcription factors and cell stressors. There are several NDRG1 transcription splice variants in human and only two of them encode the same protein, with the variant-1 (V1) representing the longer transcript. Variant-2 (V2) uses an alternate splice site in the 5' UTR. In view of the existing evidence, we hypothesized that variation in the 5'-UTR sequence of NDRG1 derived from alternative splicing may contribute to a not yet understood regulation of expression and physiological function.

Methods: The MCF-7 and HepG2 cells were used in this study as they have shown paradoxical prognosis regarding increased NDRG1 expression. Cells were plated in 96-well plates at 16000 cells/well for MCF-7 and 9000 cells/well for HepG2, and incubated for 24 h at 37°C. Subsequently the medium was removed, and the cells were reincubated in a humidified atmosphere of 5% CO2 in air at 37°C for 24 h with either control medium alone or medium containing either 150 micromolar DFO, 25 micromolar phenanthroline (PHE), 500 nM trichostatin A (TSA), as a histone deacetylase inhibitor. The effect of iron chelators and other chemicals on cell number, viability and proliferation was examined by real time cell analyzer (xCELLigence, Roche). Isolated RNA was used to perform quantitative RT-PCR of NDRG1 splice variants by the TaqMan methodology. As a housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase was measured from the same cDNA samples.

Results: In MCF-7 cells, DFO and PHE revealed antiproliferative activity whereas in HepG2 cells, DFO and PHE were not effective, as evidenced by real time cell analysis based on electrical impedance signal.

The transcription of two distinct NDRG1 splice variants are all markedly upregulated in HepG2 cells by iron chelators DFO (p<0.001) and PHE (p<0.001), showing no differential upregulation. In contrast, DFO and PHE caused significant upregulation of both variants but differentially in MCF-7 cells (p<0.001), with the V2/V1 ratio being 1.8 and 2.8, respectively. TSA revealed the same mode of differential upregulation in both MCF-7 and HepG2 cells (p<0.001), V1 predominating over V2 (about 3 folds).

Conclusion: In the present study we disclose the transcription of two distinct NDRG1 splice variants, which are all markedly upregulated in MCF-7 and HepG2 cancer cells but differentially upregulated following the treatment with DFO and PHEN. To the best of our knowledge, this is the first study to show that some iron chelators can differentially regulate the expression of NDRG1 in cancer cell lines. Iron chelators like DFO and PHE that can specifically upregulated one of the variants over other might be used as an adjunct to HDAC inhibitor therapy to reduce tumor growth rate and metastasis and to induce differentiation.

C-34

Comparison of DR-70TM with CYFRA 21-1 as a practical tumor marker for lung cancer

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Background: Lung cancer is one of the most lethal cancers in which development of effective tumor markers is urgently needed. DR-70™ immunoassay measures serum fibrin degradation product which could serve as a pan-tumor marker. We evaluated the clinical significance of DR-70™ in patients with lung cancer and compared its performance with the relatively well-established biomarker for lung cancer, CYFRA 21-1.

Methods: Serum samples of 193 patients with lung cancer drawn on the day of surgery and 84 healthy subjects were obtained from KIRAMS Radiation Biobank while those of 106 patients with benign respiratory diseases from Soonchunhyang Biobank. Male : female ratios and mean ages (SD) of lung cancer group were 7.8 : 1 with 56.9 (16.4) and those of non-cancerous control group were 4.3 : 1 with 45.4 (13.7). Commercially available DR-70™ ELISA kit (AMDL, Tustin, CA, USA) and CYFRA 21-1 electrochemiluminescence immunoassay system (Roche Diagnostics GmbH, Mannheim, Germany) were used for the measurement of both markers.

Results: Means (SD) of serum DR-70™ in lung cancer, benign respiratory diseases and healthy subjects were 35.01 (229.02) μg/mL, 0.53 (0.41) μg/mL, and 0.69 (1.03) μg/mL whereas those of CYFRA 21-1 were 4.49 (6.67) ng/mL, 1.46 (0.75) ng/mL and 1.36 (0.92) ng/mL, respectively. Both DR-70™ and CYFRA 21-1 were significantly higher in lung cancer patients than non-cancerous subjects (by sex- and age-adjusted *t*-test, $P < 0.05$). Receiver operating characteristic curve (ROC) of DR-70™ immunoassay discriminating patients with lung cancer from non-cancerous subjects showed clinical sensitivity of 85.5% and clinical specificity of 75.3% with the optimal cut-off of 0.65 μg/mL (area under the curve-AUC: 0.866). ROC curve of CYFRA 21-1 showed clinical sensitivity of 76.7% and clinical specificity of 73.7% (AUC: 0.831) with the optimal cut-off of 1.65 ng/mL. There were no significant differences in DR-70™ and CYFRA 21-1 according to sex, tumor stage and pathologic cancer type with the exception of significantly increased CYFRA 21-1 in squamous cell carcinoma.

Conclusions: DR-70™ is comparable tumor marker to CYFRA 21-1 for lung cancer with slightly better clinical performance.

C-35

Evaluating the measurement of free immunoglobulin light-chains in urine samples on The Binding Site SPAPLUS analyser

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Serum free light chain measurement by polyclonal assays is recommended in international guidelines for the detection and monitoring of patients with B cell dyscrasias. Here we assess the potential of the polyclonal assay in measuring FLC in urine. All work was carried out in accordance with the relevant CLSI standards and results are presented on the table. The SPAPLUS specific normal urine range was constructed using 120 normal urine samples from healthy adult donors. Comparison was made to the Roche Modular P analyser using 87 samples for kappa and 60 samples for lambda with each sample set including 30 normal sera and the remainder being samples from patients with prior diagnosis of multiple myeloma. The comparison results were compared using linear regression for the correlation coefficient (R^2) and Passing-Bablok regression for the slope and intercept. Total precision was assessed at three urine FLC levels on 3 kappa and lambda FLC batches tested in duplicate across three analysers over 21 days. Linearity was assessed using serially diluted monoclonal urine samples across concentrations of 1.10 - 324.5mg/L for kappa FLC and 2.21 - 405.3mg/L for lambda FLC. The linearity results were compared with calculated expected values by linear regression. Possible interference from co-existing substances was tested by adding haemoglobin, bilirubin, albumin and ascorbic acid to urine samples at the concentrations shown. Interference results were compared to an equivalent sample blank. This study shows that the SPAPLUS FLC assays provide a precise method of measuring FLC in urine and show good agreement with existing assays.

Test		CLSI Standard	Kappa FLC	Lambda FLC
SPAPLUS Normal Range (120 Normal samples)		C28-A3	0.012 – 32.71mg/L	<4.99mg/L`
Comparison of SPAPLUS urine assay vs. current Modular P urine assay		EP9A-2IR	1.14x + 0.04, R^2 = 0.998	1.00x + 5.56, R^2 = 0.970
Total Precision	Low level precision	EP5-A	10.90% (5.82 mg/L)	7.80% (7.54 mg/L)
	Mid level precision		7.80% (44.63 mg/L)	6.40% (48.97 mg/L)
	High level precision		8.90% (143.65 mg/L)	9.40% (149.55 mg/L)
Linearity	Low Level Urine (1/1 Analyser Dilution)	EP6-A	y = 0.9643x – 0.3596, R^2 = 0.9914	y = 0.9939x – 2.4756, R^2 = 0.9919
	High Level Urine (1/10 Analyser Dilution)		y = 0.9872x + 0.7538, R^2 = 0.9977	y = 0.9619x + 1.909, R^2 = 0.9926
Interference	Haemoglobin (240mg/L)	EP7-A2	8.2% @ 32mg/L	5.1% @ 5mg/L
	Bilirubin (40mg/L for kappa, 200mg/L for lambda)		-4.9% @ 32mg/L	-5.4% @ 5mg/L
	Albumin (1.3g/L for kappa, 5g/L for lambda)		15% @ 32mg/L	8.9% @ 5mg/L
	Ascorbic acid (200mg)		-9.1% @ 32mg/L	-5.0% @ 5mg/L

C-36

Determination of cutoff values for Carcinoembryonic Antigen (CEA), Alpha-Fetoprotein (AFP), and Cancer Antigen 19-9 (CA19-9) concentrations in peritoneal fluid to distinguish between malignant and benign etiologies

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Background: Ascites is the fluid accumulation in the peritoneal cavity caused by several conditions, predominantly liver disease and portal hypertension accounting for approximately 85% of cases; but it also could be due to malignancy accounting for 7% of cases. Differentiating malignant from benign etiology is crucial for management of ascites.

Objective: To establish cut-offs for CEA, CA19-9 and AFP in ascites to differentiate malignant from benign etiologies.

Methods: Residual ascites samples from 137 unique patients undergoing paracentesis for cytology examination at the Mayo Clinic Rochester between 05/2011-12/2011 were included. The cause of the ascites was classified as benign (n=83) or malignancy-related (n=54) based on cytology, imaging studies and medical record review. Concentrations of CEA, CA19-9 and AFP were measured by a chemiluminescent immunoenzymatic assay on the Beckman-Coulter UniCel DxI 800 (Beckman-Coulter, Brea, CA). Statistical analysis was performed using JMP (SAS Institute, Cary, NC). Receiver operating characteristic (ROC) curve analysis was performed to determine high-specificity cut-offs to differentiate between benign and malignancy-related ascites.

Results: A method validation for CEA, CA19-9 and AFP in ascitic fluid was performed prior to this study, the results from which are shown in Table 1. Cut-off values were set at 6.0 ng/mL for CEA, 32 U/mL for CA19-9 and 6.0 ng/mL for AFP. These cut-offs were used to determine the clinical sensitivity and specificity of each marker as shown in Table 1. When the tumor markers were used in combination with cytological findings, there was an increase in sensitivity compared to cytology alone which demonstrated 44% sensitivity at 100% specificity.

Conclusions: Analysis of CEA, CA19-9 and AFP is most useful in differentiating benign from malignancy-related ascites when used in combination to cytology. All markers showed high specificity and improved sensitivity when used with cytology.

Validation and Diagnostic Criteria for Analysis of Tumor Markers CEA, CA19-9 and AFP in Ascites

	CEA	CA19-9	AFP
Accuracy	> 90%	> 94%	> 90%
Intra-assay CV	< 6%	< 6%	< 7%
Inter-assay CV	< 10%	< 8%	< 14%
Functional Sensitivity	0.7 ng/mL (CV <10%)	5 U/mL (CV <10%)	0.6 ng/mL (CV <10%)
Reportable Range	0.7 - 850 ng/mL (R2=0.999, Slope = 1.03)	5.0 - 1800 U/mL (R2=0.999, Slope = 1.01)	0.6 - 2800 ng/mL (R2 = 0.999, Slope = 1.00)
Cut-off Concentration	> 6.0 ng/mL	> 32 U/mL	> 6.0 ng/mL
Marker Sensitivity	29.63%	44.44%	14.81%
Marker Specificity	98.80%	92.77%	96.39%
Marker + Cytology Sensitivity	59.26%	64.81%	54.41%
Marker + Cytology Specificity	98.80%	92.77%	96.39%

C-37

Evaluation of caspase-3 enzyme as a surrogate molecular marker for the assessment of response to chemotherapy in advanced breast cancer

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Background: Caspase-3 is a critical mediator of apoptosis. It is a potential marker for predicting response to chemotherapeutic agents in breast cancer. The study was aimed at assessing tumor response at molecular level to anthracycline-based chemotherapy by measuring activated caspase-3 which is chosen as an index for activated apoptotic activity; to correlate pathological and clinical response to chemotherapy with caspase-3 fold increase; to assess the relationship between pre-chemotherapy hormone receptor status to caspase-3 fold increase; analyze caspase-3 fold increase basing on the menopausal status in advanced breast cancer patients.

Methods: Eighty patients aged between 18 and 75 years presenting with advanced breast cancer were enrolled for the study. FAC regimen was chosen. Tumor samples were obtained through core needle biopsy and caspase-3 was assessed at three stages in the whole study; prior to chemotherapy, before 2nd cycle and at surgery/before 4th cycle. Caspase-3 activity was measured by caspase-3/CPP32 Fluorometric Assay Kit, Biovision Ltd., pathological response by using the Criteria of Japanese Breast Cancer Society, clinical response by RECIST criteria and hormone receptor status by Horse Raddish Peroxidase-Polymer method.

Results: Patients were categorized into 6 groups as per their caspase-3 fold increase: less than basal caspase-3, 0-<1, 1-<2, 2-<3, 3-<4 and 4-≥4. The study showed a high probability of predicting chemoresistance/sensitivity before 2nd cycle in patients showing pathological grade 0 response and clinical progressive disease with less than 1-fold increase and pathological grade 3 and clinical complete response with more than 4-folds increase in caspase-3. Probability of occurrence of the same response at surgery/before 4th cycle for pathological grade-0 is 74%, clinical progressive disease is 100%; pathological grade-3 is 80% and clinical complete response is 100% for the same chemotherapy regimen. It suggests that a change over in chemotherapy regimen is required at 2nd cycle for the patients showing pathological grade 0 response and clinical progressive disease with less than 1-fold increase in caspase-3 while continuation of the same chemotherapy regimen for the patients showing pathological grade 3 and clinical complete response with 4 and above 4-folds increase. There is no correlation between caspase-3 fold increase and pre-chemotherapy hormone receptor status. There is no statistically significant influence of the menopausal status on the chemotherapy response both before 2nd cycle and at surgery/before 4th cycle of chemotherapy.

Conclusions: The study recommends caspase-3 as a possible surrogate marker for assessing chemoresistance/sensitivity. It is possible to identify a patient's prognosis before 2nd cycle and there is a sign of possibility to individualize treatment prior to 2nd cycle. This study enables a clinician to take a decision regarding the further chemotherapy prior to 2nd cycle itself without wasting much valuable time which may impact the survival of the patients. In patients belonging to poorer prognosis categories, further chemotherapy with non-cross resistant chemotherapeutic agents can be offered while patients in the good prognosis categories do not require a change in chemotherapy. However, further studies are required to provide an evidence base for such an approach. Nevertheless, the study provides the basis for the consideration of such an approach.

C-38

Evaluation of a single, latex-enhanced assay for combined measurement of kappa and lambda free light chains in serum (CombyLite) on the Binding Site SPA PLUS turbidimetric analyser

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Recently studies in disparate disease cohorts have indicated that quantitating combined polyclonal serum free light chains (FLC, kappa-FLC+lambda-FLC; cFLC)

may have clinical value both in predicting mortality (chronic kidney disease, chronic lymphocytic leukaemia) and disease severity (systemic lupus erythematosus). The cFLC measurements are likely to serve as surrogate markers for renal function and to provide information on adaptive immune system regulation. Here we describe and evaluate a single, latex-enhanced, turbidimetric assay (CombyLite) for the combined measurement of both kappa and lambda FLC.

Monospecific, polyclonal antibodies directed to both kappa and lambda FLC were bound to latex microparticles. Assay parameters were generated for the resultant reagent on the Binding Site SPA PLUS turbidimetric analyser. The analyser was programmed to construct a calibration curve from a 6 point calibration set. Curves were validated by assay of control fluids. Samples were initially measured at a 1/10 dilution and, if out of range, the instrument automatically re-measured the samples at a 1/30 dilution. All dilutions were made with the instrument's on-board pipetting system. The measuring range was from 6.25 - 200mg/L at a 1/10 sample dilution, with a reflex range of 18.75 - 600mg/L at 1/30, and sensitivity of 0.625mg/L at neat. The assay time was 10 minutes and was read at end-point.

Precision studies (CLSI EP5-A2) were 12.23 mg/L were assessed for total, within-run, between-run and between-day precision, using three different reagent lots on three analysers. The coefficients of variation were 5.5%, 2.0%, 2.6% and 4.4% for the high sample, 5.5% 2.1%, 2.9% and 4.2% for the medium sample and 14.4%, 4.1%, 6.7% and 12.0% for the low sample respectively.

Linearity was assessed by mixing a high sample pool with a low sample pool according to CLSI EP6-A over a range of 6.05 - 223 mg/L. The assay showed a high degree of linearity when expected values were regressed against measured values $y=1.003x - 3.363$, $R^2=0.9991$.

No significant interference (+/-4%) was observed when hemoglobin (500mg/dL), bilirubin (20mg/dL) or Chyle (1500 FTU's) were added to serum samples of known total FLC concentrations.

Comparison was made with summated values from the kappa and lambda FreeLite kits for the SPA PLUS (Binding Site Group Ltd) by measuring samples from normal healthy donors (n=132) and patients with: SLE (n=280), Rheumatoid Arthritis (n=332), Lymphoma (n=37), known monoclonal light chain (n=19) and chronic kidney disease patients (n=515). The values ranged from 6.19 to 259.10 mg/L. Data was analysed using Analyse-it: Passing-Bablock fit was $y=0.98x -1.57$, linear fit was $y= 0.95x -0.80$ with R^2 of 0.96.

In conclusion the CombyLite assay provides a rapid, cost-effective means of measuring cFLCs and shows good agreement with summated values from existing assays.

C-39

p.R337H mutation in gene TP53 in breast cancer patients

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Background: Germline p.R337H mutation in gene TP53 is highly prevalent in sporadic adrenalcortical tumors (ACT) in southern Brazil, a region where a high incidence of this kind of tumor has been observed. Until now, p.R337H mutation has not been evaluated in breast cancer (BC) in this region of Brazil. In the present study we investigated the presence of germline p.R337H mutation in the TP53 gene in BC patients.

Methods: Patients who underwent removal of BC in UOPECCAN (União Oeste Paranaense de Estudo e Combate ao Câncer), from March 8th, 2010 to October 31st, 2010, were invited to take part in the study. A total of 147 women with BC were included in the study. Age ranged from 31 to 89 years old (mean age of 56 years old). The control group included 191 women, 35 years of age and above (a mean age of 58 years old, ranging from 35 to 91 years old) and were diagnosed as a non-carriers of BC or any other kind of neoplasm and/or had suggestive familial history of cancer. Written informed consent was obtained from all participants, and the study was approved by the Institutional Review Boards of participating centers.

The p.R337H mutation was investigated in blood by means of restriction enzyme analysis and sequencing of exon 10 in the TP53 gene. The genomic DNA was extracted with the commercial kit QIAamp DNA Blood Mini Kit (Qiagen, USA). Amplification of the exon 10 was carried out in 10 µl of PCR mix containing 100-200 ng of genomic DNA; 1 µl of 10x buffer; 1.5 mM $MgCl_2$; 1.2 µM of each primer (5'-TTG AAC CAT CTT TTA ACT CAG G-3' (forward) and 5'- ATG AAG GCA GGA TGA GAA TG-3' (reverse)); 0.2 mM deoxynucleotide triphosphate (DNTP); 1.25U Taq DNA Polimerase (Invitrogen, USA) and water to 10 µl. GeneAmp PCR System 9700 (Applied Biosystems, USA) thermocycler was used accordingly to the manufacturer's instruction. The amplicon was cleaved by HhaI endonuclease and evaluated over a UV light. Digested fragments harboring wild-type sequences showed two bands (82 and 177 base pairs), whereas alleles with p.R337H mutation were not cleaved and showed only one band (259 base pairs). Those amplicons that were suggestive of p.R337H mutation were confirmed by sequencing reactions using the BigDye Terminator Sequence v3.1 (Applied Biosystems, USA). The tumor DNA from those patients who carry the germline mutations were also evaluated for the p.R337H mutation.

Results: The cleavage pattern for the heterozygous p.R337H mutation was observed in two patients for both blood and tissue, and this mutation was then confirmed by sequencing. Both patients presented a familial history compatible with Li-Fraumeni syndrome (LFS). The mutation was not observed in any of the samples collected from the control group.

Conclusions: We may conclude that the p.R337H mutation in gene TP53 is not associated to sporadic breast cancer, but associated with LFS in patients from southern Brazil.

C-40

Investigation of Potential Interferences in an Automated Assay for Chromogranin A on the ThermoFisher Scientific BRAHMS KRYPTOR Compact PLUS

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Background: Chromogranin A (CGA) is commonly used as a neuroendocrine tumor marker. Non-tumor related CGA elevations have been associated with chronic kidney disease (CKD), atrophic gastritis, liver disease, congestive heart failure (CHF), and proton pump inhibitor (PPI) use. Additional interferences common in immunoassays include heterophile antibodies, rheumatoid factor (RF), renal dialysis and non-linearity of dilution.

Objective: Our goal was to investigate the effect of interferences by measuring CGA in residual samples from selected patient populations.

Method and Patient Groups: All samples were analyzed in a recently developed automated CGA immunoassay on the ThermoFisher Scientific BRAHMS KRYPTOR compact PLUS (KC+). In this study we examined 2204 samples from 1231 patients.

Patient samples included: (i) 71 with impaired renal function of various degrees, (ii) 205 with elevated gastrin levels, (iii) 427 from hepatocellular carcinoma, testicular carcinoma, and liver disease patients, (iv) 50 with elevated aspartate aminotransferase (AST) values, (v) 952 collected during a CHF study, and (vi) 26 with highly elevated NT-Pro B-Type Natriuretic Peptide levels. To test for common immunoassay interferences, 367 samples were assayed for CGA before and after treatment with Heterophile Blocking Tubes (HBT, Scantibodies). In addition, 29 RF positive samples and paired sera from 24 patients collected before and after dialysis were tested. 28 samples (~0.16% of 25684 CGA samples tested in one year) identified as having non-linear dilutions in our in-house manual CGA assay were assayed on the KC+. Some of the latter were also run in the CisBio Chromoa™ CGA ELISA kit, an assay that is claimed to be less prone to non-linearity due to CGA fragments. Finally, medical histories from all patients were examined to determine PPI use, as well as other co-morbid conditions. Generalized estimating equations were used to estimate the association of each possible interference with CGA values while adjusting for multiple CGA results per patient.

Results: In 1760 samples, PPI use was found to be associated with a mean CGA increase of 757 ng/mL (95% CI: 589, 925, $p<0.0001$); testicular cancer 189 ng/mL (95% CI: 12, 366, $p=0.04$); elevated gastrin 545 ng/mL (95% CI: 3, 1087, $p=0.049$); and renal disease 471 ng/mL (95% CI: 217, 724, $p=0.0003$). The effect of liver disease was non-significant at 499 ng/mL (95% CI: -78, 1077, $p=0.09$). CHF, hepatic cancer, and autoimmune disease did not have independent effects on CGA (all $p>0.16$). In addition, CGA did not change significantly following dialysis (24 patients; $p=0.32$).

No difference was seen in the 367 samples after HBT treatment ($y = 0.98x + 2.25$; $r = 0.9996$). Eight of the 28 non-linear dilution samples were also found to be non-linear in the KC+ assay (mean % difference 317.4%). Six of these 8 were also tested in the CisBio assay, which also showed non-linearity (mean % difference 220.5%).

Conclusions: Increased levels of CGA were found with PPI use, testicular cancer, elevated gastrin levels, and renal disease. There was a decrease in the incidence of samples with non-linear dilution with the BRAHMS Chromogranin A KRYPTOR assay.

C-41

Comparison of a serum polyclonal antibody based free light chain assay (Freelite™) with a new monoclonal antibody based test (N Latex FLC) for the detection of patients with acute kidney injury secondary to multiple myeloma

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Quantification of serum free light chains (FLCs) using Freelite™ has led to a paradigm shift in the diagnosis and monitoring of patients with plasma cell dyscrasias; highlighted by their acceptance into international guidelines. Acute kidney injury (AKI) secondary to multiple myeloma (MM) has a profound impact on patient morbidity and mortality. Rapid diagnosis is aided by serum FLC assessment. This, alongside disease specific treatment and light chain removal, has been shown to improve renal recovery. Recently, immunoassays using monoclonal antibodies against FLCs have become commercially available. Here we performed a direct comparison of these two assays utilising stored patient sera from 28 patients (17 λ, 11 κ). Serum κ and λ FLC concentrations were measured retrospectively on a Siemens BN™II Analyser, using Freelite (The Binding Site Group Ltd, UK) and N Latex FLC (Siemens GmBh, Germany). The absolute values reported by the assays showed poor agreement for both κ (R^2=0.87) and λ (R^2=0.3), neither reaching the required R^2=0.95 CLSI guidelines (CLSI EP09-A2). Utilising FLC=500 mg/L cut off for AKI, 5/28 (18%, table 1) of patients were misclassified using the N Latex FLC assay (median 221 mg/L, range 1-493 mg/L). In 1/17 patients the N Latex FLC assay failed to identify monoclonal lambda light chain (Freelite=1810mg/L vs N Latex FLC=0.5mg/L), even when run at higher dilutions.

Patient	Multiple myeloma type	Freelite			N Latex FLC			Missed by N Latex FLC	Misclassified by N latex FLC as <500mg/L
		κ	λ	Ratio	κ	λ	Ratio		
2	Free λ	2.00	7010.0	0.0003	1.02	322.00	0.003	No	Yes
7	IgA λ	8.05	1810.0	0.0044	4.19	0.52	8.058	Yes	Yes
10	IgG λ	8.47	1080.0	0.0078	8.64	64.30	0.134	No	Yes
13	IgG λ	9.25	572.0	0.0162	9.31	225.0	0.041	No	Yes
23	IgG κ	796.0	6.33	125.75	493.00	16.60	29.699	No	Yes

Rapid assessment of AKI patients can aid in renal recovery, key to the assessment with respect to MM is identifying patients with physiological levels of light chain, which could cause renal damage. The N Latex FLC assay does not clearly identify patients with AKI (i.e. above 500mg/L) and may miss monoclonality in lambda patients.

C-44

Comparison of Freelite and N Latex FLC utilising diagnostically relevant samples

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Serum free light chains (FLC) as measured by sheep polyclonal immunoassays (Freelite™, Binding Site, Birmingham UK) have been included in international guidelines. Monoclonal FLCs are unique and highly variable, therefore the ability of the assay to recognise all FLCs tested relies upon a broad range of antigens and the variable nature of the polyclonal response. Recently, assays utilising monoclonal antibodies (recognising a single epitope per antibody) have been produced (N Latex FLC, Siemens, Munich, Germany). Here we assess the performance of the two assays and comment on the suitability of the N Latex FLC assay as a replacement for the established Freelite assay. Assay comparisons were performed utilising 20 normal human sera and 144 monoclonal gammopathy patient sera (: n=82; : n=62) on the BN™II nephelometer. Using FLC as the standard assay, the samples tested had a broad range of FLC concentrations (levels from 0.36-18,500 mg/L; levels from 1-18,000 mg/L). In 20 normal samples the correlation between the two assays was poor comparing kappa assays (Passing-Bablock (PB) =0.8) but acceptable comparing lambda assays (PB=1.0). However, there was poor agreement between the assays when comparing monoclonal protein results.

Specificity & samples		No.	Passing-Bablok slope
κ	Normal sera	20	0.8
	All monoclonals	82	0.58
	Monoclonals <1200 mg/L	67	0.66
λ	Normal sera	20	1.0
	All monoclonals	62	0.56
	Monoclonals <1200 mg/L	46	0.44

Furthermore, antigen excess was identified in 2.5% kappa and 5% of lambda samples using the N Latex FLC assay compared to 1% kappa and 0% lambda using Freelite. N Latex FLC has poor agreement with Freelite, potentially reflecting monoclonal antibodies single epitope recognition. Despite antigen excess protection the N Latex FLC assay is prone to antigen excess which may result in missed monoclonal proteins. The poor agreement excludes the N Latex FLC assay from being used to measure FLC in place of FreeLite; therefore, the N Latex FLC assay cannot be used as directed by international guidelines.

C-46

Use of classical cytogenetic as a tool for the diagnosis and risk assessment of acute myeloid leukemia

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Background: Acute myeloid leukemia (AML) is characterized by abnormal proliferation of myeloblasts and represents 90% of cases of acute leukemia in adults. The American Southwestern Oncology Group (SWOG) and Medical Research Council (MRC), defined criteria for the categorization of risk of AML patients according to chromosomal abnormalities. The aim of this study was to demonstrate the importance of the study of classical cytogenetic as in the AML diagnosis and their categorization into risk groups for prognosis and treatment.

Methods: We performed the karyotype of 283 patients with clinical suspicion of AML, and/or monitoring of treatment of AML and/or after bone marrow transplantation (BMT) for AML, which came into our service in the period from 5/1/10 to 28/12/11. Samples were processed according to standard protocol (ISCN2009) and SWOG and MRD for categorization of AML patients.

Results: The present casuistic showed 51% were men and 49% women with mean age 41.3 years, other studies below ~ 65. Abnormal karyotypes were found in 26.5% (75/283) of cases, normal karyotype was observed in 54.4% (154/283) of cases and in 18.4% (52/283) there was no satisfactory cell growth, 2 (0.7%) patients were evaluation after BMT and showed complete chimerism. According to 229 karyotype analysis was found: 8% (19/229) positive patients at risk of 72% (164/229) with an intermediate risk, 14% (32/229) to unfavorable risk and 6% (14 / 229) with unknown risk. In favorable risk, the most frequent abnormality was the presence of t (15; 17) in 73% of patients, followed by the t (8; 21) in 16% and inv (16) in 11%. In the intermediate risk group the normal karyotype was observed in 94% of patients, followed by complete trisomy of chromosome 8 (3.6%) and monosomy X or Y chromosome nulissomia (2.4%); normal karyotype is the most common finding in AML patients and although to stratify patients at intermediate risk group some translocations or small mutations such as FLT3, NPM1 and cKIT, can change the prognosis in these cases. In the unfavorable risk group, complex karyotype was the most frequent finding (34.4%), followed by rearrangements with chromosome 11 (18.7%), Philadelphia chromosome - Ph (12.5%); the -7/deletion chromosome 7 (12.5%), changes in chromosome 3 (9.4%) and -5/deletion of chromosome 5 (9.4%) and clone monossomal 3.2%. It is reported that the frequency of monossomal karyotype increases with age, as the average of our patients were less than 60 years, this finding is consistent with the literature.

Conclusions: Our data are compatible with literature information reference, except the mean age of patients. Some findings allowed to guide the physician regarding treatment, because some patients were suspected to AML. Due to the high percentage of normal karyotype in AML, since mid-2010 we are guiding the clinician to request the research molecular (FLT3, and NMP1 cKIT) in order to further evaluate this portion of patients in relation to risk category. We believe that our data may be representative of incidence in the context of AML according to the age of our country.

C-48

The diagnostic role of preoperative tumor markers and serum amyloid-A in early stage endometrial cancer

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Background: The purpose of the present study is to evaluate the prognostic and predictive efficacy of the tumor markers (HE4, CA 125, CA 15-3, CEA, CA 19-9), serum amyloid A (S-AA), eotaxin, e-selectin levels in different stages of endometrial cancer.

Methods: A total of 64 women with defined stage and grade of endometrial cancer were compared with 64 women who were operated with benign uterine diseases and with 34 healthy subjects as control group. Determinations of tumor markers

and hormones (prolactin, FSH and LH) were done by E170 autoanalyzer. S-AA concentrations were measured by particle-enhanced immunonephelometry. Eotaxin and e-selectin levels were determined by ELISA method.

Results: Preoperative serum HE4 and S-AA levels were significantly higher in endometrial cancer patients than in controls (p=0.001, p=0.023, respectively). In early-stage endometrial cancer patients, only HE4 levels showed increment compared to controls (p=0.05), whereas SAA and CA 15-3 levels were also high in addition to HE4 in advanced-stage disease. Increased levels of HE4 and CA 125 were detected at advanced stages compared to stage 1A (p=0.001,p=0.013, respectively). The best cut-off points were determined to be 59.6 pmol/L for HE4 with 71 % sensitivity and 70% specifity; 8.0 mg/L for SAA with 69% sensitivity and 59% specifity .

Conclusions: Serum HE4 and S-AA may be of value in early detection of endometrial cancer. These markers are associated with the stage and grade of endometrial cancer. E-selectin and eotaxin levels were not found altered in cancer patients.

Mean values of biochemical parameters in study groups							
Tumor markers	Healthy (34)	Benign Disease (64)	EndometrialCa (64)	StageIa (40)	StageIb> (24)	GradeI	Grade II-III
Ca125 (U/mL)	18.9 (9.3-42.2)	22.5 (4.2-77.7)	26.6 (5.3-178)	19.3 ‡ (5.3-107)	37 ‡§ (8.3-178)	20 ‡ (5.4-107)	36 ¥ (6.7-178)
Ca 19-9 (U/mL)	15.2 (0.6-32.6)	14.3 (3.8-39)	23.7 (0.6-329)	14.2 (0.6-101)	36.8 (0.6-329)	14.8 (0.6-101)	36 (0.6-329)
Ca 15-3 (U/mL)	15.3 (4.7-27.2)	16.2 (5.5-31)	21 (4.6-103)	16.7 (4.6-36.4)	26.6 * (6.9-103)	15.7 (4.6-31)	27.6 ¥*‡ (7-103)
CEA (ng/mL)	1.52 (0.4-3.1)	1.5 (0.2-5)	2.1 ‡ (0.2-15.6)	1.7 (0.2-5.6)	2.7 ‡ (0.3-5.6)	1.8 (0.2-5.6)	2.5 ‡ (0.2 -15.6)
S-AA (mg/L)	11.0 (3.8-45.7)	16 (3.2-124)	14 * (3.2-56)	12.8 (3.2-54)	15.7 * (3.7-56)	12 (3.3-49.7)	17 ¥*‡ (7.2 -56)
HE4(pmol/L)	61.1 (35.4-152.7)	57 (30.7-141)	155 *‡ (38.5-1059)	89 *‡ (42.8-294)	243.4 *‡§ (38.6-1059)	88.6 *‡ (38.6-294)	246 ¥*‡ (50-1059)
Hormones							
LH (mIU/mL)	15.3 (1.5-70)	15.3 (1.5-70)	26.6 * (2.0-80)	25 (2.0-80)	26.6* (2.7-80)	19.5(2.0-80)	30 * (2.7-80)
FSH (mIU/mL)	22.5 (2.4-123)	22.5 (2.4-123)	42.6 * (2.3-95.8)	42 (2.3-96)	46* (2.4-79)	31(2.3-78)	46.5 * (2.4-96)
Prolactin (ng/mL)	18.6 (3.8-71)	19.5 (5.7-55)	17.3 *‡ (1.9-79)	19.2 (1.9-79.6)	14.6 ‡ (5.8-65)	19.7(1.9-80)	14.5 ‡ (5.4-65)
Chemokines							
Eotaxin-2 (pg/mL)	213.9 (143-330)	204 (93.5-503)	229 (106.3-468)	217 (106-427.6)	245 ‡ (106.3-428)	216(126-427.6)	245.4 (106-468)
E-selectin (pg/mL)	63.6 (22.8-88)	63.3 (21.9-164)	65.3 (17.3-141)	65.7 (23-120)	64.6 (17.3-141)	73.5(23-141)	53.4 ¥ (17.3-120)

C-49

CA 15-3, CA 125, CA 19-9, AFP and NSE measured using Roche E170 and Diasorin LIAISON XL; a comparison using the software StatisProTM (CLSI-Analyse-it)

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Background: CLSI standards are used all around the world in the clinical practice by many laboratorians but very often they require complex calculations not easily performed by standard commercial softwares. This is quite unfortunate because limits a very effective tool for quality improvement. Recently CLSI developed in conjunction with Analyse-it a Software (StatisPro™) which friendly carries out all the calculations required by relevant CLSI standards: EP10-A3, EP09-A2-IR, EP15-A2, EP05-A2, EP06-A, EP17-A, C28-A3 and we are using quite frequently in daily activity. The aim of our study was to compare the results yielded by Roche Modular E170 and DiaSorin LIAISON XL in the measurement of CA 15-3, CA 125, CA 19-9, AFP and NSE using EP9A3 CLSI standard and StatisPro™ (CLSI and Analyse-it, Wayne, PA, USA).

Methods: We measured CA 15-3, CA 125, CA 19-9, AFP and NSE using respectively Modular E170 analyzer [Roche, Mannheim, Germany (E170)] and LIAISON XL [DiaSorin, Saluggia, Italy (XL)] in at least 40 serum samples collected in patients suffering from cancer. The measurements were carried out simultaneously in duplicate strictly following the EP9A3 CLSI standard and the calculations and the graphs were carried out using StatisPro™.

Results: 1) AFP (n=41) r : 0.993; intercept= 0.02; slope =1.035; Sy.x=1.156; the difference plot shows a fair consistency under 4 ug/L, higher XL values at concentration 4-10 ug/L and great values dispersion at concentration higher than 10 ug/L; the repeatability plots demonstrate higher repeatability of E170. 2) CA 15-3 (n=43) r : 0.985; intercept= 2.17; slope =0.762; Sy.x 5.266; the difference plot shows a fair consistency under 27 KU/L, lower XL values at concentration 27-42 KU/L and much lower values at concentration higher than 42 KU/L; the repeatability plots demonstrate higher repeatability of E170 results. 3) CA 125 (n=40) r =0.994; intercept= 7.39; slope =0.892; Sy.x 5.049; the difference plot shows a fair consistency under 42 KU/L; lower XL values at concentration higher than 42 KU/L; the repeatability plots demonstrate higher repeatability of XL results. 4) NSE (N=41) r =0.959; intercept= 3.585; slope =0.843; Sy.x 6.354; the difference plot shows a fair consistence under 20 ug/L; lower XL values at concentration higher than 20 ug/L; the repeatability plots demonstrate higher repeatability of E170 results. 5) CA 19-9 (n=43): r =0.770; intercept= 17.13; slope =0.821; Sy.x 20.89; the difference plot shows a fair consistency under 40 KU/L and a mediocre consistency over 40 KU/L; the repeatability plots demonstrate higher repeatability of XL results.

Conclusions: Very often laboratorians need to compare the results yielded by the previously used analyzer and that being introduced. Reference methods for immunoassays are usually not available but the clinicians need to know the comparability between "old" and "new" assay. StatisPro™ produces all the calculations and graphs needed for demonstrating the comparability of the results along the concentration span of results. StatisPro™ allows an easy and fast comparison of methods via paired results from patients and can be employed for providing the necessary information to clinicians and for satisfying accreditation and certification requirements.

C-50

Differentiation of Premenopausal Women with Ovarian Cancer Using Multimodal Testing of CA 125 and HE4 in Serum and Urine

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Background: Ovarian cancer (OC) is the most lethal gynecologic cancer with most women undiagnosed until the disease is in advanced stages. Imaging and serum biomarker-based detection has been extensively reported, represented by the PLCO study (Buys et al, 2005) and UKCTOCS study (Menon et al, 2009). Recently, Nolen of Lokshin's Group proposed a combination of serum and urine biomarkers (Dissertation, 2011), and Urban et al reported a serum CA 125 and HE4-based multimodal screening (2011). We present a multimodal measurement of CA 125 and HE4 in urine and serum for the identification of premenopausal women with OC.

Methods: 357 matched single-point sera and urine samples from premenopausal women were used in this study, of which, 54 had OC including borderline/low malignant potential tumor (LMP) and 303 had a benign pelvic mass. The serum CA 125, HE4 and ROMA data were from databases for the three published studies (Moore et al, 2008, 2010, 2011). The urine samples were from the sample bank used for these three studies. Urine CA 125, HE4 and creatinine were measured with ARCHITECT CA 125 II, HE4 EIA and Jaffe reaction-based colorimetric measurement, respectively. A urinary predictive probability algorithm (uPP) was derived from an algorithm using urinary HE4 and CA 125.

Results: In the multimodal testing, urine uPP was calculated first as the line 1 testing and then serum ROMA was calculated as the line 2 testing on the serum samples from the urine uPP-positve subjects. The sensitivities of the multimodal testing was then compared with the individual testing of serum CA 125, HE4, ROMA, urine HE4/creatinine Ratio and uPP with a specificity at 90% as indicated in Table 1.

Conclusions: Urine uPP and serum ROMA-based multimodal testing appears to be a better option for the differentiation of premenopausal women with OC from those with a benign pelvic mass.

Table 1. Sensitivities of HE4, CA 125, uPP, ROMA and multimodality								
Performance		N	Multimodality	Serum HE4	Serum CA125	Urine HE4/ creatinine	Urine uPP	Serum ROMA
Cut-off (≥)			1.21 (uPP); 1.25 (ROMA)	77.1 pmol/L	176.9 U/mL	110.7	1.54	1.95
Sensitivity	Overall OC	54	64.8% (35)	53.7% (29)	53.7% (29)	59.3% (32)	55.6% (30)	53.7% (29)
Sensitivity	LMP	20	55% (11)	30% (6)	50% (10)	45% (9)	35% (7)	30% (6)

Sensitivity	Stage I OC	12	33.3% (4)	33.3% (4)	33.3% (4)	33.3% (4)	33.3% (4)	33.3% (4)
Sensitivity	Stage II-IV OC	19	100% (19)	94.7% (18)	73.7% (14)	94.7% (18)	94.7% (18)	94.7% (18)
Sensitivity	Unstaged OC	3	33.3% (1)	33.3% (1)	33.3% (1)	33.3% (1)	33.3% (1)	33.3% (1)
Specificity	Benign Diseases	303	90% (272)	90% (272)	90% (272)	90% (272)	90% (272)	90% (272)

C-51

Sensitivity Investigation of High Resolution Capillary Zone Electrophoresis (CZE-HR) for Slight Monoclonal Bands as Compared to HevyliteTM.

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Background. High resolution capillary zone electrophoresis (CZE-HR) for serum proteins allows clear separation in 8 well-defined fractions. Amongst many new clinical uses, CZE-HR could also increase powerfulness of serum protein electrophoresis (SPE) in monoclonal gammopathy investigation in comparison with other SPE techniques. Higher resolution and sensitivity could lead to greater detection rate of small bands or residual monoclonal proteins in post-treatment follow-up. On the other hand the new serum heavy chain/light chain (HLC) immunoassay offers sensitive follow-up by quantitative analysis of intact immunoglobulin heavy chain (IgH) and light chain kappa or lambda and provides the clinically useful IgHκ/IgHλ ratio. We challenged power of CZE-HR SPE-based algorithm for detection of thin monoclonal protein using positive patient samples as determined by the IgHκ/IgHλ ratio but all negative according to a standard SPE-based approach.

Methods. 14 samples were selected from well characterised (3 IgG, 11 IgA) multiple myeloma (MM) patients in remission after treatment. Those samples were positive for residual monoclonal band according to IgH kappa(κ), IgH lambda(λ) and IgHκ/IgHλ ratio measurement (Hevylite, The Binding Site) but negative with standard techniques (SPE and IFE, Hydrasys 2, Sebia). 3 normal samples were included. FLCs, IgGκ, IgGλ, IgAκ and IgAλ concentrations were measured on a BN™II nephelometer (Siemens Healthcare Diagnostics). All those assays were performed in the laboratories of The Binding Site, Birmingham, UK. Those 17 samples were then sent to University of Montréal Hospital Center (CHUM, Montréal, Québec) for investigation according to an algorithm based on CZE-HR SPE (Capillarys 2 running with a high resolution buffer, Sebia). If required, IFE was conducted with capillary immunosubstraction technique (IT; Capillarys 2) and/or agarose HR-IFE (Hydragel 2 IF, Sebia). Absolutely no information regarding the origin of samples, patient status or previous results were given to CHUM investigators prior to completion of whole study.

Results. All 3 normal samples were within normal when tested with CZE-HR technique. However one sample with increased transferrin peak, a second with slight haemolysis required IT which revealed normal. 3 samples had IgGκ and were correctly typed. Other tested samples (IgAκ residual band) arose from 5 patients with 1 up to 4 samples per patient. CZE-HR based approach detected IgAκ band in 3 of 4 samples from same patient (IgAκ/λ ratio of 2,66(negative) and 3,8, 3,9 & 3,9 (all 3 positive)). Another patient was correctly identified (IgAκ/λ ratio 3,9 & 4,2). One patient was easily typed (IgAκ/λ ratio of 9,1). CZE-HR-based algorithm failed to detect the IgA clone in 1 out of 2 samples from another patient (IgAκ/λ ratio 3,0(positive), 4,3(negative)) and 2 out of 2 from the last patient (IgAκ/λ ratio 3,1 & 5,0).

Conclusion. Hevylite and CZE-HR detected monoclonal proteins in 10/14 SEP/IFE negative samples from patients with confirmed MM. Hevylite detected abnormalities in the remaining 4 samples, although CZE-HR did not identify the original clone. Both assays offer a sensitive alternative to traditional tests, although further clinical studies are required to assess the impact of this increased sensitivity on patient outcomes and to investigate the discordance between the assays.

C-52

Association between an Obesity-related Gene and Breast Cancer Risk

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Background: Breast cancer (BC) is the most common cancer among women worldwide. Obesity is a well-known risk factor for BC, especially in postmenopausal women. The BMI (Body Mass Index) is a simple index used to classify overweight ($25 \leq BMI < 30$) and obesity ($BMI \geq 30$) in adults. Recently several studies have shown some genetics variants linked to increased BMI, such as the Single Nucleotide Polymorphisms (SNPs) in genes involved in food intake regulation: rs1121980 (T/C) and rs9939609 (A/T) in the *FTO* (Fat Mass and Obesity Associated) gene and the rs17782313 (T/C) in the *MC4R* (Melanocortin 4 receptor) gene. The aim of this case-control study was to investigate the association between these SNPs and BC risk and also to determine the effect of these SNPs on BMI in the control and case groups separately.

Methods: The individuals included in the current study consist of 100 BC patients (obtained prior to any radio or chemotherapy regimen) and 148 healthy women from Santa Catarina, Southern Brazil. The SNPs were genotyped using Applied Biosystems® Taqman® SNP genotyping assays. The Odds Ratio (OR) was calculated by the SPSS software (version 12.0), IC95%; $p \leq 0{,}05$. Four association analysis models were used - dominant, recessive, homozygous comparison and heterozygous comparison.

Results: The minor allele frequency in controls and cases respectively were: rs1121980 (T) 0.45/0.40; rs9939609 (A) 0.41/0.38; rs17782313 (C) 0.15/0.23. The *MC4R* rs17782313 C allele showed a 1.740 increase in risk for BC development (IC95% 1.016-2.980, p=0.044); when the C allele was analyzed in overweight postmenopausal women the risk for BC was higher in the dominant model (OR=2.772, IC95% 1.057-7.267, p=0.038) and in the heterozygous comparison model (OR=3.256, IC95% 1.109-9.557, p=0,032). Nevertheless, the *FTO* SNPs did not show any significant association with BC. We confirmed that the two *FTO* SNPs (rs1121980 and rs9939609) are in strong linkage disequilibrium, as noted in other studies in different populations. When the association with BMI was analyzed, none of the three SNPs were associated with high BMI in the control or case group, which is different from several studies in other populations.

Conclusion: This is one of the first studies to determine the *FTO* rs1121980 and rs9939609 and *MC4R* rs17782313 allele frequencies in the Brazilian population. We found an important and unpublished association between *MC4R* rs17782313 SNP and BC, suggesting a role of obesity-related genes in the increased risk for BC development.

C-53

Candidate Reference Method for measuring blood concentrations of Prostate Specific Antigen (PSA) using immuno-extraction, trypsin digestion and tandem mass spectrometry

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Objective: Develop a traceable measurement system that detects the forms of PSA which are measured by commercial immunoassays. Relevance: Harmonization is important because many clinicians utilize universal PSA decision limits; whereas PSA immunoassays produce different values even when they use the same standard.

Methodology: PSA is immuno-extracted from 200 µL serum using a combination of monoclonal antibodies directed to 3 major epitopes (3a, 5c, and 6b) recognized by commercial PSA assays. The extraction antibodies are coupled to paramagnetic beads and the antibody-antigen complexes are trypsin digested on the beads. The LSEPAELTDAVK tryptic peptide (LSE) was selected due to absence of post-translation changes, strong signal and stability. LSE is quantitated based on SRM signals at 636.8/943.8 on an API 5000 spectrometer with an internal standard at 639.9/949.5. The assay is calibrated using female sera spiked with 90% PSA-ACT and 10% free PSA and is standardized with WHO 96/670. Validation: The assay was validated using a panel of 6 calibrators, 4 sera pools, 3 commercial controls and WHO 66/670. Aliquots of this panel were frozen at -70° and thawed just prior to use. This 14 sample panel was measured on commercial PSA immunoassays in triplicate on three different days and was measured on 10 separate MS runs. The consistency of LSE recovery was validated using digests of panel samples extracted using 5 different monoclonal antibodies to epitopes 3, 6b, 3a, 6, and 5c. The LSE recovery was calculated using an isotopically labeled internal standard with an assigned value based on amino acid quantitation. The efficiency of the 3 antibody extraction system was validated by measuring immunoreactive PSA in serum samples. Validation of clinical utility and comparisons with 2 immunoassays (Roche and Beckman) were performed using frozen sera aliquots from 100 men undergoing prostrate biopsy (50 negative, 50 with cancer) and serial measures from 5 men with advanced prostate cancer. The Beckman assay was calibrated with the Hybritech calibrator.

Results: The LSE peptide produced a strong signal that was stable and consistent with five different extraction antibodies. LSE showed excellent recoveries using the 14 sample panel with averages (SE) equal to 99.7% (1.02), 106% (2.00), 105% (2.24), 99.8% (2.08), and 95.0% (1.76) for the 5 extraction antibodies. The triple-antibody extraction efficiency was >99%. The MS assay has an analytic range from 1.2 -76

ng/mL. The signal to noise for the 1.2 ng/mL standard averaged about 12. The CVs for the serum pools and controls ranged from 4.9% at 1.5 ng/mL to 5.9% at 27 ng/mL. The MS PSA values for the 125 validation sera correlated well with both the Roche Diagnostics Cobas (Roche= 0.991 x MS +0.09; R= 0.987), and the Beckman Coulter Access (Beckman= 1.09 x MS -0.14; R=0.994). All three assays showed statistically equivalent separation of prostate cancer from benign disease using ROC curve analysis, with AUCs of 0.6774, 0.6852, and 0.6759 respectively for MS, Roche, and Beckman.

Conclusions: This MS assay can reliably measure PSA concentrations in human serum and could serve as a reference standard for harmonizing PSA immunoassays.

C-54

Study of the utility of the serum Free Light Chains (sFLC) determination versus Bence Jones Proteinuria (BJP) for monitoring monoclonal gammopathies (MG)

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Background: The current techniques for monitoring patients with a monoclonal component (MC) are: SPE (serum protein electrophoresis), with low sensitivity for concentrations below 400mg/L, IF (immunofixation), serum sensitivity of 150mg/L; Bence Jones Proteinuria (BJP), affected by both renal insufficiency (RI) and sample collection difficulties; and measurement of serum Free Light Chains (sFLC), which distinguish monoclonality and light chain monoclonal gammopathies. The objective of this study is to replace the BJP by sFLC in order to increase sensitivity in monitoring malignant monoclonal gammopathies (MG), gammopathy of undetermined significance (MGUS) and Bence Jones Multiple Myeloma (BJMM).

Methods: We reviewed 47 patients with previously diagnosed MC identified by SPE and/or IF, which were being followed-up from the hematology, internal medicine and nephrology departments. During the year 2010, these patients were requested for 24h urine proteinuria (uPT), BJP, and sFLC determinations, with both serum and urine samples collected at the same time. uPT was quantified by turbidimetry (Cobas Integra 800 (Roche Diagnostic)), with pathological values >150mg/24h. BJP by nephelometry (reagent NSC; BNII analyzer (Siemens)), with pathological free κ or λ light chains >3mg/dL, and samples with values between 0,1-3mg/dL were further assessed by urine immunofixation (uIF) for confirmation. sFLC (Freelite) were quantified by turbidimetry (SPAplus Analyzer, The Binding Site), with pathological values for the κ/λ sFLC ratio (rFLC) between 0,26 to1,65.

Results: - 14/47 patients had a MC not quantifiable by SPE: 10/14 had an abnormal rFLC; 4/14 had normal rFLC and negative BJP, and corresponded to 3 MM under treatment and 1 λ-AL amyloidosis.

- 11/47 corresponded to light chain MG without MC identified by SPE.

- 31/47 patients with abnormal rFLC exhibited positive BJP. Only 1 case was positive BJP and normal for rFLC: the patient had a proteinuria of 2015 mg/24h, rFLC=0.35, BJP=71.5mg/24h, and had been diagnosed with RI and MGUS. If the sFLC renal reference range is applied (0,31-3,1) then the rFLC is also abnormal.

- 13/47 patients had positive uPT and negative BJ: 5/13 were MM with abnormal rFLC, but 1/5 MM corresponds to a MM under treatement patient with rFLC within the renal reference range normal (rFLC=3.08); 8/13 had normal rFLC (5 MGUS and 3 MM under treatement).

- The sFLC assay sensitivity (S) and specificity (E) was 91.67% (95% CI: 59.75-99.56%) and 80% (95% CI: 62.54-90.94%), respectively. The BJP assay sensitivity (S) and specificity (E) was 61.11% (95% CI: 36.1-81.74%) and 96.55% (95% CI: 80.37-99.82%), respectively. The Spearman correlation coefficients obtained were: 0.062 for 24h urinary free-κ vs sFLC-κ (95% CI: 0.310-0.895; p=0.0015); 0.767 for 24h urinary free-λ vs sFLC-λ (95% CI: 0.209-0.948; p=0.0159).

Conclusions: 1. The BJP may be substituted for the sFLC determinations for monitoring MG since the last did not result in any false negative, with the added benefit to the patient because it avoids the 24h urine collection.

2. The sFLC assay improves sensitivity for monitoring: malignant MG in remission after treatment, MGUS, and more particularly for monitoring BJMM.

3. - The uPT does not provide added value in screening for BJ proteins and monitoring MG.

C-55

Performance Evaluation of Microfluidic-based Alpha-fetoprotein-L3 and Des-γ-carboxy Prothrombin Assays using the µTASWako® i30 Immunoanalyzer.

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Background: Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide. Alpha-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) are serological markers that are helpful in identifying patients at risk for developing HCC. It has been shown that AFP-L3, a glycosylated variant of AFP, is a better marker for early HCC detection and prognosis. DCP is independent of and complementary to AFP, therefore measuring both analytes can identify a greater number of patients at risk for developing HCC. The objective of this study was to determine the analytical performance of the µTASWako® i30 AFP-L3 and DCP immunoassays.

Methods: Serum DCP and AFP-L3 were measured on the µTASWako i30 (Wako Chemicals, Richmond, VA) which utilizes a microfluidic hybrid method of electrokinetic analyte transport and capillary zone electrophoresis. De-identified, residual serum samples sent to ARUP Laboratories were used to determine analytical sensitivity, linearity, imprecision, accuracy, reference interval verification, and biomarker stability. The study was approved by the University of Utah Institutional Review Board.

Results: *AFP and AFP-L3%.* Precision was determined by measuring total AFP and AFP-L3% at two concentrations in three replicates once each day for five days. Within-run imprecision of total AFP was 1.4 and 6.9% and total imprecision was 3.4 and 7.9% at a concentration of 409 and 21 ng/mL, respectively. Analytical sensitivity of total AFP was determined to be <0.3 ng/mL by ten replicates of the AFP zero calibrator. Linearity for total AFP was determined by combining serum samples with low and high total AFP concentrations in different ratios to create a set of five samples ranging from 1.1-780 ng/mL and tested in three replicates. Linear regression produced a slope of 0.978, y-intercept of 1.11, and R^2 of 0.999. The linearity of AFP-L3% was determined similarly and produced a slope of 0.934, y-intercept of 0.0, and R^2 of 0.994. Accuracy was evaluated using 40 serum samples tested in duplicate on the µTASWako i30 and the previously validated LiBASys® immunoassay system (Wako Chemicals). For total AFP, Deming regression analysis produced a slope of 0.920, y-intercept of 1.07, and R^2 of 0.972. For AFP-L3%, Deming regression analysis produced a slope of 1.323, y-intercept of -11.31, and R^2 of 0.755. The AFP reference interval of 0-15 ng/mL was verified with 20 healthy individuals. *DCP*. Using the same methods described above, within-run imprecision was 7.2 and 2.2% and total imprecision was 8.3 and 2.7% at a concentration of 748 and 12 ng/mL, respectively. Analytical sensitivity was determined to be <0.1 ng/mL. For linearity, linear regression produced a slope of 1.012, y-intercept of 0.74, and R^2 of 0.999. For accuracy, 66 serum samples were tested and Deming regression produced a slope of 1.30, y-intercept of -10.21, and R^2 of 0.860. The DCP reference interval of 0-7.5 ng/mL was verified with 20 healthy individuals. AFP and DCP showed no significant concentration changes in samples stored at 22-25°C, 4-8°C, and -25-35°C for 24h, 7d, and 30d, respectively.

Conclusions: The µTASWako i30 reagent system demonstrates acceptable performance characteristics for quantifying AFP-L3 and DCP in human serum.

C-57

Examination of Thyroglobulin Iodination States and Quantitative Usefulness for Clinical Thyroid Cancer Diagnostics

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Background: Thyroglobulin's (Tg) capability to iodinate tyrosine residues is paramount to the end-goal of thyroid hormone production. Previous studies have associated under-iodination of Tg with thyroid cancer due to the inherent loss of the capability to control the complicated redox chemistry required for iodination within the cancerous tissue. However, a useful technique does not exist for a quantitative measure of the ratio of iodinated to non-iodinated protein. Measurement of these ratios in Tg would allow characterization of the thyroid's capability to iodinate Tg, thus allowing one to distinguish cancerous tissue from normal.

High mass-accuray mass spectrometry allows for accurate identification of specific modification locations within a protein, and once identified, tryptic fragments containing the modified loci can be monitored quantitatively on an LC-MS/MS system to give a ratio of iodinated to non-iodinated Tg, simplifying the identification

of cancerous versus non-cancerous thyroid tissue.

Objective: To elucidate iodination coverage in human Tg and utilize iodination ratios in a quantitative assay to determine cancer state in human thyroid.

Methods: For iodination coverage: Purified human thyroglobulin was reduced with DTT, alkylated with iodoacetamide and digested with trypsin, chymotrypsin, GluC, or a combination of the same proteases. Digested specimens were introduced to an AB Sciex TripleTOFTM 5600 instrument operating in electrospray mode by an Eksigent cHiPLC nanoflow LC system. IDA survey scans were performed with up to 20 product ion scans exceeding 100 counts per second. Survey data was processed using Protein Pilot Software via the Paragon Algorithm. Tyrosine and histidine residues that were mono-iodinated, di-iodinated, or converted to thyroxine (T4) or tri-iodothreonine (T3) were recorded. Peptide peak intensity was extracted qualitatively using Peak ViewTM software.

For quantitative workup: Six modified peptides identified during tripleTOF analysis were synthesized containing iodo-tyrosine, di-iodo-tyrosine, and in the respective unmodified form. Each peptide was optimized on an AB Sciex 5500 LC-MS/MS system in electrospray mode. LOD (detection of analyte with >95% confidence) was determined for each peptide and its modified variants. Standard Tg was then assayed to determine relative ratios of unmodified to modified Tg. FFPE tissue scrapes of normal thyroid tissue were then assayed in a similar manner.

Results: A total of 20 mono-iodinated tyrosine and one mono-iodo histidine, 11 di-iodo tyrosine, and 1 thyroxine sites were recorded. Those that were studied for relative iodination percentage were SYEASVPSVPISTHGR, TAFYQALQNSLGGEDSDAR, ENILLEPY, VSPGYVPACR, SHGQDSPAVYLK, and YSLEHSTDDYASFSR.

For individual peptides, LOD ranged from 30fmol to 180fmol of digested thyroglobulin on column. Total iodination percentage in normal thyroglobulin varied widely between locations. Percentages ranged from 2.4% to 45.0% for mono-iodinated tyrosines and from 1% to 12% for di-iodo tyrosine. Tissue scrape ratios for normal thyroid tissue matched iodination percentages from standard reference material to within 7% in the initial specimens assayed.

Conclusions: We have identified site specific locations of iodination in human thyroglobulin utilizing a high mass accuracy tripleTOF instrument. Utilizing the loci, a quantitative method for determination of iodination percentage in thyroglobulin was developed. The described method will potentially aid in clinical diagnostic accuracy in determination of the malignancy of thyroid nodules based on iodination state.

C-58

Serum Free Light Chain rate reduction after hemodialysis with an High Cut-Off membrane

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Background: The incidence of acute renal failure (ARF) associated with Multiple Myeloma (MM) is of 12-20%, and survival is associated with the renal function recovery after serum Free Light Chains (sFLC) reduction. From 2007 to 2012, 11 patients underwent High Cut-Off hemodialysis (HCO-HD), resulting in sFLC reduction rates of over 60%.

Objective: Analyze the effectiveness of the HCO-HD through the estimation of the reduction rate of sFLC (molecular weight below 45 kDa).

Methods: A total of 11 patients were hemodialysed with a HCO-HD membrane (6 sessions in average, 8 hours/patient), with the sFLC determinations preformed in pre-and post-hemodialysis samples. Albumin and creatinine concentrations were also assessed. The sFLC levels (Freelite) were quantified by turbidimetry SPAplus analyzer (The Binding Site), and the albumin and creatinine by spectrophotometry on a P analyzer (Roche Diagnostic).

Results: Patients with ARF associated with sFLC kappa overproduction (n=7/11) underwent an average of 5 sessions, achieving an average reduction in sFLC of 61.098% (SD +/-15.54%). The maximum concentration pre-HCO-HD was of 54800 mg/L. The rate of reduction of creatinine was 52.41% (SD + / -16.20%). An average of 7 sessions was done in patients with ARF associated with sFLC lambda overproduction (n=4/11), with an average reduction of sFLC of 62.47% (SD +/-3.95%). The maximum concentration pre-HCO-HD was 21505 mg/L. The average rate of reduction of creatinine was 59.86% (SD +/-8.16%). The highest sFLC reduction (86.83%) was observed in the patient with higher sFLC concentration pre-HCO-HD (54,800mg/L), with a creatinine reduction of 75.31% (creatinine pre-HCO-HD = 11.18mg/dL). However, this patient required a greater number of sessions (8 HCO-HD sessions) and he did not achieve a full recovery of the renal function (MDRD 17mL/min). For 14 HCO-HD sessions, the sFLC concentrations were analyzed at 4 hours and 8 hours after starting the HCO-HD, with the average reduction rate at 4 hours 52% (SD +/-26.25%) and 8 hours 49% (SD +/25.24%), and a correlation coefficient of 0.95 (p<0.001). The recovery rate of albumin post-HCO-HD was of 89.69%. The survival rate was 73% (3 exitus/11 patients).

Conclusions: HCO-HD is an effective tool in the adjuvant treatment in Multiple Myeloma (MM) for the rapid reduction in sFLC levels causing acute renal failure (ARF), facilitating a faster renal function recovery and survival. The High Cut-Off technology allows to discriminate low molecular weight molecules (MW<45kDa), without decreasing the albumin levels (MW=67kDa). There were no significant differences (P <0.001) in the sFLC levels reduction from halt-time (4hr) to the end (8hr) of HCO-HD sessions.

C-60

Immunoglobulin's Specific heavy/light chains pairs in patients with monoclonal gammopathy of undetermided significance.

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Introduction: Monoclonal gammopathies (MG) are a heterogeneous group of pathologies that can range from neoplastic malignant diseases, as multiple myeloma which requires active treatment until benign identities as monoclonal gammopathy of undetermined significance (MGUS) that generally don't require a clinical intervention. All identities of MG have in common the presence of a monoclonal immunoglobulin in serum and/or urine However, multiple myeloma develops at the rate of about 1-2% a year, so clinicians recommend monitoring it yearly. Recently the international myeloma working group (IMWG) have established guidelines in order to stratify the risk of progression to multiple myeloma (MM). In this model, the amount and type of monoclonal protein together with ratio of serum free light chains (sFLC) are risk factors for progression. Recently, new assays that allow the identification and quantification of specific immunoglobulin heavy/light chains pairs (IgGk, IgGλ; IgAk, IgAλ; IgMk, IgMλ) have been developed. The aim of the present work is to study if MGUS patients also present specific heavy/light chains (HLC) alterations.

Material And Methods: A group of MGUS patients (N=59) was risk stratified according to the IMWG guidelines. All the patients had the serum M-spike quantified by serum protein electrophoresis, identified by serum immunofixation (sIFE) and serum free light chains (Freelite™, sFLC) were quantified by nephelometry. Immunoglobulin specific heavy/light chains pairs (IgGk, IgGλ; IgAk, IgAλ; IgMk, IgMλ) were also requested for all the study participants. The inclusion risk factors(IMWG) were: M-spike > 1,5 g/dL; isotype different from IgG, sFLC ratio < 0,26(λ) or > 1,65(k)) and patients were classified as High, High- intermediate, low-intermediate and low risk of progression according to the number of altered risk factors (3, 2, 1 or 0 respectively). The correlation between M-spike and monoclonal HLC pair was also established.

Results: Among the selected MGUS population (2 biclonal; 32 IgG; 12 IgA; 13 IgM), 41% of the patients presented a low-intermediate risk of progression, 26 % had a low risk and a 33% presented an high-intermediate risk for progression. HLC ratios were altered in all except 2 IgG low risk patients. 30/32 IgG(94%); 11/12 IgA(92%); 12/12 IgM (100%) presented increased the monoclonal HLC. 15/32 IgG (47%); 9/12 IgA (75%) and 7/11(64%) MGUS pts presented the uninvolved HLC isotype immunosupressed. When was compared the M-spike quantification vs quantification of the monoclonal HLC pair, we found a moderate correlation for IgGk and IgAλ (r^2=0,51; r^2=0,69), and very good correlations for IgGλ, IgAk, IgMk, IgMλ (r^2=0,81; r^2=0,83; r^2=0,80; r^2=0,93).

Conclusion: Due to the high sensitivity of the HLC ratio to indicate monoclonality, HLC assays could be of great utility to quantify monoclonal components, special those hidden by other proteins in patients with an IgA or IgM M-spike. Larger studies are need it to determinate the value of HLC assays as risk factor for progression however, immunosupresion of the uninvolved monoclonal isotype is seen frequently in MGUS patients and could play a role as progression marker.

C-61

Sensitive Multiplex KRAS/BRAF Mutation Detection Assay in a Single-well Reaction

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Background: Anti-EGFR monoclonal antibodies have been used or evaluated for treating some cancers, such as non-small cell lung cancer (NSCLC), colorectal

cancer (CRC), etc. However, some activating point mutations of KRAS and BRAF, which are downstream from EGFR in the MAPK -signaling pathway, can render these monotherapies ineffective. A well designed SNP assay for the KRAS & BRAF mutation detection is thus critical.

Methods: A multiplex KRAS/BRAF assay was designed around the PCR/capillary electrophoresis (CE)-based ICEPlex platform, where all primers for detection of the major KRAS (G12S, G12R, G12C, G12D, G12A, G12V, G13S, G13R, G13C, G13D, G13A, G13V) and BRAF (V600E/D) mutations are included in one single well. A primer for the wild-type KRAS was also designed and included as an internal control. To reduce the common non-specific and cross-talk problems, primers were designed with two domains: 1) target-specific core sequences at the 3' ends; and 2) heterogeneous tail sequences at the 5' ends (for raising PCR annealing temperature, reduction of primer cross talk, and size differentiation on CE). Individual and mixtures of purified DNAs obtained from KRAS/BRAF wild type or mutant cell lines and FFPE specimens were used for testing on specificity and sensitivity.

Results: To test the specificity of the assay, individual mutants with available cell line DNAs (KRAS G12S, G12R, G12C, G12D, G12A, G12V, G13C, and G13D, and BRAF V600E) were included in the assay. All the tested mutants were positive for respective specific mutant signals, while the wild-type DNA was negative for all the mutant signals. To test the sensitivity (selectivity), mutant DNAs were individually tested in the background of wild-type DNA at 1:100 ratio. Each individual mutant signal was specifically and simultaneously generated with the wild-type signal.

Conclusions: We have developed a sensitive one-well multiplex qPCR assay to accurately detect the KRAS (codons 12 and 13) and BRAF (V600E/D) mutations. The results presented here demonstrate many benefits of the single-well multiplex format when coupled with automated detection, including conservation of precious specimens, saving on the cost and labor, increase in assay throughput, and reduction in turn-around time. This assay is for research use only.

C-62

Specific immunoglobulin heavy/light chain pairs: IgM normal ranges in two diferent platforms

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Introduction: The detection and quantification of monoclonal proteins by serum protein electrophoresis is the most used technique for the screening of monoclonal gammopathies. However this can often be difficult, especially in cases where the paraprotein is of low amount and in cases where the band is hidden. Immunofixation (IFE) improves sensitivity to the detection protocol but is not quantitative. A new assay is now available that allows the quantification of specific heavy chain/light chain pairs (HLC) (IgAk, IgAλ, IgGk, IgGλ, IgMk, IgMλ) and it is our aim to determine normal IgM ranges both by nephelometry and turbidemtry in healthy individuals considering that the use of ratios help us improve the diagnostic and follow-up of monoclonal gammopathies.

Material And Methods: We measured IgM HLC immunoglobulin specific pairs (IgMk; IgML) in blood donor sera by turbidimetry (SPA+) and nephelometry (BNII). 70 samples have been used to calculate the normal range by nephelometry and 79 samples have been used to calculate the normal range by turidimetry.

Results :

	IgM Kappa (n=70) BNII	IgM Lambda (n=70) BNII	IgMK/ IgML (n=70) BNII	IgM Kappa (n=79) SPA+	IgM Lambda (n=79) SPA +	IgMK/IgmL (n=79) SPA+
Median (95% range)	0,64 0,26-1,53	0,40 0,14-0,80	1,73 1,17-2,44	0,66 0,315-1,83	0,35 0,10-0,84	2,03 1,38-3,54
Median (rango 95% TBS)	0.63 0,19-1,63	0,35 0,12-1,01	1,81 1,18-2,74			

Conclusions: The IgM HLC assay presented similar normal ranges for each of the specific HLC pairs in the different platforms, and it may be a valuable tool to follow-up IgM monoclonal components. Compared to immunofixation (currently the gold standard technique), HLC is quantitative, automated and could optimize the follow-up of these specific monoclonal protein that often co-migrates with other serum proteins, a phenomenon also observed with IgA MC and that makes very difficult the MC quantification.

C-63

Hevylite: A New Valuable Assay For Multiple Myeloma Patients Follow-Up And Response Assessment

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Background: The analysis of immunoglobulin heavy chain/light chain pairs (HLC) has been proposed as a new tool for monitoring monoclonal protein (MP) production in monoclonal gammopathies. The aim of this study was to evaluate the usefulness of the heavylite assay (Binding site) for diagnosis and follow-up of multiple myeloma (MM) patients.

Methods: Multiple serum samples (mean n=7, range 4-12) from 10 MM patients (2 Ig Gκ, 4 Ig Gλ, 3 Ig Aκ, 1 Ig Aλ,) were analyzed. Serum protein electrophoresis (SPE) and immunofixation (IFE) were performed on a Sebia Capillarys and a Sebia Hydrasys, respectively, in accordance to manufacturer´s instructions. Total Ig G and Ig A concentrations were measured on an Immage 800 (Beckman Coulter). Serum Free Light Chain and Heavy/light chain analysis (The Binding site) was performed on a BN II analyser (Siemens). In-house generate HCL normal ranges were used.

Results: Ten newly patients diagnosed with MM have been prospectively followed-up for an average of 475 days (min: 66; max: 919). All diagnostic samples had an altered HLC ratio (rHLC), increased involved HLC (iHLC) levels and uninvolved HLC (uHLC) decreased levels.

During follow-up, 28 samples with MP by SPE had an altered rHLC and a decreased uHLC, 22 of them with an increased iHLC as well. 29 samples were negative by SPE and were further analyzed by serum IFE. IFE and rHLC results were in agreement in 23 of them (20 negative and 3 positive samples by both assays). 4 samples had a negative IFE profile and a slightly abnormal rHLC, and 2 samples had positive IFE and a normal rHLC (although IFE was difficult to interpret).

The HLC assay has been proven particularly interesting for two patients follow-up described afterwards.

Patient 2: after induction chemotherapy (MEL-PRED-VEL) and autologous stem cell transplant, the rHLC, iHLC and uHLC as well as sFLC ratio were within normal ranges. The patient achieved a complete response. 529d after transplantation both iHLC and uHLC were still within normal ranges but the rHLC became slightly abnormal. After 94d the rHLC was substantially more abnormal and the serum IFE was positive for IgA-κ. Total IgA was within normal range. The rHLC was the most sensitive laboratory test to indicate disease relapse.

Patient 9: treatment with MEL-PRED-VEL started 30d after diagnosis. rHLC normalized 211 days after diagnosis, the iHLC normalized at day 126 and the uHLC normalized at day 316. IFE was negative until day 316, although they were difficult to interpret after day 211. Therefore, the rHLC identified faster the degree of treatment response.

Conclusions: The inclusion of the HLC determinations to the routine follow-up of MM patients under treatment has been shown to be highly valuable for assessing the response level and determine disease relapse.

Wednesday AM, July 18, 2012

Poster Session: 10:00 AM - 12:30 PM

Mass Spectrometry Applications

C-67

Association of Arginine Derivatives with hsCRP in a Selected Patient Population

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Background: Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are turnover metabolites of arginine (ARG). ADMA is an endogenous inhibitor of nitric oxide synthase and has previously been found to be associated with cardiovascular disease. The objective was to determine the relationship between plasma levels of ARG, ADMA, SDMA, or ratios of these analytes and a known cardiac biomarker (high sensitivity C-reactive protein [hsCRP]) in a clinical population.

Methods: This study was approved by our Institution Review Board. The leftover plasma samples with hsCRP values were collected and separated into three categories based on relative risk for cardiovascular disease with low (< 1.0 mg/L, n=27), medium (1.0 to 3.0 mg/L, n=50) and high (>3.0 mg/L to <10mg/L, n=25). All samples were analyzed for ADMA, SDMA, and ARG using a published liquid chromatography-tandem mass spectrometry method. Analysis of variance was used to examine the association between each biomarker or the biomarker ratio and the hsCRP category. The Pearson correlation was calculated by treating hsCRP as a continuous variable. A P-value of <0.05 indicates the significance.

Results: ARG, ARG/SDMA and ARG/ADMA had significant negative correlation and ADMA had a significant positive correlation with hsCRP with the strongest correlation being ADMA and ARG/ADMA, while SDMA and SDMA/ADMA were found not to be significantly associated with hsCRP (Table 1).

Conclusions: ADMA and ratios of these derivatives were found to be significantly associated with an established cardiovascular biomarker (hsCRP) in the selected patient population. Further research is warranted to explore the clinical applications of these biomarkers.

Table 1. Association of arginine derivatives with hsCRP

	All (n=102)	Low (n=27)	Middle (n=50)	High (n=25)	P-value (category)	Correlation with continuous hsCRP (95% CI)
ARG	48.36 (19.70)	54.44 (23.49)	49.06 (17.54)	40.38 (17.26)	0.033*	-0.244 (-0.418, -0.052)
SDMA	0.75 (0.53)	0.63 (0.09)	0.75 (0.55)	0.86 (0.73)	0.298	0.243 (0.051, 0.418)
ADMA	0.56 (0.09)	0.52 (0.05)	0.56 (0.08)	0.59 (0.12)	0.014*	0.339 (0.154, 0.500)
ARG/SDMA	75.23 (36.05)	89.40 (42.14)	75.58 (31.52)	59.22 (32.00)	0.009*	-0.322 (-0.486, -0.136)
ARG/ADMA	88.46 (37.15)	106.45 (45.38)	87.73 (30.42)	70.47 (31.39)	0.002*	-0.337 (-0.499, -0.153)
SDMA/ ADMA	1.31 (0.71)	1.22 (0.18)	1.30 (0.75)	1.44 (0.97)	0.533	0.202 (0.008, 0.382)

C-68

Highly Selective Measurement of Underivatized Methylmalonic Acid in Plasma and Serum by Liquid Chromatography-Tandem Mass Spectrometry

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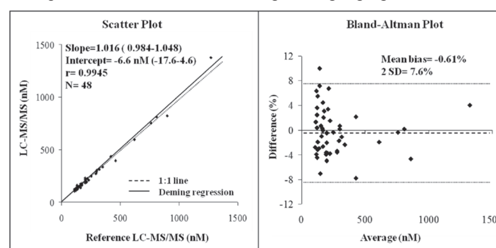
Background: Methylmalonic acid (MMA) is a functional biomarker of vitamin B12 deficiency. Accurate measurement of plasma MMA is challenging and several technologies have been applied for this purpose, among which liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered the method of choice. However, current LC-MS/MS methods suffer from lengthy sample preparation, long chromatographic run time, inadequate sensitivity or interference from succinic acid (SA), which has limited their widespread adoption in clinical labs. Our objective was to develop an LC-MS/MS method for the quantitation of underivatized MMA with simple sample preparation, high sensitivity and high selectivity for both serum and plasma samples.

Methods: Sample purification involved solid phase extraction followed by online extraction using mixed ion exchange turbulent flow chromatography. Then, MMA was transferred to an organic acid column for chromatographic separation. Total chromatographic run time was 6 min. A quantifier (117.1 to 73.1 m/z) and a qualifier (117.1 to 55.2 m/z) transition were monitored for MMA to increase selectivity.

Results: The method was free from interferences and linear from 26.2 to 26010.0 nM with an accuracy of 98-111%. Total coefficient of variation was less than 4.6% for three concentration levels tested. Comparison with a reference laboratory LC-MS/MS method showed a mean difference of -2.3 nM (-0.61%) and the Deming regression showed a slope of 1.016 (95% CI: 0.984 to 1.048), intercept of -6.6 (-17.9 to 4.6), standard error of estimate of 25.3 nM, a R of 0.9945, and diagnostic agreement of 100%.

Conclusions: The validated LC-MS/MS method presented here offers highly sensitive and selective quantitation of MMA with simple sample preparation.



C-69

Fast and sensitive amino acid analysis by LC-MS/MS without derivatization.

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Background: The diagnosis of inherited metabolic disorders of amino acid metabolism is based on quantitative analysis of amino acids, mainly in blood, urine or cerebrospinal fluid. Until recently, the most widely used method was ion-exchanged chromatography followed by post-column derivatization with ninhidrin. The emergence of tandem mass spectrometry coupled to liquid chromatography has made possible the measurement of both amino acids and their metabolites in different body fluids. The objective of this work is to present greatly improved amino acid analysis by shortening the analysis time and lowering the limits of detection and quantitation.

Methods: The LC-MS/MS method requires prior protein precipitation. An internal standard for each amino acid was created using labeled reagent. Thirty eight amino acids and metabolites were resolved using an HPLC column with an overall runtime of 7,5 minutes. Another 2,5 minutes of runtime is needed to resolve 4 more amino acids and their metabolites. Detection of compounds were achived with Agilent 6460 Triple Quad LC-MS.

Results: Using a gradient elution and ESI+ mode, 38 amino acids and their metabolites have been completely baseline-separated in a run time of 7,5 minutes. Whereas the rest of the amino acids and their metabolites have been separated by using isocratic elution and ESI+ mode. Limit of detection (LOD) and limit of quantitation (LOQ) studies revealed 0,03-6,40 umol/L and 0,09-21,50 umol/L respectively. Interassay and intrassay precision studies were also evaluated. Accuracy in two different levels were found to be less than ±15%, whereas intrassay precision levels were less than 5%.

Conclusions: An LC-MS/MS method was succesfully developed for quantitative measurements of amino acids in human samples. The method proves to be precise, accurate and easy to perform in high-throughput routine laboratories.

C-70

Glucose determination in SRM 965b by isotope-dilution mass spectrometry using liquid chromatography tandem mass spectrometry

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Objective: This study examines the use of ion exchange (Pb^{2+}) chromatography to separate glucose in a higher order method using isotope-dilution mass spectrometry

via liquid chromatography tandem mass spectrometry (LC-MS/MS). Glucose measurement by MS/MS is confounded by the existence of multiple carbohydrates of the same mass. Current methods rely on specific carbohydrate derivitization prior to gas chromatography (GC-MS). LC-MS/MS must separate the carbohydrates prior to MS/MS and is utterly dependent upon chromatography for this purpose. LC offers few choices for the complete separation of carbohydrates hindering the analysis of glucose by LC-MS/MS. However, the development of carbohydrate specific ion exchange resins has provided one means for the separation. This study undertook to analyze glucose in a certified reference material (NIST SRM965b) using LC-MS/MS considering aspects such as bias, recovery and potential cross-interference from a variety of carbohydrates.

Methodology: Four bottles of NIST SRM965b Level 2 were analyzed in triplicate to allow for within- and between-bottle analysis using [$^{13}C_6$]-glucose (Cambridge Isotopes Laboratories) as internal standard and externally calibrated with NIST SRM 917c. Spikes of calibrant were added to one additional sample from each bottle for recovery analysis. Three potentially confounding isobaric carbohydrates (galactose, fructose and mannose) were also added to separate samples to evaluate potential interference. Equal volumes of each sample and internal standard were weighed into tubes and allowed to equilibrate overnight with added sodium azide. Samples were treated with acetonitrile to precipitate the protein prior to analysis by LC-MS/MS. Calibrants were run interspersed among the samples and the results of multiple ion transitions and injections were combined to provide a single value estimate.

Validation: Accuracy validation was provided by comparing against the certified value of glucose in SRM965b (118.5 mg/dL - 95% confidence range ± 1.7 mg/dL) obtained by GC-MS. The five point calibrant curve yielded a linear regression equation of $y = 1.266x - 0.1808$ ($r^2 = 0.9966$) over a ratio range of 0.8 to 1.1 mg calibrant/ mg internal standard. Recovery was estimated to be 99.9 % (n = 4, %CV = 0.001). Estimates of glucose in single samples containing spikes of galactose, fructose and mannose produced values of 116.8 mg/dL 116.2 mg/dL and 121.0 mg/dL which compared well with the assigned value range.

Results and Conclusions: The average value of glucose in NIST SRM 965b, Level 2 was found by LC-MS/MS to be 117.9 mg/dL with a %CV of 0.6 % and range of 1.4 mg/dL. The bias relative to the certified value was low (-0.5 %), well within the inner half of the 95% confidence range. The within-bottle averages ranged from 117.3 mg/dL to 118.7 mg/dL. Within-bottle variances (range of %CV from 0.9% to 3.3 %) were greater than between-bottle demonstrating the improvement in precision obtained by replicate samples. Additions of potentially confounding isobaric carbohydrates did not affect the results of glucose measurement in those samples. This study demonstrates that LC-MS/MS analysis of glucose in serum achieves results comparable to established methods when using Pb^{2+} ion chromatography as a means of separation.

C-72

Feasibility of Quantitative Serum Apolipoprotein Profiling using SID-MRM-MS

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Background and objectives: Apolipoproteins AI, B, CI, CII, CIII and E all play a role in lipoprotein metabolism and have potential as key lab diagnostic biomarkers of cardiovascular disease. From a standardization viewpoint, quantification of defined apolipoproteins is preferred above that of heterogeneous lipids and macromolecular lipoprotein complexes. Mass spectrometry offers the possibility for sensitive, selective measurement of proteotypic peptides obtained after tryptic digestion. Our objective was to develop an accurate, quantitative and multiplex method for measurement of all six serum apolipoproteins using LCMS.

Methods: For each apoliprotein two proteotypic peptides were selected and synthesized together with their heavy labeled internal standards (HL-IS). An Agilent 1290 LC system and a 6490 mass spectrometer were used for measurement of peptides by stable isotope dilution multiple reaction monitoring MS. Serum proteins in 1 µl pool serum were digested with 2 µg trypsin and diluted to a total volume of 400 µl before analysis. Concentration ranges of the corresponding peptides were calculated based on published reference values of the apolipoproteins. Linearity was evaluated for all peptides simultaneously, with the IS at half maximal concentration.

Results: Product ion scans were obtained for all peptides and used to build a spectral library for their positive identification and to select the Precursor (Prec) and Product (Prod) ion transitions that were used as quantifiers at the optimal collision energies (CE). Eleven out of twelve peptides were linearly recovered in the defined concentration range with $R^2 > 0.99$ for the correlation between concentration and signal in relation to IS. All peptides were also detectable in the normal pool serum after trypsin digestion, with CVs (n=5) ranging from 1.6-10.3%.

Conclusions: A practical LCMS method was developed that enables simultaneous measurement of six apolipoproteins in 1 µl serum in an 18 min chromatographic run.

Table: Characteristics of MS measurements of the apolipoprotein peptides.

Apo/pept			CE	Rt	Range	R2	CV
+ HLV or L (IS	Prec	Prod	V	min	nmol/L		≤%
AI:DYVSQFEGSALGK	700.8	1023.4	19	7.9	0-100	0.998	2.4
AI:VQPYLDDFQK	626.8	228.1	18	5.2	0-100	0.999	1.6
B:FPEVDVLTK	524.4	450.7	20	8.0	0-5	1	3.3
B:VSALLTPAEQTGTWK	801.4	1017.4	24	7.0	0-5	0.997	1.6
CI:LKEFGNTLEDK	647.3	1052.4	24	3.7	0-10	0.995	10.3
CI:TPDVSSALDK	516.8	466.2	19	3.9	0-10	1	5.1
CII:ESLSSYWESAK	643.8	870.4	19	5.2	0-10	0.996	1.7
CII:STAAMSTYTGIFTDQVLSVLK	1117.1	1149.7	36	12.2	0-10	n.d	-
CIII:DALSSVQESQVAQQAR	858.9	573.3	26	4.7	0-20	0.996	6.6
CIII:GWVTDGFSSLK	598.8	244.0	18	7.9	0-20	0.992	1.9
E:LGPLVEQGR	484.8	588.4	20	4.1	0-5	0.999	2.4
E:SELEEQLTPVAEETR	865.9	902.5	28	6.1	0-5	0.998	6.3

C-74

Direct Identification of Microbes in Urine Specimens Using Mass Spectrometry

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Background: Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid and accurate method for identification of microbial isolates growing on solid culture medium. However, direct identification of microorganisms from clinical specimens with this method has rarely been pursued due to two limiting factors: 1) the inability of this method to resolve polymicrobial specimens; and 2) the fact that relatively large quantities of organisms are required. Direct detection of organisms in the setting of urinary tract infection (UTI) is an ideal model for MALDI-TOF-based detection, as UTIs are most frequently monomicrobial with a high organism burden. To date, identification of the causative pathogen in UTI has relied on culture-based methods, with an average turn-around time of 24 to 48 h. We sought to develop sample processing methods that could reliably identify microorganisms above the clinically relevant threshold of ~10^5 to 10^6 CFU/mL.

Methods: For method development, known concentrations of the common uropathogens *Escherichia coli* or *Pseudomonas aeruginosa* were added to sterile urine. For MALDI-TOF analysis, urine was briefly centrifuged to remove large particulates. The supernatant was desalted, fractionated and concentrated using a diafiltration device. The concentrate was then centrifuged at 15,000 *g* for 2 min to pellet intact microorganisms. The pellet was resuspended in buffer and spotted onto a MALDI target plate. Use of 4 or 15 mL of urine consistently achieved a limit of detection of 10^7 and 10^6 CFU/mL, respectively. Subsequently, patient specimens were utilized to compare the MALDI-TOF method to conventional urine culture. Fresh urine specimens were processed for MALDI-TOF concomitant with conventional cultures.

Results and Conclusions: In the pilot study, the MALDI method correctly identified urine specimens positive for uropathogens (such as *Escherichia coli* and *Klebsiella pneumoniae*), and correctly classified all specimens negative for uropathogens. The MALDI-TOF method failed to detect one positive specimen, a urine with *Candida albicans* at 10^5 CFU/mL by culture. The promising negative predictive value (93%) of this new method suggests it could be used to quickly rule out UTI and reduce unnecessary antimicrobial therapy. In specimens positive for uropathogens, preliminary results demonstrate that as little as 4 mL of urine yields positive identification of organisms near clinical thresholds. Strikingly, the time to microbial identification using MALDI-TOF was 2 h, a drastic improvement in turn-around time over conventional culture. A larger trial is underway to establish the analytical performance characteristics of this new method.

C-75

A rapid LC-MS/MS method for measuring everolimus

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Background: Everolimus (42-O-[2-hydroxyethyl)sirolimus]) is an immunosuppressant drug used to prevent rejection of organ transplants, and has been FDA-approved for treating renal cell carcinoma and subependymal giant cell astrocytoma under certain conditions. Our objective was to develop a simple, rapid LC-MS/MS method for quantifying everolimus that could be added to existing methods for measuring immunosuppressant drugs.

Methods: Whole blood samples, standards or controls were extracted in 50uL of 25mM zinc sulfate with the internal standard 32-desmethoxyrapamycin (5 ng/mL). Samples were mixed with a solution of methanol:acetonitrile in a ratio of 70:30 and centrifuged at 13000 rpm for 10 minutes. The supernatant was injected into an Aria TLX-2 (Thermo Fisher Scientific) for online cleanup (Thermo: Cyclone-P 0.5x50mm column) at flow rate of 3 mL per min in 15mM ammonium acetate/0.1% formic acid:methanol (80:20). Elution (Thermo: Hypersil GOLD 50 x 3 mm column) was performed at a flow rate of 0.75 mL per min in 100% methanol with 15mM ammonium acetate. An API-5000 (AB Sciex) mass spectrometer utilizing atmospheric pressure chemical ionization was used for analysis (dwell time: 25-70 msec; curtain gas: 15 psi; ion source gases: 35 and 15 psi; collision gas: 9 psi; nebulizing current: 5 uA; entrance potential: 10V, temperature: 480 C). The MRM transition for everolimus was monitored at m/z 975.5/908.6. Total analysis time was 5 minutes. We evaluated linearity, within-run precision, day-to-day precision, and functional sensitivity. Percent recovery was determined by spiking calibrators into whole blood samples from patients who were not taking everolimus. We also assessed potential interferences by testing 1000 samples from patients taking cyclosporine A, sirolimus or tacrolimus. Finally, we compared our method to a second LC-MS/MS method at a collaborating institution using 20 samples from patients taking everolimus.

Results: The method was linear to 50 ng/mL, and had a functional sensitivity (CV ~ 20%) of 0.5 ng/ml. Within-run precision (%CV) ranged from 2.9% to 10.6% whereas day-to-day precision ranged from 5.7 to 10.4%. Recovery after spiking at concentrations of 3, 10 and 20 ng/mL was 92%, 100% and 101% respectively. There was no apparent detection of everolimus in the 1000 samples from patients taking other immunosuppressants. The correlation between our method and the second LC-MS/MS method was 0.99, whereas the slope was 0.89, and intercept was 0.42.

Conclusions: The LC-MS/MS method developed here adds the capability to measure everolimus to our existing LC-MSMS method for sirolimus, tacrolimus and cyclosporine. This further enhances the efficiency of the "multi-plexed" method allowing simutaneous determination of immunsuppressant drug concentrations, while reducing sample volume requirements, time and cost.

C-76

Detection and Quantitation of Aripiprazole and its Metabolite Dehydroaripiprazole in Human Serum by LC-MS/MS

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Background: Aripiprazole (Abilify®) is a second generation antipsychotic for the treatment of schizophrenia, bipolar disorder, and clinical depression. Aripiprazole (ARP) is metabolized by CYP3A4 and CYP2D6 and its major active metabolite is dehydroaripiprazole (DARP). Drug serum/plasma concentration monitoring of ARP and DARP can be helpful to improve efficacy, or to detect an over-dosage, thus optimizing therapy.

Methods: In this study, a rapid, accurate, and sensitive LC-MS/MS method for the detection and quantification of ARP and its main metabolite DARP, in human serum/plasma samples, was developed and evaluated. The calibration curves of ARP and DARP using 0.1mL serum were linear from 20-1000 ng/mL and 10-1000 ng/mL, respectively. Serum levels of ARP and DARP were quantitated, and the sum of ARP and DARP and the metabolic ratio DARP/ARP were calculated. Protein precipitation was used as the extraction method. Aripiprazole-d_8 was chosen as the internal standard. The analytical HPLC system used a Pinnacle DB Biphenyl column with a binary mobile phase consisting of an aqueous phase of water and formic acid and an organic phase of acetonitrile and formic acid, coupled with positive ESI. The protonated analyte was detected in MRM mode in API 4000 system. A gradient time program was used and a second transition was monitored to provide a qualifying ion ratio to ensure component identity. The following mass transitions were used: *m/z* 448.4>285.2 and 448.4>176.2 for ARP, 446.4>285.2 and 446.4>174.2 for DARP, and 456.4>293.2 for internal standard aripiprazole-d_8.

Results: A subset of 110 randomly selected samples sent to our lab for ARP testing were de-identified and used in the study. Of the tested samples, 23% were <20 ng/mL ARP. The ARP (mean ± standard deviation) serum concentration was 221±207 ng/mL (range, 21-1120 ng/mL). The DARP mean serum concentration was 67±59 ng/mL (range, 0-300 ng/mL). The sum of ARP and DARP mean serum concentration was 282±261 ng/mL (range, 21-1420 ng/mL). For the ARP serum levels, the DARP/ARP ratios (in %) were calculated. The ARP serum level <50 ng/mL had DARP/ARP mean ratio 4% (range 0-15%); ARP 50-100 ng/mL had mean ratio 22% (range 0-50%); ARP 100-200 ng/mL had mean ratio 32% (range 0-59%); ARP 200-300 ng/mL had mean ratio 33% (range 7-56%); ARP 300-400 ng/mL had mean ratio 24% (range 12-39%); ARP >400 ng/mL had mean ratio 27% (range 18-47%).

Conclusions: From the literature it is known that improvement may be best in patients with 150-300 ng/mL aripiprazole, with no or mild side effects at 110-249 ng/mL. Data obtained from our study showed that the ARP serum levels between 50-300 ng/mL had variability in DARP/ARP ratio ranging from 0-59%, showing biggest inter-individual variations at these levels. Thus, measuring serum concentrations for both ARP and DARP may be more beneficial compared to only measuring ARP concentration for personalized treatment with aripiprazole.

C-77

Development and Validation of a Bioanalytical Method for the Quantification of 25-hydroxyvitamin D2, 25-hydroxyvitamin D3, 3-epi-25-hydroxyvitamin D2 and 3-epi-25-hydroxyvitamin D3 by LC-MS/MS

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An LC-MS/MS method has been developed to allow quantitative measurements of 25OHD and the C-3 25-hydroxy epimers for both vitamins D2 and D3

Introduction: Recent publications and internal data have demonstrated that low but significant levels of the C-3 epimer of 25-OH-vitamin D2 or D3 (3-epi-25OHD2 and 3-epi-25OHD3) are present both in young children and also in some adult populations. The downstream metabolite of 25OHD3, 3-epi-1,25-(OH)2D3, has altered activity relative to 1,25-(OH)D3. No comprehensive clinical studies have addressed the relative activities of 1,25(OH)2D3, 1,25(OH)2D2, 3-epi-1,25(OH)2D2 and 3-epi-1,25(OH)2D3. As a result, it is not clear whether the corresponding 3-epi-25OHD metabolites should, or should not, or be measured in clinical assays of vitamin D sufficiency. To study this phenomenon well, a method is needed which resolves all four 25OHD metabolites in such a way that will enable the quantification of low concentrations of 3-epi-25OHD metabolite in the presence of normal to elevated 25OHD metabolite. In the work presented here, we have developed an LC-MS/MS method to quantify serum levels of all four metabolites.

Methods: An analytical method was developed using a Thermo/Cohesive TX-4 HPLC system (Thermo-Fisher/Cohesive Technologies) with Agilent® 1200SL pumps (Agilent Technologies, Inc.) and an AB Sciex® 5000 (AB Sciex PTE. LTD.) triple quadrupole mass spectrometer. A Supelco® PFP analytical column was used (100 x 2.1mm, 2.7um, 100A) with a water:methanol gradient to achieve full baseline chromatographic separation of epimer and non-epimer. Independent calibration curves were prepared for all four metabolites (25OHD2, 25OHD3, 3-epi-25OHD2, 3-epi-25OHD3). Verification was performed using UV/Vis spectrophotometry and calibration was verified using reference materials from NIST as well as reputable 3rd party vendors. Sample preparation consisted of isotope dilution using the internal standards 25OHD2-2H3, 25OHD3-2H3 and 3-epi-25OHD3-2H3 (3-epi-25OHD2-2H3 is not commercially available), protein precipitation and liquid-liquid extraction.

Validation Data: Analytical sensitivity (LLOQ) was defined as 0.5 ng/mL for 3-epi-25OHD2 and 3-epi-25OHD3, and 1.0 ng/mL for 25OHD2 and 25OHD3 from 100uL of serum. Overall bias relative to traditional LC-MS/MS method was -0.1% and average precision was found to be 4.5% (inter-assay). Dynamic range was up to 250 ng/mL (2,750 ng/mL with dilution). Correlation with routine clinical 25OHD LC-MS/MS assay was excellent (R2 = 0.94, slope 0.957 + 1.5 ng/mL, n=184). In adults, the epimer was present on average as 4.2% of the total 25OHD, whereas in children <1 year, the epimer was present on average as 16% of the total 25OHD.

C-78

Liquid chromatography-tandem mass spectrometric assay for simultaneous determination of urinary conjugated metanephrine and normetanephrine: a comparison with high-performance liquid chromatographic assay with a commercial reagent kit

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Background: Simultaneous quantification of urinary conjugated metanephrine (MN) and normetanephrine (NMN) as biomarkers for the diagnosis of pheochromocytoma is usually performed using commercial reagent kits by high-performance liquid chromatography with electrochemical detection (HPLC-EC) in clinical laboratories. However, cost of the reagent kits is expensive and interfering substances often obscure the peaks of interest in HPLC chromatograms. The aim of this study is to demonstrate an availability of a fast, sensitive, specific, cost-effective liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay as an alternative method to HPLC-EC with a reagent kit for simultaneous determination of urinary conjugated metanephrine and normetanephrine.

Methods: For LC-MS/MS assay, fifty microliters of urine samples were acidified with 50 µl of 0.1 M HCl and hydrolyzed at 90°C for 20 min. Ten microliters of each stock solution of MN-d_3 and NMN-d_3 as internal standards were added to the hydrolyzed samples, which was neutralized with 900 µl of 10 mM sodium phosphate buffer (pH 8.0), followed by solid-phase extraction. The eluates reconstituted in mobile phase (5 mM NH_4Ac:ACN = 40:60, pH 5.5) were analyzed by LC-MS/MS (API 4000, AB/Sciex, Forster City) in multiple-reaction monitoring mode after separations on a HILIC HPLC column. For HPLC-EC assay with a kit (Bio-Rad, CA), two milliliters of urine samples were acidified, hydrolyzed, and neutralized. All the procedures were performed strictly according to the manufacturer's instructions.

Results: The LC-MS/MS assays showed good linearity from 10-3000 ng/ml for NMN and 10-5000 ng/ml for MN. Intraassay and interassay CVs for MN at 100, 1000, and 5000 ng/ml were 1.4, 2.6, and 2.6 %, and 6.2, 3.6, and 1.2 %, respectively. Intraassay and interassay imprecision values (CVs) for NMN at 100, 750, and 3000 ng/ml were 4.3, 2.3, and 2.9 %, and 1.6, 3.9, and 3.9 %, respectively. The mean recoveries of MN and NMN were 101.7%, and 102.3%, respectively. The LC-MS/MS measurements were highly correlated with the HPLC measurements for both MN (y = 1.1487x + 18.42, r^2 = 0.9962) and NMN (y = 1.17206x - 11.14479, r^2 = 0.98202) from 24-h urines of healthy subjects (n=7) and patients (n=2) with pheochromocytoma.

Conclusions: The LC-MS/MS method requires only one-fortieth urine volume needed by the HPLC assay with a commercal reagent kit. Furthermore, the LC-MS/MS assay will provide a fast, accurate and specific alternative with much lower costs of consumables to the HPLC assay for the simultaneous quantification of conjugated metanephrine and normetanephrine in urine.

C-79

Radioactive Iothalamate versus Non-radioactive Iothalamate by Liquid Chromatography-Tandem Mass Spectrometry for the Determination of Glomerular Filtration Rate

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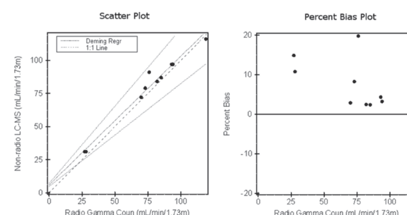
Introduction: Glomerular filtration rate (GFR) is commonly determined by measuring radioactivity in serum/plasma and urine after infusing radioactive iothalamate. Our objectives were to develop a simple and fast liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to measure non-radioactive iothalamate in serum/plasma and urine for GFR calculation and to compare the resulting GFR results with those by a routine radioactive iothalamate method.

Methods: After consent, the subjects (n = 10) were infused with radioactive ^{125}I-sodium iothalamate in one arm and non-radioactive iothalamate meglumine in the other at the same time, followed by bracketed collection of blood (n = 3/patient) and urine (n = 2/patient) samples.

Results: The LC-MS/MS method was free from ion suppression, carryover or interference, and was linear from 1.7 to 61.8 µg/mL and 2.6 to 378 µg/mL in serum and urine, respectively. Recovery ranged from 83.6% to 95.9% for both matrices. Total coefficient of variation was less than 7.2% at three different levels tested for both matrices. Comparison of the GFR results with those by the radioactive iothalamate method showed a mean difference of 3.8 mL/min/1.73m^2 and the Deming regression showed a slope of 0.971, intercept of 5.9 and R of 0.9872 (Figure 1).

Conclusions: The LC-MS/MS method presented here is a simple, fast and reliable replacement of the radioactive method.



C-81

LC-MS/MS Assay for Precise, Simultaneous Evaluation of Testosterone in Men, Women and Children

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Background: Leydig cells of the testes primary produce the androgen steroid hormone, testosterone; however, it is also produced by the ovaries and adrenal glands in women and children. A variety of anabolic and androgenic effects, including, bone metabolism and remodeling, and sexual differentiation are effected by testosterone levels. Circulating testosterone measurement is clinically relevant in the diagnosis of androgen disorders in humans such as hypogonadism in adolescent boys and men, and hyperandrogenism which can be related polycystic ovarian syndrome, androgen secreting tumors and late onset congenital adrenal hyperplasia in women. It is also important in the treatment and gender assignment of infants with ambiguous genitalia and to diagnose pituitary or hypothalamic disease in girls. Unfortunately, the standard immunoassay frequently utilized in the clinic have been shown to be unable to accurately measure testosterone levels from women and children due to lack of both sensitivity and specificity brought on by the nonspecific binding of antibodies to other steroids. As a result, significant effort has been spent to develop liquid chromatography- tandem mass spectrometry (LC-MS/MS) assay by reason of their superior analytical sensitivity and specificity. We have developed an in house LC-MS/MS method that allows for simultaneous, reliable quantitation of testosterone levels from men along with women and children.

Method: Dueterated internal standard was added at 5 ng/dL and liquid-liquid extraction with 90% Hexane; 10% MTBE was used to extract testosterone from serum samples. The samples (20uL) were injected onto a HPLC system (LC-20AD Prominence, Shimazu) separated by C18 reversed phase (Kinetex, 50X2.1mm, phenomenex) coupled a 5500 triple quadruple mass spectrometer (AB Sciex). The column was eluted with a 5 minute gradient from 30% - 95% of a buffer containing 80% methanol, 20% acetonitrile, 0.1% formic acid at a rate of 0.45 mL/min. The mass spectrometer was set to ionize the samples using atmospheric pressure chemical ionization and to detect in selective ion monitoring mode. Concentration was calculated with peak area ratios of testosterone (m/z 289.2 ->97.1) to internal standard (m/z 292->97.1).

Results: The validation of our testosterone LC-MS/MS method was preformed according to the CLSI guidelines. Linearity and precision testing showed the assay was linear from 0 to 1000 ng/dL with a mean CV of 7.6% for intra-day precision and 8.4% for inter-day precision with a LLOQ of 4 ng/dL. Our assay showed an average bias of 0.245% across the linear range and no ion suppression or carryover was observed. Additionally, none of the substances test interfered with the analysis of testosterone.

Conclusions: Our LC MS/MS method using APCI ionization is an accurate, precise, reproducible method to simultaneously evaluate the levels of testosterone from men, women and children.

C-82

Total and Allele-Specific Quantitation of Alpha-1-Antitrypsin by Mass Spectrometry

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Background: Genetic alpha-1-antitrypsin (A1AT) deficiency results from decreased concentrations of circulating A1AT. Although numerous allelic variants exist, the M (wild type) allele is the most common non-deficiency allele, while the S and Z alleles account for 95% of the variants detected in affected patients. Simultaneous

A1AT phenotyping and quantitation using tryptic peptides of A1AT has recently been published. This method utilizes the mass shift caused by the mutations to detect the S and Z peptides and a non-varying proteotypic peptide for total quantitation. An interesting feature of this method is the ability to separately quantitate A1AT variants. In heterozygote S or Z patients, it is not known how much of the serum A1AT is wild-type compared to mutant protein. Allele-specific protein quantitation may be useful in clinical evaluation and identifying patients at risk for specific clinical manifestations.

Objective: (1) To compare A1AT quantitation by mass spectrometry to quantitation by immunoassay. (2) To establish a method for allele-specific quantitation of A1AT. (3) To quantitate wild-type and mutant circulating A1AT protein in heterozygous samples.

Methods: Serum (252 M/M, 61 M/Z, and 63 M/S) or purified A1AT (for construction of proteotypic, non-Z and non-S peptide standard curves) was combined with labeled internal standards for non-S, non-Z, and proteotypic peptides, denatured with trifluoroethanol, reduced with dithiothreitol and treated with iodoacetamide. The proteins were digested with trypsin at 37°C for 30 minutes and inactivated with formic acid. Peptides were separated on reverse phase C18 liquid chromatography and detected using standard MRM with an AB-Sciex API 5000. Total and allele-specific A1AT quantitation was performed by comparison to the standard curve for each peptide (proteotypic, non-Z, and non-S). A1AT quantitation of each patient sample was performed using a Siemens Dade Behring BN II nephelometer and Siemens reagent sets.

Results: Standard curves from purified A1AT were constructed for proteotypic, non-Z and non-S peptides ($R^2 > 0.97$). Comparison between total A1AT quantitation by mass spectrometry and immunoassay revealed a slope of 1.24, with evidence of both proportional and constant biases, and an R^2 of 0.64, with the mass spectrometer quantitating higher concentrations than the immunoassay. Comparison of A1AT concentration in M/M patients calculated using proteotypic vs. non-Z and proteotypic vs. non-S yielded slopes of 1.12 (R^2=0.86) and 0.93 (R^2=0.80), respectively, indicating consistent quantitation by mass spectrometry between the 3 peptides. In M/S patients, circulating protein consists of, on average, 63% M (95th percentile range 43-83%) , while in M/Z patients the composition is, on average, 82% M (95th percentile range 55-100%). These ranges for the M concentration in M/Z and M/S heterozygotes are significantly different ($p<0.001$).

Conclusions: This is the first method to demonstrate quantitation of individual A1AT allele expression. Total A1AT quantitation by mass spectrometry could potentially be added to phenotyping by mass spectrometry to multiplex the orderable assays, as both results are necessary to establish a diagnosis of A1AT deficiency. In addition, elucidating allele composition of the circulating A1AT in heterozygous patients has the potential to reveal correlations between composition and development and/or severity of clinical symptoms.

C-83

Differential Expression of Red Blood Cell Membrane Proteins in Myelodysplastic Syndrome by Nano-Ultra Performance Liquid Chromatography-Quadrupole-Time of Flight

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Background: Myelodysplastic syndromes (MDS) are clonal disorder of hematopoietic stem cells that may eventually lead to leukemia. The ultrastructural defects in erythrocyte membranes may have a role in early red cell destruction within circulation. We hypothesized that the dyserythropoiesis could be due to changes in the level of the protein components of the RBC membrane. To examine this, we performed a proteomic analysis of RBC membrane proteins of MDS subjects and normal controls by tandem mass spectrometry (MS/MS).

Methods: We studied peripheral blood specimens from 5 MDS patients showing anisopoikilocytosis and 6 normal controls. After extraction of RBC membrane proteins, proteomic analysis was performed. Tryptic peptides were analyzed with nano-ultra performance liquid chromatography (nano-UPLC)-MS/MS (quadrupole-time of flight, Q-TOF). The analytical column was BEH ACQUITY C18, and a trap column was used for desalting. Gradient of 0.1% formic acid in water/acetonitrile was used as mobile phase. Enolase from *Saccharomyces cerevisiae* (P00924) was spiked for absolute quantification. The run time was 2 hours. Data-independent mode was used to analyze the *m/z* of the peptides. Each sample was analyzed in triplicate. PLGS 2.4 and excel 2010 were used for data analysis, and Uniprot database for human was used for identifying the proteins.

Results: The number of proteins identified by using UPLC-MS/MS and database searching software ranged between 38 and 44. Spectrin beta chain, brain 2 and glucose-6-phosphate isomerase were found to be 7.6, 2.6 fold up-regulated in MDS patients when compared to normal controls (p=<0.001, 0.02, respectively). Using Mann-Whitney U test, CD59 glycoprotein was found to be 0.76-fold down-regulated and RhCE polypeptide was 1.29-fold up-regulated in MDS patients when compared to normal controls (p=0.028, 0.024, respectively). Considering ‘not detected’ protein as zero, plasma membrane calcium transporting ATPase 4 and Ras related protein Rap 1A were down-regulated in samples with MDS patients (p=0.013, 0.037, respectively). The MS/MS data from specimen of subjects with increase or decrease in those proteins showed that CD59 glycoprotein was identified in all the experiments and had a significant change in the level of RBC membrane proteins. The others were not detected in all experiments analyzing both MDS patients and normal controls.

Conclusions: Some proteins which were not detected in all experiments may have too low level in their concentration which may have an effect on analytical precision. Two-dimensional fractionation would be needed to solve these problems. Decrease in the level of CD59 implicates the fact that paroxysmal nocturnal hemoglobinuria (PNH) also develops in MDS and explains why there appears to be a higher rate of leukemia in PNH. The results suggest that proteomic analysis of RBC membrane proteins using nanoUPLC-Q-TOF provides a potential link between the alterations in RBC membrane proteome in MDS subjects and MDS pathology. Therefore, this method will be helpful in the diagnosis of MDS and could detect differences between subjects in the MDS and those with other diseases causing anemia if well adjusted.

C-84

Improving the selectivity of endogenous testosterone analysis using differential mobility spectrometry

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Background: Testosterone is one of the most commonly measured steroid hormones and is present in very low concentrations in females and some pediatric male samples. Due to other endogenous compounds that are very similar in structure and molecular weight, testosterone must be chromatographically separated from these in order to obtain specific and accurate quantification by tandem mass spectrometry. Additionally, the gel in serum separator tubes in which blood samples are routinely collected causes interference in testosterone MRM transitions and so clinical research laboratories are required to collect the sample in an alternative tube type unless they run a long chromatographic method.

Methods: Five male and 5 female serum samples were obtained from different blood collection devices, as was a pool of pediatric samples (<7 years old) in order to create a standard. These samples were extracted using two different methodologies: liquid-liquid extraction with 90/10 hexane/ethyl acetate and protein precipitation using acetonitrile. An ABSCIEX 5500 QTRAP® in ESI positive mode conjugated with SelexION TM was utilized coupled to a Shimadzu Prominence UPLC. A Kinetex 2.6 µm, 100x3 mm column was used with gradient elution of water and methanol with 0.1% formic acid, a flow rate of 0.3 mL/minute and run time of 7 minutes. A Kinetex 2.6 µm, 50x2,1 was used for short gradient with 2.5 minutes run time.

Results: Differential ion mobility demonstrated significant removal of interferences from matrices and interferences from collection devices. As a result, more accurate ion ratios and concentrations of testosterone can be detected and reported at low levels with simple protein precipitation and a fast gradient. The curve constructed in pediatric samples with the same collection protocol demonstrated linearity up to the highest standard with no evidence of deviation, and accuracy from 96-105% and R= 0.999. There was no significant peak area difference between samples extracted by liquid-liquid extraction or protein precipitation when differential ion mobility spectrometry was applied. With the interferences removed by differential ion mobility mass spectrometry, a short HPLC gradient method was developed and applied for samples with interferences.

Conclusions: A novel testosterone analysis method with differential ion mobility mass spectrometry to remove interferences was developed for human serum sample.

C-85

A Quantitative and Selective Analysis of Aldosterone and Cortisol in plasma by LC-MS/MS

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Background: Liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) is ideally suited for the rapid, simultaneous analysis of multiple analytes. A highly sensitive and specific method has been developed for the quantitation of two mineralocorticoids for clinical research - Aldosterone and Cortisol at the required ranges and functional sensitivity required. The analysis is achieved through the use of ultra high performance liquid chromatography (UHPLC) to quickly separate compounds of interest from any interference. Two different sample preparation procedures are evaluated and compared.

Method: An LC-MS/MS method was developed using a QQQ mass spectrometer with electrospray ionization (ESI) using negative mode. Chromatographic separation was achieved using a C18 Poroshell column and a mobile phase comprised of methanol and water containing 2 mM ammonium acetate. The mobile phase was run through a gradient from 20% to 70% methanol over 4 minutes and held at 90% for 2 minutes. Two internal standards were used to achieve accurate and precise quantitation. Aldosterone-D8 was used for the quantitation of Aldosterone and Cortisol-D4 for Cortisol. LLE (liquid-liquid-extraction) procedure is compared with SLE (solid-liquid extraction).

Results: The proposed method can accurately and reliably quantitate two mineralocorticoids simultaneously. Limits of quantitation (LOQ) for the compounds spiked in plasma ranged from 50 pmol/L to 6500 pmol/L for Aldosterone and 10 nmol/L to 1450 nmol/L for Cortisol with excellent reproducibility (CV < 10%). All calibration curves displayed excellent linearity with an R2 > 0.995.

Conclusion: A robust method for quantifying Aldosterone and Cortisol with excellent reproducibility and accuracy has been developed. A comparison of LLE and SLE sample preparations shows that both methods are suitable for clinical research. However, SLE helps to simplify and improve the extraction procedure.

C-88

Optimization of a sample preparation in the supported liquid extraction for the measurement of serum testosterone using LC/MS/MS

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Background: ISOLUTE supported liquid extraction (SLE) plates (Biotage®, Uppsala, Sweden) offers an alternative to traditional liquid-liquid extraction (LLE) of serum sample preparation for testosterone assay, eliminating emulsion formation and reduced sample preparation time. However, the method had a reported LOD of 100 ng/dL, which is not sufficient for measurement of the low concentrations characteristics of females and children. We investigated ways to improve the sensitivity of the SLE method.

Methods: We diluted 300 uL of serum with 100 uL of distilled water and loaded the 400 uL into the wells of the ISOLUTE SLE+ 96-well plate. The plate was eluted with ethyl acetate/hexane (3:2) in to a deep well collection plate, which was evaporated to dryness using EZ-2 personal solvent evaporator (Genevac, UK), then each well was reconstituted in 100 uL of methanol/water (75:25). For instrumental analysis, 10 uL of the reconstituted extract was injected in to the API 4000 HPLC tandem mass spectrometer in the multiple-reaction monitoring (MRM) mode.

Results: The CV at 15 ng/dL levels was 6.3% (n=6). The overall analyte recovery was 82%. The limit of quantification was 15 ng/dL and the limit of detection was 3 ng/dL. The assay was linear (R2=0.994) over the range of 6.5-839 ng/dL tested with mixing high and low level serum samples (n=11). In a comparison study using 12 serum samples (ranged from 3 to 326 ng/dL), SLE method showed a good agreement with the traditional LLE method (Deming regression line with the equation: y = 1.08x - 0.44 ng/dL, r = 1.0; Sy|x = 3.84 ng/dL).

Conclusions: The optimization of sample preparation improves the quantification sensitivity of the serum testosterone assay using supported liquid extraction followed by LC/MS/MS.

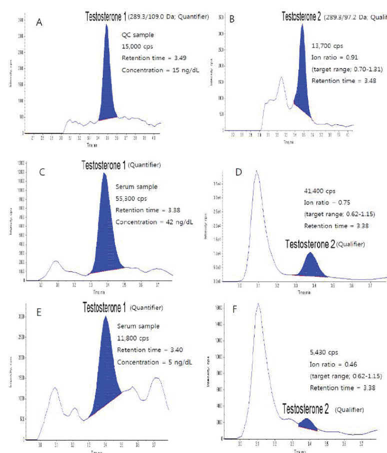

Fig. 1. Multiple-reaction monitoring chromatograms of extracted samples containing 15 ng/dL testosterone (A and B; QC sample), 42 ng/dL testosterone (C and D; serum sample), 5 ng/dL testosterone (E and F; serum sample).

C-89

Accurate Mass Analysis of Monoclonal Immunoglobulin Light Chains in Serum Using Electrospray Ionization-LC-Time-of-Flight Mass Spectrometry

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Objective: Develop a robust, high throughput electrospray ionization (ESI)-liquid chromatography (LC)-time of flight (TOF) mass spectrometry (MS) method to determine the accurate mass of immunoglobulin light chains for detecting monoclonal immunoglobulins

Population: Residual waste serum from controls and from patients with multiple myeloma.

Relevance: Monoclonal immunoglobulins are routinely characterized and quantitated by protein electrophoresis (PEL). The fundamental principle of detection of monoclonal immunoglobulins from the polyclonal immunoglobulins is their relatively restricted mobility which results in a distinct spike, often referred to as an M-spike. Alternatively, a plot of the distribution of immunoglobulin masses could be used to in a similar manner. The light chain portion of the immunoglobulin is an attractive target for characterizing the antibody by MS due to its smaller size (22,000 KD) and lack of post translation modification. Manufacturers of recombinant antibodies have successfully analyzed the light chain portion of the immunoglobulin by ESI-LC-TOF MS for quality control purposes. We have explored analyzing the light chain mass distribution from patients who have large M-spikes (monoclonal immunoglobulins) in a method similar to the techniques employed by manufacturers of recombinant immunoglobulins. As expected, a single, unique mass was observed for the light chain from myeloma patients as compared to a distribution of masses observed in normal controls.

Methods: Immunoglobulins were purified using a Melon Gel kit per the manufacturer's protocol (Pierce Biotechnology, Rockford, IL). Specifically, 50 uL of serum was diluted with 450 uL of Melon gel buffer and applied to the gel. The sample was mixed with the gel for 5 minutes then the purified immunoglobulins were collected in the flow through. Purified immunoglobulins were reduced with 20 mM DTT for 30 minutes at 55 degrees C then desalted using a 3 kDa cut-off microcentrifuge filter. The reduced immunoglobulins were analyzed by ESI-LC-TOF MS by injecting them onto a 0.3 x 50 mm C18 column flowing at 10 uL/min. An AB Sciex 5600 Q-TOF mass spectrometer was scanned from 400-3,000 m/z in 250 ms at a resolution of 32,000 at 800 m/z and an external mass calibration of 5 ppm at 800 m/z. Scans were summed at the top of the eluting peak and were analyzed using BioAnalyst software.

Conclusions: ESI-LC-TOF MS is capable of determining the unique mass of the monoclonal light chain present in serum from patients with multiple myeloma versus the wide distribution of masses from polyclonal light chains in normal controls. By identifying the exact mass, the ESI-LC-TOF MS is superior to migration patterns of PEL for characterizing immunoglobulin clonality. In addition, ESI-LC-TOF MS can be used to quantify the abundance of light chain present as well as track mutational drift in plasma cell proliferative disorders.

C-90

Quantification of Plasma Carnitine and Acylcarnitines with Simple Sample Preparation and Liquid Chromatography-Tandem Mass Spectrometry Methods

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Background Measurement of plasma carnitine and acylcarnitine species is used to diagnose inborn errors of metabolism, including disorders of mitochondrial fatty acid beta-oxidation and some organic acidemias. Commonly used tandem mass spectrometry methods are limited by either lack of specificity for some isobaric species or long analytical procedures involving the use of in-house-synthesized compounds. The objective of this study was to develop a sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with simple sample preparation to quantify plasma carnitine and acylcarnitine.

Methods Free and total carnitine were measured using 20 µL of plasma. After addition of deuterium-labeled internal standard (IS), the plasma sample was divided. One portion underwent precipitation of protein, with the supernatant used for measuring free carnitine; the other portion was alkaline-hydrolyzed prior to protein precipitation for the measurement of total carnitine. LC-MS/MS analysis was performed using HILIC chromatography and multiple reaction monitoring mass spectrometry with a 5 min run time. Quantification of acylcarnitine species also used 20 µL of plasma, and the IS was a mixture of 11 deuterium-labeled acylcarnitines (Cambridge Isotopes, Andover, MA). Sample preparation included protein precipitation and derivatization with n-butanol. LC-MS/MS analysis was performed on the same instrument with a solid-core reverse phase column, and the run time was 15 minutes. Acylcarnitine concentrations were calculated using NeoScan software (ThermoFisher Scientific, Waltham, MA), and quantification was based on IS concentration and analyte/IS peak height ratio.

Results For free and total carnitine, linearity was determined to be 1.2-289.9 and 1.3-296.9 µM, respectively. Inter-assay and total CVs were <3.0% for the 3 levels tested. No carryover was observed up to 148.0 µM. Quantitative comparison (n=20) with a reference LC-MS/MS method yielded a Deming regression slope of 0.980, an intercept of -0.486 µM and a correlation coefficient of 0.9404 for free carnitine, and a slope of 0.867, an intercept of -5.1 µM and a correlation coefficient of 0.8863 for total carnitine. In acylcarnitine analysis, 31 species of interest, including some structural isomers (e.g butyrylcarnitine and iso-butyrylcarnitine), were chromatographically resolved. Lower limits of quantification of five representative species ranged from 0.8-6.0 nM. Carryover limits of 15 selected species ranged from 4.5 to 380.0 fold of their corresponding upper reference limits. Inter-assay and total CVs were within 30% for all species for the three levels tested except for hexadecenoylcarnitine (42% and 32% at 9 and 42 nM, respectively). No interference from lipemic, uremic, icteric or hemolyzed samples was detected. Plasma samples for acylcarnitine determinations were stable for 2 hours, 18 days, and 60 days at room temperature, 4 °C and -20 °C, respectively. Quantitative comparison (n=28) of four abundant acylcarnitine species with a reference LC-MS/MS method yielded similar results (slopes were 0.712-0.945, intercepts were 0.02-0.53 nM, and correlation coefficients were 0.8805-0.9708). Comparison of clinical diagnoses using an additional 40 patient samples (both normal and abnormal) yielded 100% concordance.

Conclusion This validated LC-MS/MS method is simple, sensitive, and specific. It can be easily adopted by other laboratories as all reagents and standards used in this study are commercially available.

C-91

Differences in insulin resistance stratification using HbA1c and the metabolic markers alpha-hydroxybutyrate, linoleoyl-GPC, and oleate

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Conventional methods for assessment and stratification of insulin resistance (IR) rely on measurement of hemoglobin A1c (HbA1c) and fasting plasma glucose to stage patients at risk of dysglycemia. Prior studies have identified metabolic changes in the plasma concentrations of alpha-hydroxybutyrate, linoleoyl-glycerophosphocholine, and oleate as markers of IR. When coupled with body mass index (BMI), these metabolites form the basis of the Quantose™ IR Test (Metabolon, Inc) and provide an additional assessment of IR. This study aimed to compare HbA1c to the Quantose test in classifying insulin resistant subjects from an uncontrolled population. Following IRB review, plasma was removed from 216 random whole blood patient samples previously tested for HbA1c (Ortho Vitros 5600) within 10 hrs of collection and stored at -80°C. Samples were stratified as consistent with normoglycemia (<5.7%), impaired glycemia (5.7-6.4%), or hyperglycemia (>6.4%) during the past 3 months using HbA1c. Gender, BMI, and pharmacotherapy with insulin or metformin were documented. Samples were submitted for targeted mass spectrometric analysis of AHB, L-GPC, oleate, and results were coupled with BMI to estimate the glucose uptake rate, Q_M, as a prediction of IR risk. IR was defined as a HbA1c >6.4%, or Q_M <5.6. Data were analyzed using pharmacologic intervention, HbA1c and Q_M results. As expected, increases in IR correlated with decreases in Q_M scores for the untreated subjects between the normal, impaired and hyperglycemic groups, respectively. The average and median values for Q_M decreased in the normoglycemic (6.64, 6.06), impaired (5.77, 5.37), and hyperglycemic (4.80, 4.88) HbA1c groups as the risk of insulin resistance increased. However, discrepant classification of IR between the two methods occurred in a substantial portion of untreated patients. Forty-one percent (35/85) of subjects with normoglycemic HbA1c concentrations had Q_M values <5.6, compared to 77% (10/13) of subjects with HbA1c >6.4% suggesting HbA1c alone may be insufficient for identifying patients in early stages of IR and at risk for type 2 diabetes progression. The sensitivity and specificity of HbA1c to detect IR as defined by the Q_M score was 45.1% and 75% respectively in untreated subjects. Drug-treated subjects showed a similar decrease in IR between the normoglycemic and impaired glycemic groups, respectively; however, a slight improvement in the average insulin sensitivity was observed for those hyperglycemic subjects on drug therapy compared to those in the impaired glycemic group. Of those patients receiving insulin or metformin monotherapy, or both, 54% (6/11) with normoglycemic HbA1c were insulin resistant using the Quantose test. Within the drug treated group, 40% (37/97) of those with elevated HbA1c were classified as insulin resistant. For patients with HbA1c >6.4%, average and median Q_M values were higher, suggesting lower IR risk in the drug-treated group (5.43, 5.47) relative to the untreated group (4.80, 4.88). The sensitivity and specificity of HbA1c to detect IR as defined by the Q_M score was 86% and 15.7% for treated patients. In conclusion, these data suggest that novel metabolic biomarkers may identify patients with increased risk of developing insulin resistance despite having normal HbA1c levels.

C-92

Quantifying the impact of ion suppression on the performance of a clinical LC-MS/MS assay.

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Background: Ion suppression results when chromatographically unresolved components co-elute with the analyte of interest and compete for ionization and/or suppress the efficiency of ionization of the analyte. This phenomenon could adversely impact assay precision, accuracy, and sensitivity. Therefore, the extent of ion suppression and its impact on the assay must be characterized. Ion suppression can be minimized with rigorous sample preparation and carefully designed chromatographic techniques prior to mass spectrometric analysis. Much of the imprecision arising from variations in ion suppression between samples can be removed by the use of an isotopically labeled internal standard. However, labeled internal standards may not be available or may be cost prohibitive. Often, to meet demands for rapid turnaround times, clinical laboratories perform routine therapeutic drug monitoring using fast and "crude" sample preparation and separation techniques that could suffer from ion suppression. In these cases, the need to characterize of ion suppression may be even more important. We developed an approach to characterize the impact of ion suppression on assay performance. In this approach, analytical recovery, ion suppression, and precision of the analytical recovery are determined for a set of 20 patient samples and the degree of correlation (p-value) between ion suppression and analytical recovery is used to estimate the potential impact of ion suppression on assay performance. This approach is successfully demonstrated for an LC-MS/MS assay used for routine therapeutic drug monitoring of a panel of immunosupressants in our hospital.

Methods: Each analyte (cyclosporin A, tacrolimus, sirolimus and everolimus) and internal standards (cyclosporin D and ascomycin) were separately spiked into negative patient samples (N=20), extracted whole blood samples of the same patients (N=20), and water (N=4). MRM peak areas for analytes extracted from each matrix were used to determine % ion suppression ($A_{whole\ blood}/A_{water}$), % recovery ([A]measured/[A]spiked), and % extraction efficiency ($([A]/[IS])_{spiked\ in\ whole\ blood}/ [A]/[IS])_{spiked\ in\ extracted\ whole\ blood}$). Imprecision of analyte recoveries and extraction efficiencies across the 20 samples for each analyte and internal standard were determined and each performance characteristic was tested for correlation with ion suppression.

Results: Mean ion suppression (N=20) or enhancement of <10% was determined for all analytes except cyclosporine D (46% suppression). In order to examine if ion suppression compromised the accuracy or imprecision of the assay, we demonstrated good recovery (range 84-102%) and extraction efficiency (range 82-119%) for each analyte including cyclosporine D and observed high consistency in analytical recoveries (5-13% CV) and extraction efficiencies (3-12% CV) across 20 patient samples. A significant improvement in the consistency of cyclosporine A recovery across the 20 samples ($CV_{peak\ area}$ = 15.6%) was observed when normalized by cyclosporine D ($CV_{response}$ = 6.6%) and the absence of correlation between ion suppression and recoveries ($p \geq 0.25$) for any analyte or internal standard across the sample set clearly indicated that the potential contribution of ion suppression on assay performance is minimal, if present.

Conclusions: This approach can be used to characterize ion suppression and its impact on any clinical mass spectrometry assay, which is especially important for those that do not employ isotopically labeled internal standards.

C-94

Determination of Argininosuccinic Acid and its Anhydrides by Tandem Mass Spectrometry

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Background: Argininosuccinic acid (ASA) lyase deficiency is a urea cycle defect characterized by early onset hyperammonemia and accumulation of ASA as well as citrulline in blood and urine. Deficiency of the ASA lyase gene (ASL) product on chromosome 7 affects approximately 1:100,000 newborns and is characterized by encephalopathy, seizures, vomiting, hepatomegaly, and dry, brittle hair. ASA exists in multiple forms in biologic fluids. In addition to the tricarboxylic acid, ASA may also exist as two different ninhydrin-positive anhydrides (anhydride I and II) and one deaminated lactam formed from anhydride I. In mass spectrometry (MS) procedures employing butylation, multiple derivatives of ASA may be formed, complicating quantitation. Our previously published LC-MS/MS protocol utilizes limited butylation and underestimates ASA concentrations by 50-60% because it detects only the monobutyl derivative of the tricarboxylate. Detection of other butyl esters unacceptably lengthens chromatography time.

Methods: In the present study, we describe the development of a flow-injection tandem MS method employing methanol extraction and more extensive butylation (15 min at 65°C) for simultaneous assessment of multiple derivatives of ASA using homoarginine as an internal standard. A mobile phase consisting acetonitrile/water (90/10 v/v) containing 0.1% formic acid was employed at a flow rate of 300 microliters/minute. Collision-induced dissociation of ASA yields a fragment of m/z 70 likely derived from the aliphatic carbon chain of arginine and the α-amino group following loss of butylformate and the guanidinium group. We employed homoarginine as an internal standard because it yields a similar fragment of m/z 84 containing an additional methylene group.

Results: In aqueous solution (pH 7-8), tri-butyl ASA was the predominant molecular species followed by di-butyl ASA, mono-butyl ASA, di-butyl ASA-anhydride I/II, the sodium adduct of tri-butyl ASA, mono-butyl ASA-anhydride I/II, di-butyl deaminated ASA, mono-butyl deaminated ASA, the sodium adduct of di-butyl ASA, and underivatized ASA. The ten most abundant ASA derivatives were used to assess linearity, recovery, and imprecision of the assay in blood. Quantitation was linear to at least 1250 micromoles/L, and average ASA recovery across the dynamic assay range was 113%. Imprecision of the technique was monitored in 24 analytic runs over 10 days yielding CV's of 8.9% and 5.9%, respectively, at ASA concentrations of 125 micromoles/L and 625 mirocomoles/L. Split-sample comparison of our method to a second, commercial LC-MS/MS procedure yielded the following linear regression statistics: ASA = 2.2 x (alternate method) + 11, r = 0.988, Syx = 42. The analyte was found to be stable in plasma for at least 4 days and 21 days at 4 °C and -20 °C, respectively.

Conclusions: This method promises to improve the accuracy of ASA quantitation in biologic samples submitted for the diagnosis and followup of urea cycle disorders.

C-95

Evaluation of bioMerieux Vitek MS Maldi-TOF Technology for Identification of Clinical Significant Bacterial and Candida Strains

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Background: Currently, new techniques for identifying and classifying microorganisms are emerging in an attempt to speed up the results, reduce costs and reach more precise identifications. One of these is mass spectrometry (MS), specifically the Matrix-assisted laser desorption/ionization time-of-flight (Maldi-TOF/MS). MS can be applied in the identification of microorganisms such as bacteria and fungi as it analyzes a combination of biomolecules such as proteins, carbohydrates, lipids, DNA, RNA, among others. This mixture of molecules will generate a mass spectrum that is characteristic of each species. Objectives: To evaluate the BioMerieux Vitek MS for use in a clinical Microbiology laboratory.

Methods: Evaluation was performed using 124 samples, being 14 from non-fermenting Gram-negative rods, 45 Gram-negative rods from *Enterobacteriaceae* family , 17 Gram-positive cocci from *Staphylococcus* genus, 22 Gram-positive cocci from *Streptococcus* and *Enterococcus* genus, 04 Gram-negative cocci and rods, including *Haemophilus* spp. and *Neisseria* spp, and 22 *Candida* spp. All these strains were identified conventionally mainly using the bioMerieux Vitek 2 system.

Results: All identifications were in agreement, except for one urine strain that had been identified as *Yersinia pestis* by Vitek 2 and as *Serratia marcenscens* by the Vitek MS. We could not perform complementary identification tests on that strain and the final identification could not be determined.

Conclusions: The results showed a very high concordance between the conventional and the Maldi-TOF/MS identification. The turnaround time of the Vitek MS is much better than the conventional identification, with a final result being possible just a few minutes after the identification of the plate growth. Variable costs of Vitek MS are very efficient when compared with the Vitek 2 costs. Also, the database is open for inclusion of new groups of bacteria, what permits a continuous improvement of the system.

C-96

Determination of Pipecolic Acid by UPLC-MS/MS.

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Background: Pipecolic acid is an imino acid derived from lysine metabolism. Pipecolic acid is oxidized by a peroxisomal oxidase to form Δ^1-piperideine-6-carboxylate/α-aminoadipic semialdehyde. These metabolites are metabolized to aminoadipic acid, which is converted to acetyl-CoA to fuel the citric acid cycle. However, pipecolic acid is markedly elevated in patients with peroxisome biogenesis disorders, in which several peroxisomal functions are deficient. Pipecolic acid is also elevated in patients with pyridoxine dependent epilepsy and, to a lesser degree, in chronic liver dysfunction.

Objective: The goal of this study was to develop and validate a rapid UPLC-MS/MS assay for measuring pipecolic acid in human plasma, urine, and CSF to aid in the diagnosis of peroxisomal disorders and of pyridoxine responsive seizures.

Methods: Acetonitrile (200 µL) containing deuterated pipecolic acid as internal standard was added to 20 µL of sample (plasma, urine, CSF) and centrifuged. An aliquot of the supernatant was dried and derivatized with *n*-butanol. Derivatized samples were dried, reconstituted in water/methanol (70:30) and injected into an ACQUITY UPLC System. Separation was achieved by elution on an ACQUITY BEH-C_{18} column (2.1x100mm, 1.7µm) at 40°C using gradient elution. The UPLC was interfaced directly to the electrospray source of a Waters Xevo TQ MS system. Pipecolic acid eluted at 1.68 min and d_9-pipecolic acid at 1.67 min in a total run time of 7 minutes Analysis was performed in SRM mode using the following transitions: m/z 186.2>84.2 and 186.2>130.2 for pipecolic acid and m/z 195.2>93.2 and 195.2>139.2 for the internal standard.

Results: The method was linear to 200 µmol/L, with a limit of detection of 0.05 µmol/L, and lower limit of quantitation of 0.30 µmol/L. The accuracy was evaluated by comparing results obtained with this method with proficiency testing samples. The concordance was excellent with a slope of 0.95 calculated using Deming regression. The recovery was 99-115%, over the analytical measurement range (AMR) of 0.3-200 µmol/L. The total imprecision was 3.2 %CV over the AMR. Plasma specimens collected from patients with peroxisomal biogenesis disorders, showed markedly

increased concentrations of pipecolic acid, thus confirming the clinical utility of this assay.

Conclusions: We have successfully developed and validated a reverse phase UPLC-MS/MS method for determining the concentration of pipecolic acid in human plasma, urine and CSF. This method can be used for the laboratory evaluation of patients with peroxisomal disorders and with pyridoxine responsive seizures.

C-98

Proteomic Approach to Study Inter-/Intra-Subject Biological Variations of Human Urine Proteome

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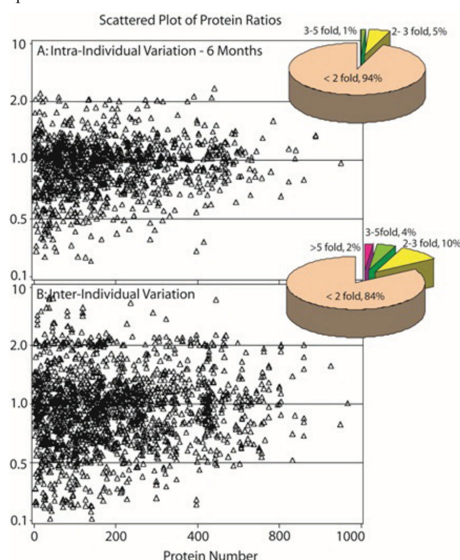
Background: Several unique features of urine make it an important fluid for clinical proteomics and biomarker discovery in human diseases. Understanding biological variations of urinary proteins in healthy individuals is very essential in identify potential biomarkers. This study applied proteomic approach to demonstrate intra-person variation over a period of six months as well as inter-person differences among a diverse group of individuals.

Methods: Six healthy individuals of widely diverse age, gender and race participated in this study. Five to ten mL of urine collected from each individual over times ranging from one day (morning and afternoon urine), one, two, three-week to six-month was concentrated to 1mL using a centrifugation filtration system(MW cutoff = 5KDa). The samples were dialyzed against 20 mM NH4HCO3, frozen and lyophilized. The lyophilized proteins were labeled by four-channel iTRAQ® (isobaric Tags for Relative Quantification) technology, which were subsequently desalted and purified using MCX Oasis® solid phase extraction before they were subjected to mass spectrometry analysis (QSTAR® Pulsar i ESI-QqTOF).

Intra-person variations were calculated relative to protein levels at time zero. Six pair-wise comparisons within one iTRAQ® were used to indicate the inter-person variation.

Results: Over 900 proteins were identified with >95% confidence. The proteins were mainly distributed among cytoplasm (36%), extracellular space (28%), and plasma membrane (30%). Temporal-individual protein variation suggested stability over six months with an average coefficient of variance of 30%. Only Six percent of the proteins showed more than a two-fold intra-individual difference and instances of higher than three-fold difference were very rare. Inter-person variations were significantly larger with an average coefficient of variance of 69%. Sixteen percent of proteins showed greater than a two-fold inter-individual difference and 6% greater than three-fold.

Conclusions: Proteins that were consistent among individuals may offer more sensitive biomarkers. Study of temporal-individual variations may provide great insights into personalized medicine.



C-99

High Resolution LC-MS/MS Screen for Psychiatric Drugs in Serum

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Background: The object of this project was development of a semi-quantitative drug screen on the Thermo Scientific Exactive Orbitrap mass spectrometer for a variety of psychiatric medications. Potential applications include drug compliance monitoring in psychiatric patients which can help reduce incidence of admission/readmission to psychiatric facilities. The assay could also be a screen for patients presenting at the hospital with altered mental status to help determine if a psychiatric issue is present. Advantages of using serum over urine include a more real-time picture of drugs present, an important consideration in emergency medicine, and decreased sample tampering in compliance monitoring. The semi-quantitative nature of the assay provides possible rough determination if drugs are present in potentially toxic or subtherapeutic amounts.

Methods: A semi-quantitative assay for a variety of psychiatric drugs (N = 31) from several classes, including benzodiazepines (N = 14), barbiturates (N = 3), anti-psychotics (N = 6), anti-convulsants (N = 3), and anti-depressants (N = 5), was developed. The compounds were extracted from serum by protein precipitation and separated on a Waters X-Terra C18 2.1 x 100mm, 3.5 micron analytical column by gradient elution (mobile phase A: 5mM ammonium formate, 0.05% formic acid in water; mobile phase B: 0.05% formic acid in methanol) over 13.5 minutes at a flow rate of 0.5 mL/min. Compounds were detected by a Thermo Scientific Exactive Orbitrap high resolution mass spectrometer in both positive and negative modes. Alternating full MS and higher energy collisionally activated dissociated (HCD) scans were used to collect parent ion data at high (50,000) resolution for semi-quantitation and product ion data at enhanced (25,000) resolution for confirmation, respectively.

Results: Compounds were tested over the range of 10 to 100 ng/mL and all were determined to have a lower limit of detection of at least 10 ng/mL. Two stable labeled internal standards were used to determine relative retention times of each compound. Precision, as measured by C.V. of peak areas, was determined for concentration levels 10, 50, and 100 ng/mL for each compound. C.V.'s were: anti-convulsants, 3.67% to 14.0%; anti-psychotics, 3.85% to16.8%; barbiturates, 3.72% to 24.2%; benzodiazepines, 2.19% to 18.2%; and anti-depressants, 4.02% to 17.4%. The intraday precision of the absolute retention times ranged from 0.0200% to 1.19%. Confirmation ions were determined for the majority of compounds, an exception being the barbiturates which were run in negative mode and yielded the lowest responses of the compounds tested. Positive results are considered to be accurate mass within 10 ppm, relative retention time match with a known standard, and presence of a characteristic confirmation ion(s) when appropriate.

Conclusions: A simple semi-quantitative high resolution LC-MS/MS assay for a variety of psychiatric medications in serum was developed using the Thermo Scientific Exactive Orbitrap system. In addition to accurate mass and retention time information, confirmation ions were included when possible to yield extra confidence in identification of compounds. High resolution instrumentation allows collection of rich data sets which can be further interrogated for compounds not already included in the screen, another advantage in emergency medicine.

C-100

Development of a Liquid Chromatography Tandem Mass Spectrometry Method for the Quantitation of the Antiseptic Chlorhexidine in Serum

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Background: The topical antiseptic chlorhexidine (MW = 504.20 Da) has become an important tool in preventing healthcare-associated infections in hospitalized patients. Chlorhexidine is generally applied as a one-time skin scrub or a full body bath and the safety and efficacy of such treatments have been extensively studied. While skin irritation is the most commonly reported adverse effect, other side effects have also been noted including severe anaphylactic shock, ototoxicity, and blood cell cytotoxicity. Despite these investigations, there is limited data on the use and safety of chlorhexidine in neonates and particularly in preterm infants. As part of a multidisciplinary team, we are exploring whether chlorhexidine is absorbed through the skin into the bloodstream in premature infants less than 32 weeks gestation after a single skin preparation prior to central venous catheter placement. Here we report an assay developed by our group in order to quantify chlorhexidine in human serum samples.

Methods: Following a protein precipitation from human serum, chlorhexidine and its internal standard chlorhexidine-d4 (Toronto Research Chemicals) were injected, separated, and eluted from a Hypersil Gold C18 column using a Transcend liquid chromatography system (Thermo Fisher Scientific). Elution occurred with a gradient of water and methanol with 0.1 % formic acid. Analytes were detected over a 6.25 minute run time using a TSQ Quantum Vantage tandem mass spectrometer (Thermo Fisher Scientific) with a heated electrospray-ionization source in positive ionization mode with selected reaction monitoring. For chlorhexidine the collision energy was 17 V and the m/z 253.12 ◊ 170.00 transition was selected for monitoring; for chlorhexidine-d4 the collision energy was 15 V and the m/z 257.12 ◊ 174.10 transition was monitored.

Results: The described method was determined to be linear from 12.5 ng/ml to 100 ng/ml. Inter-day precision was evaluated at 12.5 ng/ml, 25 ng/ml, and 50 ng/ml, and yielded %CVs of 6.2, 2.8, and 3.3, respectively. Carryover studies at 100 ng/ml resulted in minimal carryover of < 3 %.

Conclusions: We have developed a robust LC-MS/MS method for the quantification of chlorhexidine in human serum. Our method shows minimal carryover, and can be used to monitor chlorhexidine levels in infants as part of a quality and safety measure.

C-101

A comprehensive validation of an LC-MS/MS method for the quantification of 25-hydroxyvitamin D2/D3 in human serum in the range of 5 ng/mL to 100 ng/mL.

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Background: Vitamin D is a potent fat-soluble molecule with pleiotropic physiological activity including the regulation of calcium homeostasis, melanocyte physiology, and maturation of progenitor cells. Deficiency in vitamin D has been associated with many diseases including cancer, heart disease, and CNS diseases. The concentrations of 25-hydroxyvitamin D2 and D3 (25(OH)D2, 25(OH)D3) serve as the best indicator of vitamin D body stores due to their longer half-lives and higher circulating levels (ng/mL). An assay which utilizes liquid-liquid extraction (LLE) followed by LC-MS/MS analysis was developed and validated to quantify the concentration of 25(OH)D2/D3 in human serum samples in the range of 5 ng/mL to 100 ng/mL.

Methods: The internal standard (IS) 25(OH)D3-d6 was added to spiked 25(OH)D2/D3 standards and quality control samples (QCs) prior to LLE with zinc sulfate, methanol, and hexane. Following centrifugation, the supernatant was transferred to separate extraction vials and evaporated under nitrogen. The standards and QCs were reconstituted in 50% methanol and RP-LC-MS/MS was acquired on a LC-20AD XR (Shimadzu) connected to a 5500 QTRAP (AB Sciex). 25(OH)D2/D3 were separated with a C18 column (Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 µm) with a water/methanol gradient and quantified by monitoring one MRM transition per analyte and IS.

Results: The method was linear over the range of 5 ng/mL to 100 ng/mL 25(OH)D2/D3 with a weighting factor of 1/x. The LOD for 25(OH)D3 and total 25(OH)D was 0.4 ng/mL and for 25(OH)D2 was 1 ng/mL. The LLOQ of 5 ng/mL was established for both 25(OH)D2 and 25(OH)D3. The overall accuracy at LQC (15 ng/mL), MQC (50 ng/mL), and HQC (80 ng/mL) for both D2 and D3 was between 0.4% and 4.6% with total errors all less than 21.4%. The intra-assay and inter-assay precision estimates for 25(OH)D2 at the three levels of QCs were within 9%, with the total precision within 12%. The intra-assay, inter-assay, and total precision estimates for 25(OH)D3 at the three levels of QCs were within 9%. No linear drift or interferences from lipids, hemoglobin, or bilirubin were observed. Matrix factor, extraction efficiency, and process efficiency were 83.0%, 100.3%, and 83.2%, respectively, for 25(OH)D2, and 87.9%, 97.4%, and 89.2%, respectively, for 25(OH)D3. Spiked serum samples are stable for up to 24 hours when stored at room temperature and for 3 freeze-thaw cycles and up to 28 days when stored at -20°C ± 5°C. Extracted serum samples are stable at 2-8°C and in the instrument's autosampler for up to six hours and stock solutions of 25(OH)D2/D3 and 25(OH)D3-d6 are stable at room temperature for up to 24 hours.

Conclusions: An efficient and robust method for determining the concentration of 25(OH)D2/D3 in human serum by LC-MS/MS in the range of 5 ng/mL to 100 ng/mL has been developed. The results herein lay a strong foundation for more precise correlation between disease pathophysiology and Vitamin D levels. Furthermore, the Berg method development allows for more relevant monitoring of those who require supplementation while ensuring that serum levels remain optimal for health.

C-103

Ultraperformance Liquid Chromatography/Tandem Mass Spectrometry Assay for Erythrocyte Metabolites and Intermediates

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Background: Deficient enzyme activity in the erythrocyte may result in abnormalities that lead to premature destruction and hemolytic anemia. Conventional quantitiative erythrocyte enzyme assays for the confirmatory diagnosis of enzyme deficiency are time-consuming, expensive, and require additional multiple steps. We tried to develop the simple multiplexing enzyme assay using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). To do this, we first developed and evaluated a quantitative analysis of erythrocyte metabolites and intermediates which were the enzyme reaction products using UPLC-MS/MS.

Methods: A Waters Acquity UPLC and Waters Xevo TQ were used for UPLC-MS/MS analysis. Separation was achieved with a Acquity UPLC High Strength Silica(HSS) T3 (100 mm x 2.1 mm, 1.8 µm) column employing a flow rate of 0.2 mL/min and mobile phases, 0.1% aqueous ammonium hydroxide and 0.1% ammonium hydroxide in acetonitrile, respectively. Total run time is 3.5 min. Negative electrospray tandem mass spectrometric methods (ESI-MS/MS) were conducted for 15 compounds using MRM transitions optimized by direct infusion. We evaluated the assay performances such as imprecision, linearity and ion suppression.

Results: The MRM transition of 15 compounds such as pyruvate, lactate, phosphoenolpyruvate, glucose, 2,3-phosphoglycerate, ribulose-5-phosphate, cystidine, fructose-6-phosphate, glucose-6-phosphate, inosine, 6-phosphogluconate, 2-glutathione, fructose-1,6-bisphophate, adenosine monophosphate, and glutathione disufatide were 87.00>43.00 m/z, 89.00>43.00 m/z, 166.90>79.00 m/z, 178.90>89.00 m/z, 184.90>97.00 m/z, 228.90>97.00 m/z, 242.00>109.00 m/z, 258.90>97.00 m/z, 258.90>198.80 m/z, 267.00>135.00 m/z, 274.90>256.90 m/z, 306.00>143.00 m/z, 338.90>97.00 m/z, 346.00>134.00 m/z, and 611.10>306.00 m/z, respectively. All compounds were clearly separated in UPLC-MS/MS system within 3.5 min. Within-run imprecision were 2.67-7.46 % in low level and 1.77-5.73 % in high level. And those of between-run 2.20-7.38 % and 2.72-10.38 % respectively . Calibration curves are linear over the range 0-50 µg/mL. The effect of ion suppression was rarely shown.

Conclusions: We developed a rapid method to measure erythrocyte metabolites and intermediates concentration utilizing UPLC-MS/MS successfully. Our study may be the cornerstone of novel confirmatory tests for RBC metabolic disorders.

C-105

Discovery of'cell-derived'circulating peptide biomarker in blood and LC-MS/MS assay development for liver disease

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Background: Although serum peptides may serve as surrogate markers for cancer detection, there is no convincing report of valuable low abundance peptides of cellular origin identified as potential biomarkers. More than 170 million individuals are now affected with HCV worldwide, and in the coming decades many of these cases will progress to chronic hepatitis (CH), liver cirrhosis (LC), and eventually HCC. Here we established a quantitative peptidomic strategy for 'peptidomic' biomarker discovery and used it to identify such 'masked' peptide biomarkers in circulation of chronic liver disease.

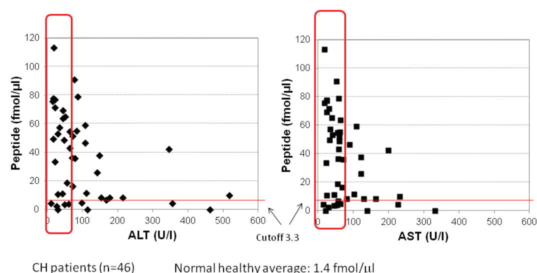
Methods: We established a quantitative peptidomic methodology using MS to screen for masked peptide biomarkers in circulating blood. We comprehensively analyzed more than 180 serum samples by quantitative 2D-LC MALDI-TOF MS/MS, and developed UPLC-MS/MS assay and immunoMS assay for these novel biomarkers.

Results: We identified 12 cell-derived peptides associated with cancer and a glycosylated peptide with high sensitivity and specificity for the diagnosis of chronic liver disease. By efficient peptide extraction and extensive fractionation, we identified twelve cell-derived peptides and a glycosylated peptide with high diagnostic potential for disease. NPY1R peak intensity showed significant differences between CH vs. HCC (P= 0.000072), LC vs. HCC (P=0.015), and normal vs. HCC (P=0.000000023) comparisons, and 73% sensitivity in HCC which are negative for known tumor markers. Glycosylated ITIH4 peptide showed remarkable diagnostic potential in the CH vs. normal (under area curve, 0.88) and was confirmed by an immunoprecipitation-MS assay. We verified this novel glycosilated peptide biomarker using 108 biopsy-proved NAFLD (53 non-NASH and 55 NASH). Furthermore, we developed an UFLC-MS/MS assay to rapidly (within minutes) quantify the biomarker

peptides for clinical applications.

Conclusions: Our findings indicate that peptides circulating in blood, particularly those of cellular origin, are potentially valuable biomarkers that could impact molecular diagnostics and therapeutic intervention in cases of chronic liver disease including non-alcoholic fatty liver disease and hepatocellular carcinoma.

High concentrations of glycosylated peptide marker in ALT and AST negative-CH indicate usefulness of this assay in screening of hepatitis



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C-107

Integration of an Automated Sample Preparation Workstation for the Analysis of 25-OH Vitamin D2 and D3 by LC-MS/MS

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Background: (For Research Use Only. Not for Use in Diagnostic Procedures.) Liquid chromatography-tandem mass spectrometry technology provides laboratories with a powerful tool for robust, accurate, sensitive detection of a wide variety of analytes. However, in the absence of complete method automation, results are susceptible to human error at many different stages, including preparation of calibration standards, sample preparation, and data processing. Not only does method automation eliminate human error, thus increasing reproducibility, it also eliminates subjectivity during data processing, and furthermore has the potential to save a great deal of time.

Methods: In the work presented here, an LC-MS/MS method for the analysis of 25-OH Vitamin D2 and D3 has been developed, making use of commercially available plasma calibrators and controls. In addition to manual preparation, all steps of sample processing could be automated using a BioMek NX^P platform. The sample preparation consisted of a liquid-liquid extraction in hexane, followed by evaporation to dryness under N_2 gas, and reconstitution in 50:50 v/v MeOH and H_2O. Samples were loaded in test tube format and the final samples were prepared in a 96-well plate format, and then transferred to the LC-MS/MS system for analysis of 25-OH Vitamin D2 and D3.

Results: The LC-MS/MS data acquisition, processing, and reporting were performed using the Cliquid® software. Upon completion of data acquisition, the software automatically performed quantitation for the target analytes included in the method, and automatically generated and printed out reports. The reproducibility of the automated protocol versus manual protocol was assessed by preparing and analyzing replicates of each calibration standard. The measured CVs were at least equivalent between protocols over the entire concentration range covered by the assay. The method displayed good linearity for all analytes, with R>0.999.

C-108

A Novel LC-MS/MS Method for the Ultra-Sensitive Detection of 1,25(OH)2-Vitamin D3 in Serum

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(For Research Use Only. Not for Use in Diagnostic Procedures.) Owing to the relatively poor ionization efficiency of 1α,25-$(OH)_2$-Vitamin D3, and the very low concentration of this compound in plasma, the intrinsic sensitivity is quite low when this compound is measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The challenge is to improve the high sensitivity and selectivity of LC-MS/MS analysis, without resorting to chemical derivatization strategies, by using ultra-sensitive mass spectrometric detection.

In the work presented here, an ultra-sensitive LC-MS/MS method has been developed for the analysis of 1α,25-$(OH)_2$-Vitamin D3 (DHVD) in serum. The method employs a 2D chromatographic separation followed by MRM detection of the DHVD species as their lithium adducts, which allows for low levels of detection without the use of derivatizing agents thereby greatly simplifying the analytical workflow. Online sample clean-up of the protein precipitated sample is accomplished using a POROS column, which minimizes the need for offline, manual sample preparation. Analytical chromatographic separation is accomplished using dual Phenomenex Onyx Monolithic C-18 columns to enhance the separation of complex biological matrices while providing extremely low back pressures. The flows in the preparative and analytical columns are regulated by the use of an auxilary Valco 10-port valve assembly.

Using a simple acetonitrile protein precipitation step followed by centrifugation, the method readily achieves a limit of detection of 10 pg/mL of 1α,25-$(OH)_2$-Vitamin D3 in serum. To achieve this level of sensitivity, it was important to enhance the ionization efficiency of the target analyte by forming the lithium adduct, and to also use an ultra-sensitive tandem mass spectrometer. The linear dynamic range for the method ranged from 0 to 250 pg/mL in serum. The method has been demonstrated to be robust and reproducible, and the target analyte is effectively separated from other matrix componenents with minimal interferences in the ion source.

C-109

Analysis of Estrogens in Plasma Samples with a Reduced Sample Preparation Afforded by the Use of SelexION Ion Mobility Technology

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Introduction: (For Research Use Only. Not for Use in Diagnostic Procedures)

The two estrogens, Estrone (E1) and 17β-Estradiol (E2) are the primary female sex hormones. An accurate analytical method to detect for Estrone (E1) and 17β-Estradiol (E2) with a very easy and time-saving sample preparation was the mission. However, analysis of these compounds by LC-MS/MS is challenging due to the high number of interferences in addition to high chemical noise. In the present work we evaluated the use of differential mobility spectrometry (DMS) to improve the selectivity of the MRM detection to reach lower LODs for the analysis of the estrogens in plasma samples after protein precipitation.

Methods: Anonymized plasma samples were precipitated with acetonitrile. After centrifugation the supernatant was directly injected on a 2D chromatographic separation system using a C8 clean up column (10 µm, 50 x 4.6 mm) and a Phenomenex Luna C8 separation column (5µm, 100 x 2.1 mm). This was coupled to two Agilent 1200 SL Systems utilizing a 10 minute gradient using methanol and water containing 0.2 mM NH_4F. The mass spectrometric detection of the analyte was done on an ABSCIEX QTRAP® 5500 system with Turbo V™ source in positive ion mode. For a better selectivity and sensitivity the instrument was equipped with the SelexION™ Technology differential mobility device.

Preliminary Data: The analysis of estrogens by liquid chromatography tandem mass-spectrometry in plasma requires a very time consuming and extensive sample preparation which is also very costly. With the use of a tandem mass-spectrometer coupled to DMS device associated with on-line sample extraction and clean up, the sample preparation has been reduced to a simple protein precipitation step. With the additional gained selectivity using the SelexION™ Technology, a significant reduction of the background interferences was achieved. With this approach it was possible to detect an Estrone (E1) concentration below 1 ppt and an 17β-Estradiol (E2) concentration below 5 ppt in plasma.

C-110

Selectivity Enhancement in High Throughput Analysis of Testosterone using Differential Ion Mobility Coupled to LDTD MS/MS

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(For Research Use Only. Not for Use in Diagnostic Procedures.) Laser Diode Thermal Desorption (LDTD) ionization source technology has been coupled to a mass spectrometer equipped with a differential ion mobility device, enabling a high throughput capacity for the analysis of testosterone in biological matrix. The innovative source design allows rapid laser thermal desorption of samples at atmospheric pressure, followed by APCI type ionization. The addition of the differential ion mobility device between the source and the tandem mass spectrometer provided enhanced selectivity, and effectively allowed the separation of the isobaric analytes. The method described here enabled sample-to-sample run times of less than 10 seconds, a significant improvement over LC-MS/MS run times which are typically

3-5 minutes.

A Laser Diode Thermal Desorption (LDTD) ionization source was utilized to perform rapid, high throughput analysis of testosterone in human plasma by tandem mass spectrometry. To enable the separation of isobaric species prior to mass spectrometric detection, a SelexION differential ion mobility device was inserted between the source and the mass spectrometer. The ion mobility device was optimized for the detection of testosterone by tuning the Compensation Voltage (CoV) parameter to a value of 4V. Pre-analytical sample preparation consisted of a simple liquid-liquid extraction of human plasma using MTBE or 90:10 hexane:ethyl acetate, in a 1:4 v/v ratio. 2 µl of the upper layer was deposited directly into the 96 well plate and allowed to dry prior to analysis.

When the analysis was performed with the ion mobility device turned off, the lower limit of quantitation for the LDTD-MS/MS determination of testosterone was limited by the blank interference arising from the presence of isobaric analytes. However, when the LDTD source and SelexION ion mobility device were used in tandem to analyze spiked serum testosterone calibrators, the interference in the blank was reduced from 65% to nearly zero, resulting in a 10x improvement in the lower limit of quantitation. The method described here easily permitted the measurement of down to 1 ng/ml, and the assay displayed excellent linearity, with $r^2 = 0.99972$, over 4 orders of magnitude. Accuracy and reproducibility were well within the acceptable values. Using this approach the sample-to-sample run time was only 7 seconds. In comparison, the equivalent analysis using conventional LC-MS/MS would typically require approximately 3-5 minutes per sample.

C-111

Development of a Method for the Analysis of 3-epi-25-OH Vitamin D and 25-OH Vitamin D by LC-MS/MS

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(For Research Use Only. Not for Use in Diagnostic Procedures.) Due to the similarity in chemical structure, 25-OH Vitamin D3 and 3-epi-25-OH Vitamin D3 display very similar chromatographic retention properties, and produce the same MS/MS fragment ions. Since it was not possible to distinguish these epimers by tandem mass spectrometry alone, it was essential to resolve these epimers chromatographically. Chromatographic separation cannot be achieved using a standard C18 HPLC column, however we have developed an LC-MS/MS method utilizing a Phenomenex Kinetex PFP column that achieves baseline separation of 25-OHD3 and 3-epi-25-OHD3. The total run time is less than 7 minutes, using a shallow gradient consisting of water and methanol, with ammonium formate and formic acid additives. 'Blank' stripped serum and spiked serum samples have been analyzed to determine LOD and LOQ for the method.

An analytical method was developed using an AB SCIEX liquid-chromatography/tandem mass spectrometry system. A Phenomenex Kinetex PFP analytical column was used (100 x 3mm, 2.5um, 100A) to achieve the chromatographic separation of 25-OH-vitamin D_3 and 3-epi-25-OH-vitamin D_3, in a total run time of 10 minutes. Analytical standards for each compound were purchased from Cerilliant, as 1.0 mg/mL solutions in acetonitrile. Sample preparation consisted of the addition of 200uL of internal standard solution to 200uL of spiked serum, followed by liquid/liquid extraction (LLE) using hexane. Supernatant was transferred to a clean tube, evaporated under nitrogen, and reconstituted in 150uL of 50:50 methanol:water, prior to injection onto the LC-MS/MS system.

The method developed here permits the independent measurement of both 25-OH Vitamin D3 and 3-epi-25-OH Vitamin D3 in a single LC-MS/MS analysis. The estimated Limit of Quantitation is better than 2 ng/mL for both analytes, and the method displayed good accuracy, reproducibility, and linearity over a concentration range from 0-100 ng/mL.

Wednesday AM, July 18, 2012

Poster Session: 10:00 AM - 12:30 PM

Immunology

C-112

Toll Like Receptor 3 Expression as a Novel Predictor of Response to Treatment in Chronic Hepatitis C Virus Patients

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Background: this work was carried out to study the level of pretreatment hepatic expression of Toll Like Receptor 3 *(TLR3)* among chronic HCV patients, aiming to determine if there are consistent differences in gene expression, between those who show complete early virological response (cEVR) at week-12 of Pegylated-Interferon α-2a plus ribavirin treatment and others who are not responding to this combination, also if this could be used to predict treatment outcomes.

Methods Sixty one chronic hepatitis C patients were enrolled in the study. For all of them, baseline hepatitis C virus (HCV) viral load was determined and *TLR3* gene expression was examined in their hepatic percutaneous needle biopsy specimens using a real time -polymerase chain reaction technique. Hepatic *TLR3* was also traced using immunohistochemistry. All patients followed 12-weeks regimen of Pegylated-Interferon α-2a plus ribavirin then post-treatment viral load was assayed.

Results Hepatic expression of *TLR3* was significantly increased in non-responder group as compared to those who showed complete early virological response to the used treatment regimen. Using receiver operator characteristic curve (ROC) curve analysis, a cutoff level of 1.5 was set for *TLR3* expression to distinguish responders from non-responders to the 12-weeks treatment regimen used.

Conclusion, measuring the hepatic expression of the interferon stimulated gene; *TLR3* could provide a new molecular marker for pre-treatment prediction of complete early viral clearance, thus helping in patients' selection and optimizing treatment outcomes.

C-113

microRNA146a expression in lupus patients with and without renal complications

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Background: microRNA (miRNA) expression profiles are likely to become important diagnostic and prognostic tools in many disease aspects. This work aimed to study the expression of miRNA146a in lupus patients with and without renal complications and to assess its association with disease activity. Patients enrolled in the study included 52 females affected by systemic lupus erythematosus (SLE) and another 60 females with lupus nephritis. Forty eight age-matched healthy females were enrolled as a control group.

Methods: miRNA146a expression was assessed using real-time PCR.

Results: In SLE patients, miRNA146a was under-expressed as compared to healthy controls and lower levels were detected among lupus patients with renal affection. In addition, miRNA146a expression was low and serum Interferon-alpha (IFN-α) level was high in patients with active lupus as compared to those with inactive disease. miRNA146a expression was inversely correlated to serum IFN-α level and to anti ds-DNA titer in the three studied groups.

Conclusion, miRNA146a might be implicated in lupus pathogenesis. Moreover, miRNA146a expression correlates negatively to lupus activity and lupus nephritis, while serum IFN-α has a direct correlation to both disease activity and nephritis, hence both miRNA146a expression and serum IFN-α could be potentially important diagnostic biomarkers and potential novel strategies for therapeutic interventions which may possibly be implied to enhance the sensitivity and specificity for the prediction of flares and prognosis in SLE patients.

C-114

Evaluation of a newly developed screening test for connective tissue disease (Phadia EliA CTD screen) in clinically defined patients

H. Hwang, H. Yang, G. Kim, H. Jeong. *Kosin university college of medicine, Busan, Korea, Republic of*

Background: The purpose of this study is to evaluate the Phadia EliA connective tissue disease (CTD) screen test (Phadia, Sweden), a screening test for CTD, by comparing it with the Hep-2 cell-based anti-nuclear antibody (ANA) screening test, an indirect immunofluorescence assay (IFA), in clinically defined patients.

Methods: A total of 211 patient samples were used; 62 from CTD patients and 148 from patients with non-CTD. CTDs consisted of systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren's syndrome, rheumatism, and systemic disorders of connective tissue. Non-CTDs consisted of allergic urticaria, autoimmune hepatitis, viral hepatitis, other liver disease, pneumonia, dermatitis, nephrotic syndrome, renal failure, other kidney disease, spondylopathy, gonarthrosis, polyarthritis, other forms of arthritis, fracture of the femoral neck, autoimmune pancreatitis, stomach and duodenal ulcer, cancer, hematologic disorder, polyneuropathy, other neurologic disorder, vulva ulceration, cystitis, urinary tract infection, hyperplasia of the prostate, vitreous opacities, zoster, and other viral diseases. Antigens represented on the wells of the Phadia EliA CTD screen plates were human recombinant U1-snRNP (RNP70, A, C), Ro-60, Ro-52, La, Scl-70, Jo-1, CENP-B, Sm proteins, fibrillarin, RNAP III, ribosomal-P, PM/Scl, PCNA, Mi-2 proteins, and purified ds DNA. A ratio of test sample response-to-calibrator higher than 1, 0.7 to 1.0, and less than 0.7 on the Phadia 250 instrument were interpreted as positive, equivocal, and negative, respectively. ANA was tested by IFA using Hep-2 cell line slides (MBL, Japan). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were compared between IFA and Phadia EliA CTD screen. The degree of agreement between the two tests was calculated with kappa statistics. Comparisons of parameters between Phadia EliA CTD screen and IFA were performed in 1:80, 1:320, and 1:640 titrated sera.

Results: The sensitivity was highest in the 1:80 titrated sera in IFA (73.0% and 66.7% in IFA and Phadia EliA CTD screen, respectively); on the other hand, specificity, PPV, NPV, and accuracy were better in the Phadia EliA CTD screen (86.5%, 67.7%, 85.9%, and 80.6%, respectively). The degree of agreement between IFA and Phadia EliA CTD screen was highest in the 1:320 titrated sera ($k = 0.389$, $p = 0.000$); however, the strength of agreement between them was just acceptable. Phadia EliA CTD screen showed a fair degree of agreement with IFA in all titrated sera.

Conclusions: Although a higher sample number and more kinds of CTD-positive samples need to be tested to reach a more concrete conclusion, the results of this study demonstrate that the Phadia EliA CTD screen test seems to be appropriate to screen for CTD and superior to IFA in specificity, PPV, NPV, and accuracy.

C-115

ANA pattern determination in autoimmune hepatitis

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Background and Aims: Type 1 autoimmune hepatitis (AIH) is characterised by ANA and/or ASMA positivity detected by indirect immunofluoroscence . No pattern of ANA i.e. homogenous, nucleolar,speckled or nucleolar has been described in patients of autoimmune hepatitis, thereby limiting the development of liver specific immunoblots in Indian setting. The aim of the current study was to exploit the database in Indian setting to determine the patterns of ANA in confirmed cases of autoimmune hepatitis .

Methods: 78 female patients ,>18 years with ANA (1:80)positivity, ALP:AST ratio of <1.5, serum IgG level >40g/L, non reactive viral hepatitis markers and no history of drug or alcohol intake were taken (definite AIH by revised IAHG scoring system) to evaluate pattern of ANA by hep2 cell line (Euroimmune).

Results: Homogenous pattern was seen in 33 of 78 patients , with speckled pattern in 39 of 78 patients. Nucleolar pattern was seen in 6 patients with no patient having centromere pattern. More than 90% of definite AIH patients had either homogenous or speckled pattern.

Conclusions: Homogenous pattern which signifies dsdna,histones and ssdna ; speckled pattern which signifies SSA,SSB, ro-52,nRNP,nuclear dots,Scl-70,CENPb, PCNA (extractable nuclear antigens) are prevalent in Indian setting. Liver specific Immunoblots consisting of extractable nuclear antigens and dsdna,ssdna and histones can be developed which can diagnose cases of autoimmune hepatitis objectively without the application of user dependent immunofloroscence microscope.

Keyword: ANA pattern autoimmune hepatitis immunoblot

C-117

Effects of radioiodine therapy on immune status in papillary thyroid carcinomas with/without autoimmune thyroid diseases

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Background: Immunological tolerance in papillary thyroid carcinomas (PTC) and autoimmune thyroid diseases (AITD) has opposite effects: in PTC patients it stimulates the growth of the cancer and in AITD patients may stop the attack by autoantibodies. Little is known about the immunological tolerance in PTC associated with AITD (PTC+AITD) patients. The thyroidectomy followed by radioiodine (I-131) ablation of the residual thyroid tissue, after thyrotropin (TSH) stimulation, is considered the ideal treatment for PTC with/without AITD patients. The radioiodine therapy triggers a variety of physiologic responses in thyroidian cells, including immunosuppressive effects and inflammation. The aim of this study was to evaluate the effects of therapeutic irradiation with I-131 on peripheral blood lymphocyte subsets and on the regulation of cytokine release in PTC with/without AITD patients.

Methods: We selected 37 patients with PTC (5M/32F, mean age 45.1±17.6 years) and 29 with PTC+AITD (2M/27F, mean age 37.2±18.7 years), who received the same dose of I-131 (3.7 GBq, first dosage after thyroidectomy). All patients had elevated serum levels of TSH (>30mU/L). PTC+AITD patients had positive titers of anti-Tg autoantibodies (TgAb). Peripheric blood samples were collected before and at 96 hours after I-131 administration. The serum levels of TSH, TgAb, IL-6, IL-10 and TNF-α were measured by ELISA and the lymphocyte subsets by flow cytometry.

Results: We found that I-131 therapy was associated with an impairment of T-cell immunity. Thus, in patients with PTC, it was observed a decrease in the percentage of CD3 T-cells (from 67.17±8.68% to 66.47±8.77%), CD19 B-cells (from 11.53±6.41% to 9.26±6.08%), CD4 T-cells (from 41.96±8.47% to 39.85±8.50%), with a consequent significant reduction in the CD4/CD8 ratio (from 1.99±0.90 to 1.71±0.73), Th1 type, such as IL-6, TNF-α (1.64-fold, 1.58-fold), the induction of CD16+56 NK cells (from 16.89±10.51% to 19.47±10.13%) and Th2 type, such as IL-10 (1.78-fold). The effects on the immune system observed in PTC+AITD patients, undergoing radioiodine therapy, include increases in CD3 T-cells (from 73.39±6.00% to 75.07±4.73%), CD4 T-cells (from 44.30±6.60% to 47.73±6.07%), CD4/CD8 ratio (from 1.81±0.74 to 2.05±0.77), the ability to produce pro- and anti-inflammatory cytokines (IL-6: 1.31-fold, TNF-α: 1.29-fold and IL-10: 1.35-fold) and reduction in CD16+56 NK cells (from 10.88±5.71% to 9.16±5.60%). Also, in PTC+AITD patients I-131 therapy caused an increase in TgAb titers, which reflects an activation of autoimmunity. CD19 B-cells increased from 11.93±3.38% to 12.62±4.14% and TgAb from 175.88±46.35 U/mL to 187.9±38.11 U/mL, probably due to release of thyroid antigens by cell damage, or destruction of intrathyroidal T cells after irradiation. The correlation between TgAb titers and the percentage of B-cells increased after I-131 administration (r=-0.48 vs. r=-0.74, p<0.001).

Conclusions. The effects of radioiodine therapy on the immune system remain controversial. In patients with PTC the treatment with radioiodine leads to immunosuppresion, reduces cellular responses controlled by Th1 cells and increases humoral responses controlled by Th2 cells by triggering a shift from Th1 towards Th2 (Th1/Th2 imbalance). In patients with PTC+AITD, radioiodine administration leads to immunostimulation and increases not only Th2-related cytokines, but also Th1-related cytokines. Radioiodine is probably better considered an immunomodulator agent because modulates the production of cytokines.

C-118

Effects of radioiodine therapy on imbalance between expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in papillary thyroid carcinomas with/without autoimmune thyroid diseases

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Background: Under physiological conditions the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) is highly coordinated at the level of gene expression and this balanced expression guarantees normal tissue structure and organ function and prevents both excessive extracellular matrix (ECM) deposition and increased ECM degradation. Abnormal activity of MMPs has been implicated in many disease processes including papillary thyroid carcinomas (PTC) and papillary thyroid carcinomas associated with autoimmune thyroid diseases (PTC+AITD). In PTC with/without AITD patients, the balance between MMPs and TIMPs is broken and ECM is degraded. The thyroidectomy followed by radioiodine (I-131) ablation of the residual thyroid tissue, after thyrotropin (TSH) stimulation, is considered the ideal treatment for these patients. The aim of this study was to evaluate the effects of therapeutic irradiation with I-131 on imbalance between expression of MMP-9 and TIMP-1 in PTC with/without AITD patients.

Methods: We selected 45 patients with PTC (6M/39F, mean age 44.2±19.1 years) and 38 with PTC+AITD (3M/35F, mean age 38.2±19.6 years), who received the same dose of I-131 (3.7 GBq, first dosage after thyroidectomy). All patients had elevated serum levels of TSH (>30mU/L). PTC+AITD patients had positive titers of anti-Tg autoantibodies (TgAb). Peripheric blood samples were collected before and at 96 hours after I-131 administration. The serum levels of TSH, TgAb, transforming growth factor-beta1 (TGF-β1), MMP-9 and TIMP-1 were measured by ELISA.

Results: Because TGF-β1 stimulates collagen synthesis and alters the levels of MMP-9 and TIMP-1, we investigated the hypothesis that TGF-β1 activity after radioiodine therapy is associated with differences in decrease the imbalance between MMP-9 and TIMP-1 in PTC with/without AITD. We found that I-131 therapy of PTC+AITD patients was associated with an increase in titers of TgAb (1.18-fold), TGF-β1 (1.27-fold) and a decrease in the serum levels of MMP-9 (1.27-fold) and MMP-9/TIMP-1 ratio (1.24-fold). Increased TgAb titers are related to increased TGF-β1. The correlations between TgAb and TGF-β1, MMP-9, MMP-9/TIMP-1 ratio (r=-0.56, r=0.57, r=0.51, p<0.001), lead to the idea that the presence of TgAb partially blocks the immunosuppressive effect of radioiodine. Thus, the correlation between TGF-β1 and MMP-9/TIMP-1 ratio decreased at 96 hours after the I-131 administration (r=-0.92 vs. r=-0.24, p<0.001), due to increased TgAb titers. Before radioiodine therapy, the MMP-9 and TIMP-1 concentrations of PTC+AITD patients (635.12±370.03 ng/mL, 140.27±33.76 ng/mL) were higher than those of the PTC patients (484.98±377.50 ng/mL, 128.6±43.38 ng/mL). In PTC patients, the serum concentrations of TGF-β1 and MMP-9 decreased 1.03- and 1.72-fold after irradiation. The correlation between TGF-β1 and MMP-9/TIMP-1 ratio increased at 96 hours after the I-131 administration (r=-0.45 vs. r=-0.73, p<0.001). MMP-9/TIMP-1 ratio decreased by 45% more than in PTC+AITD patients.

Conclusions: In PTC patients the blockade of TGF-β1 signaling by radioiodine therapy has almost halved the imbalance between MMP-9 and TIMP-1 and this decrease may reduce tumor cell viability and migratory potential. In PTC+AITD patients, increased TgAb titers partially block the beneficial effect of radioiodine. These titers are associated with increased TGF-β1 concentrations and with a lower decrease of MMP-9/TIMP-1 ratio, after the radioiodine administration, than in PTC patients.

C-120

Increased levels of interleukine-6, -8, -10, G-CSF and TNF-α in Korean burn patients: relation to burn size and postburn time

H. Kim, J. Kim. *Hallym University College of Medicine, Seoul, Korea, Republic of*

Background: Major burn injury induces an inflammatory response, which is accompanied by the release of various cytokines. This study was performed to investigate changes in levels of pro-inflammatory and anti-inflammatory cytokines following burn injury over time, and to investigate the relationship between these levels and burn size, in adult Korean patients with burn injury.

Methods: Blood samples of 60 Korean burn patients were collected on days 1, 3, 7, 14, and 21 after burn injury, as well as from 9 healthy controls and concentrations of IL-6, IL-8, IL-10, TNF-α, and G-CSF were measured using the Bio-Plex Suspension Array System. Burn patients were divided into 3 groups according to burn size (15-30%, 31-50%, >51% total burn surface area) and concentration of cytokines were compared between these groups and the control group, across a time course of 3 weeks.

Results: Median (range) concentrations of IL-6, IL-8, IL-10 TNF-α, and G-CSF in burn patients during the observation period was 127.0 (0.3-10900.0) pg/mL, 42.5 (2.3-528.0) pg/mL, 15.3 (0.27-1380.0) pg/mL, 6.9 (2.07-86.2) pg/mL, and 197.0 (6.2-12000.0) pg/mL, respectively. These levels were significantly higher than in non-burn controls ($P < 0.05$): 1.4 (0-46.8) pg/mL, 3.9 (3.0-50.3) pg/mL, 1.9 (0.0-3.1) pg/mL, 3.3 (2.0-5.7) pg/mL, and 37.3 (20.9-60.0) pg/mL, respectively. Median concentrations of IL-6, IL-8, IL-10, G-CSF, and TNF-α at each time point increased with burn size, although peak levels and time to peak levels of these cytokines differed from patient to patient.

Conclusions: These findings indicate that IL-6, IL-8, IL-10, TNF-α, and G-CSF play important roles in inflammatory changes after burn injury; however, various factors, including burn size, influence the concentrations of these cytokines.

C-121

Unusual Hepatitis B Serological Results in a Veterans Affairs Medical Center [VAMC]

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Background: In July 2011 a dual positive (DP) result for HBsAg and anti-HBs was noted in our laboratory. In general positive results on these two tests are mutually exclusive with positive anti-HBs suggesting immune clearance of the HBV although occasional reports of unusual serological profiles have appeared [Viral Immunology 19:623 (2006)].

Methods: We proceeded to review all hepatitis results from January 1, 2006 to July 31, 2011. During that interval there were 13,964 assays performed for HBsAg and 13,731 assays performed for anti-Hbs. All assays were performed on the Centaur immunoassay analyzer (Siemens Diagnostics; Tarrytown, NY) according to manufacturer's directions. Assays for HBeAg, anti-HBe and HBV viral loads (HBV-VLs) were went to a reference laboratory (Laboratory Corporation of America; Burlington, NC).

Results: During the time period reviewed there were 19 DPs in 15 patients. As might be expected in a VAMC all patients were male with a median age of 59 YO (range: 47-92 YO). None of the patients had been vaccinated against HBV. Review of patient medical charts and lab data suggested that seven (46.7%) of the patients had chronic hepatitis B while four (26.7%) were in process of sero-conversion and immune clearance. One patient (6.7%) receiving hemodialysis (HD) had multiple non-reactive (NR) results for HBsAg and reactive (R) results for anti-HBs prior to the DP result and then three months later reverted to his usual pattern suggesting passive transfer via HD followed by immune clearance. Two patients were lost to follow-up after the initial DP and one patient had insufficient data to reach a conclusion as to disease status. One of the seroconverted patients although R for anti-HBs was NR for total anti-HBc and one of the chronic hepatitis B and two of the seroconverted patients were NR for both HBeAg and anti-HBe.

Conclusions: In conclusion DPs remained distinctly uncommon in our laboratory and included patients that were both chronically infected with HBV and those apparently in the process of sero-conversion. Occasional unusual results involving HBeAg, anti-HBe and anti-HBc were also noted raising the question of an aberrant immune response to HBV in these individuals.

C-124

Performance Evaluation of the IMMULITE 2000 Anti-CCP IgG Assay

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Background: Rheumatoid arthritis is a debilitating disease causing cartilage and joint destruction. Detection of anti-cyclic citrullinated peptide (anti-CCP) IgG antibodies is useful in the diagnosis of rheumatoid arthritis and in the prognosis of disease severity. Serologic testing for anti-CCP antibodies has recently been integrated into the diagnostic algorithm for detection of rheumatoid arthritis and is an important tool in the evaluation of patients suspected of having the disease.

Objective: In this study we evaluated the analytical and clinical performance of the newly developed automated chemiluminescent enzyme immunoassay* for measurement of CCP IgG levels in serum using the IMMULITE® 2000 instrument (Siemens Healthcare Diagnostics, Tarrytown, NY).

Materials and Methods: Precision was evaluated using a high control and two six-member precision panels (serum and lithium heparin) following CLSI guideline EP-5A. Relative and clinical sensitivity and specificity were determined using two-by-two tables comparing results to a predicate method (DIASTAT ELISA, Axis-Shield Diagnostics) using 457 serum samples: 200 normal donor samples and 257 rheumatoid factor-characterized patient samples. Additionally, endogenous interference was assessed using samples containing high concentrations of bilirubin (conjugated and unconjugated), human serum albumin, triglycerides, hemoglobin, and rheumatoid factor, while the cross-reactivity was evaluated using five antibody-reactive serum specimens from the following eight disease states: SLE, Sjögren's syndrome, scleroderma, polymyositis, osteoarthritis, autoimmune thyroiditis, Lyme disease, and infectious mononucleosis. All results were reported in U/mL. A result < 4.0 U/mL was considered nonreactive and a result ≥ 4.0 U/mL, reactive. The reportable range was <1.5 to 200 U/mL.

Results: Results were obtained in this study using IMMULITE 2000 Anti-CCP IgG reagents. Within-run and total precision (CV) were <11% and <19%, respectively. The method comparison between IMMULITE 2000 and DIASTAT assays obtained relative overall agreement of 94.1%, relative sensitivity of 93.5%, and relative specificity of 94.6%. Clinical overall agreement was 87.5%, clinical sensitivity was 78.2%, and clinical specificity was 99.5%. Endogenous interference testing obtained a mean percent difference across all validation lots of ≤10% of the control sample for all interfering substances. To assess cross-reactivity, the mean doses of all disease state samples tested on IMMULITE 2000 system were compared to results generated on the predicate DIASTAT assay and found to be in 100% agreement.

Conclusions: The IMMULITE 2000 Anti-CCP IgG Assay performs well both analytically and clinically.

*The IMMULITE 2000 Anti-CCP IgG Assay has not been cleared by the FDA and is not available in the U.S.

C-125

Voltage-Gated Potassium Channel Autoantibody Detection

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Background: Antibodies reactive with voltage-gated potassium channel receptors (VGKC) are associated with several autoimmune neurological disorders including peripheral nerve hyperexcitability (PNH), limbic encephalitis (LE), neuromyotonia and Morvan's Syndrome

Methods: The analytical performance of a commercial product [Voltage-Gated Potassium Channel (VGKC) Autoantibody RIA kit; Kronus, Catalog # KR5200] was evaluated by testing sera collected from 55 self-proclaimed healthy individuals and 8 VGKC antibody positive patient sera. Subsequently, serial sera and cerebrospinal fluid (CSF) specimens from a case study patient collected over a five year period and 440 consecutive patient specimens submitted to ARUP were investigated. Testing was performed according to manufacturer's instructions; in brief, 50 uL diluted patient specimen was incubated with ^{125}I-labeled digitonin-extracted VGKC receptor. Anti-VGKC antibody bound ^{125}I-VGKC was precipitated by incubation with anti-human IgG. Radioactivity in the washed pellet was measured by gamma counter and the picomole per liter (pmol/L) concentration of radio-labeled toxin calculated. Normality of result distribution was evaluated by Shapiro-Wilk W test and the interpretive threshold established by Wilcoxon Signed Rank analysis.

Results: Values were non-parametrically distributed (Shapiro Wilk $p > 0.01$) with an optimal threshold value of 30.7 pmol/L. This cut point provided a specificity of 91% and sensitivity of 75% in the 65 characterized sera. Sixty-two of 440 (14%) clinical specimens demonstrated pmol/L values above the threshold value. A slight gender bias (1.2 female-to-male ratio) was observed in VGKC antibody positive patient specimens; 33 females (median age 51, range 5-73 years), 27 males (median age 53, range 5-77 years) and two patients where gender was not provided. Median titer values (60 pmol/L and 80 pmol/L) with similar range of result values (34 - 651 pmol/L versus 32 - 691 pmol/L) were observed in sera with elevated VGKC antibody levels from female and male patients respectively. Sera from the limbic encephalitis patient shortly before onset of symptoms, when the patient was neurologically normal by clinical examination, brain magnetic resonance imaging, and formal neurological testing, demonstrated an extremely elevated VGKC antibody titer (660 pmol/L). Following initiation of methylprednisolone and intravenous immunoglobulin G antibody therapy, the VGKC titer rapidly fell to 52 pmol/L and eventually became undetectable (0.0 pmol/L).

Conclusions: Although incidence and prevalence of VGKC antibody associated disorders cannot be determined from this limited data set, especially since limited clinical information is available for most patients; nevertheless, our data suggests that antibodies reactive with VGKCs may be more common than previously thought. VGKC antibody can be detected before onset of symptomatic LE and fall to undetectable levels following treatment as demonstrated in our case study patient. Radioimmunoassay detection of VGKC antibody can provide useful diagnostic information in spite of known assay limitations. These include restriction of ^{125}I-dendrotoxin labeling of VGKC receptors subtypes (K*v*1.1, K*v* 1.2 and K*v* 1.6) and the inability of digitonin to separate two recently recognized target components of the VGKC receptor complex (leucine-rich glioma inactivated protein 1 and contactin-associated protein 2).

C-128

Functional polymorphisms in TBX21 and HLX are associated with development and prognosis of Graves' disease

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Background: We have previously reported that the severity of Hashimoto's disease (HD) is associated with high producibility of interferon-gamma and with low producibilities of interleukin-4 and transforming growth factor-beta. These suggest the important role of T helper 1 (Th1) development in the prognosis of HD. It has been indicated that TBX21 and HLX are transcription factors related to the differentiation of Th1 cells, whereas GATA-3 is master transcription factor of T helper 2 (Th2) cells. The objective of this study was to identify associations between the prognosis of autoimmune thyroid diseases and the functional polymorphisms of genes encoding TBX21, HLX, and GATA-3.

Methods: We genotyped -1514T/C (rs17250932) and -1993T/C (rs4794067) polymorphisms of *TBX21*, -742C/G polymorphism (rs2184658) of *HLX*, and -1420G/A polymorphism (rs1269486) of *GATA3* in genomic DNA samples from Japanese patients; 51 patients with severe HD and 39 patients with mild HD carefully selected from 205 registered HD patients who were positive for antithyroid autoantibody. Patients with HD who developed moderate to severe hypothyroidism before the age of 50 and who were treated daily with thyroxine were defined as severe HD. Untreated, euthyroid patients with HD, older than 50 years of age, were defined as mild HD. We also genotyped these polymorphisms in 66 patients with intractable Graves' disease (GD) and 47 patients with GD in remission cafefully selected from 251 registered GD patients who had clinical histories of thyrotoxicosis with elevated levels of antithyrotropin receptor antibody. Euthyroid patients with GD who had been treated with methimazole for at least 5 years and were still positive for TRAb were defined as intractable GD. Patients with GD who maintained a euthyroid state and were negative for TRAb for more than 2 years without medication were defined as GD in remission. 79 control subjects were also genotyped. The restriction fragment length polymorphism method was used for genotyping.

Results: The T alleles of the *TBX21* -1514T/C and -1993T/C polymorphisms were more frequent in patients with intractable GD than in those with GD in remission. Among individuals with the *TBX21* -1993TT genotype, the G allele of *HLX* -742C/G polymorphism, which correlates with low HLX expression, was more frequent in patients with intractable GD than in those with GD in remission.

Conclusions: Functional polymorphisms in *TBX21* are associated with development of autoimmune thyroid diseases and prognosis of GD, and a functional polymorphism in *HLX* in combination with the *TBX21* polymorphism is also associated with the prognosis of GD.

C-129

Smoking dose and oxidative stress interact and are involved in the development of rheumatoid arthritis.

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Background: Smoking has shown to increase the risk of developing rheumatoid arthritis (RA), but the mechanism remains unknown. Oxidative stress can be measured by lipid or protein damage biomarkers. Some studies have established association between oxidative stress and RA. The objective was to investigate the relationship between smoking dose, oxidative stress and development of RA.

Methods: A case-control study was performed. Sixty five patients with early RA (ACR, 1987 criteria) without treatment, and 65 controls matched by age, gender and present smoking habit were included. Demographic and disease related variables were registered. Pack/years index, lipid-hidroperoxides (LOOH), carbonyl-protein (CP) and malonyldialdehyde (MDA) were obtained. The individuals were classified as positive or negative for each variables using median as cut-off. In order to study the interaction, we calculate RERI (Relative excess risk due to interaction), AP (the attributable proportion due to interaction) and S (synergy index) from logistic regression using dummy variables.

Results: Rheumatoid factor (RF) [35.0 (14.0-201.0) vs 12.0 (10.0-14.0) UI/mL], anti-CCP [22.2 (3.0-198.9) vs 2.8 (1.6-5.6) U/mL], shared epitope (SE) [34/65 vs 20/65], C reactive protein (CRP) [9.2 (3.4-25.4) vs 1.0 (0.4-4.6) mg/L], pack/years index [3.0 (0.0-26.0) vs 0.0 (0.0-9.0)], LOOH [36.2 (26.7-51.2) vs 16.7 (8.4-29.2) µM] and CP [112.2 (59.1-147.0) vs 46.0 (25.1-68.2) µM] plasma levels were significantly higher in patients, while MDA [8.0 (6.7-8.6) vs 9.1 (8.5-10.1) µM] was decreased (median, p25-p75). The OR showed that LOOH+ or CP+ confer a risk 10 times to have the disease, while a pack/years>20 showed OR 8.5 times. The interaction of different oxidative stress markers and smoking showed a relative risk additional to the sum of the risks of each variable separately (Figure 1).

Conclusions:

Oxidative stress and smoking dose act synergistically predicting the risk of RA. Understanding this interaction may allow the development of novel therapeutic strategies for RA.

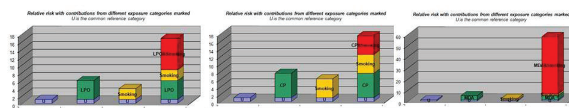

Figure 1. Interaction of smoking and oxidative damage markers. Relative risk for LOOH, CP and MDA.

C-130

Diagnostic utility of lipidhydroperoxide in recent-onset rheumatoid arthritis.

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Background: Oxidative stress is an imbalance between oxidant/antioxidant mechanisms, and can be measured by biomarkers that reflect lipid (LOOH), protein (CP) or DNA damage. Some studies have established association between rheumatoid arthritis (RA) and oxidative stress, suggesting that precedes the onset of RA and may be a factor influencing the appearance of it. The objective of this study was to determine the diagnostic utility of oxidative stress markers in RA naïve patients.

Methods: Sixty five RA patients (ACR, 1987 criteria) without DMARDs or steroids treatment and 65 healthy controls were included. Whole blood was obtained, centrifuged at 0º C to obtain fresh plasma and an antioxidant cocktail was added previous stored at - 80 °C until analysis. LOOH were measured by chemical oxidation of ferrous to ferric and measurement of absorbance at 500 nm. CP concentration was calculated by selective reaction with 2,4 dinitrophenylhydrazine, precipitation in Cl_3CCOOH and measurement of absorbance at 370 nm. Statistical analysis was performed using the STATA 10.0.

Results: Sixty five RA patients and 65 healthy controls were included. Regarding demographic and disease characteristics, there were differences between both groups on age, variables associated to RA (RF, CCP, CRP, shared epitope), antioxidant (c-HDL), pack/years index and oxidative damage markers (CP, LOOH) as higher in RA patients. To evaluate the diagnostic usefulness in total population and in nonsmokers patients, logistic regression models were constructed with classical parameters (RF, CCP and CRP) and two markers of oxidative damage were added (LOOH and CP). Comparing the models found a significant increase of AUC by adding LOOH or both at once, while adding CP alone did not provide a significant increase (Table).

Conclusions: LOOH may be a good marker of early stage disease combined with RF, CCP and CRP. Its diagnostic utility is remarkable in non-smokers patients.

Diagnostic performance of logistic models for diagnostic of RA in total and nonsmokers population.

GROUP	Parameter or model	Cut-off	Sen (%)	Spe (%)	LH+	LH-	AUC (95%)	P
TOTAL (65-65)	LOOH	22.5 mM	81.5	64.6	2.30	0.29	0.758 (0.672-0.844)	
	CP	52.8 mM	83.1	63.1	2.25	0.27	0.791 (0.714-0.869)	
	RF+CCP+CRP		78.5	92.3	10.20	0.23	0.908 (0.855-0.962)	reference
	RF+CCP+CRP+LOOH		86.2	93.8	14.01	0.15	0.951 (0.917-0.985)	0.035
	RF+CCP+CRP+CP		81.5	93.8	13.26	0.20	0.934 (0.893-0.974)	0.082
	RF+CCP+CRP+LOOH+CP		86.2	93.8	14.01	0.15	0.959 (0.928-0.990)	0.020
Nonsmokers (33-65)	RF+CCP+CRP		69.8	96.9	23.19	0.31	0.878 (0.803 -0.954	reference
	RF+CCP+CRP+LOOH		76.7	93.9	12.57	0.25	0.934 (0.885-0.982)	0.05
	RF+CCP+CRP+CP		74.4	98.5	49.60	0.26	0.911 (0.856-0.967)	0.10
	RF+CCP+CRP+LOOH+CP		79.1	93.8	12.75	0.22	0.948 (0.906-0.990)	0.02

C-132

Importance of IL28B gene polymorphisms in HIV-HCV-coinfected patients treated with pegylated interferon and ribavirin

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Background and objectives: Chronic hepatitis C virus infection is still a major cause for developing cirrhosis and hepatocellular carcinoma. The standard of care (SOC) treatment consists of (pegylated) interferon-alfa and ribavirin. It is a long-term treatment, without being free from important side effects. It may have extremely slight chances of success, depending on some factors, such as virus genotype. The impact of genetic variation near the interleukin 28B (IL28B) gene for response in HCV infected patients was shown recently. The SNP known as rs12979860 located 3 thousand bases before gene IL28B, a change of base instead of a cytosine we can find a thymine, the C/C genotype in that position of the genome has shown an association with an increased spontaneous virus clearance chance and response to treatment when compared to C/T and T/T individuals.

About one-third of people infected with human immunodeficiency virus-1 (HIV-1) are coinfected with hepatitis C virus (HCV) because of shared transmission routes. Studies report that HIV-1 complicates hepatitis C infection by increasing HCV viral load and reducing spontaneous clearance. The aim of this study was to evaluate the association between the SNP rs12979860 of the IL28B gene and the on-treatment virological responses in 42 HIV-HCV-coinfected individuals.

Patients and Method: We selected 42 HIV/HCV-coinfected individuals who had beginning a course of peginterferon-ribavirin therapy, and determine the association of IL28B SNPs with virological responses. It was assessed in univariate and multivariate analyses.

HCV RNA viral load was measured by Cobas Amplicor Monitor 2.0, real-time PCR assay Cobas TaqMan HCV test (Roche Molecular Systems) which has a lower limit of detection of 10 IU/ml.

Blood was collected into EDTA tubes. Genomic DNA was extracted using the Pure MagNa Roche Compact according to the manufacturer's instructions. IL28B variants rs12979860C>T were diagnosed from whole blood samples using the Roche polymerase chain reaction (PCR) assay (Kit LightMix® IL28B,Lightcycler 2.0 Roche). The polymorphisms results were reported as C/C, C/T, and T/T.

Results: Rate of sustained virologic response (SVR) and non-response virologic in the study population was 26,19% and 35,71% respectively. The SVR rates for the IL28B CC, CT, and TT polymorphisms were 81,81%, 18,18%, and 0%. Rate of non-response virologic for the IL28B CC, CT, and TT polymorphisms were 6,66%, 40%, and 53,33%, respectively.

Conclusions: Parameters for the prediction of sustained virologic response in patients with chronic hepatitis C before initiation of antiviral therapy are important in order to be able to estimate the potential for treatment success. IL28B CC polymorphism is a strong predictor of virological response to therapy in HIV/HCV-coinfected patients. 81,81% of all patients with SVR had the IL28B CC polymorphism and 93,33% of patients with virologic non-response had CT or TT polymorphism.

Our study demonstrates an important role for IL28B polymorphism in predicting on-treatment virological responses to pegIFNa-RBV therapy in HIV/HCVcoinfected individuals.

Knowledge at baseline of the IL28B polymorphism may assist in treatment decisions, both at the time of deciding who should be treated and during treatment.

C-133

Epstein-Barr Test Panel Evaluation on the DiaSorin Liaison

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Introduction: Epstein Barr Virus (EBV) is a member of the herpes virus family. In the United States, 95% of people between the ages of 35 and 40 have been infected. Although the symptoms of IM resolve with 6 to 8 weeks, the virus remains in a dormant state and periodically, is reactivated

Methods: EBV IgM, & IgG, and EBNA were determined qualitatively on the DiaSorin Liaison utilizing indirect chemiluminscence. This is a retrospective study looking at EBV Panel results for 1060 patients collected during 2011.

Results:

EBV Panel Results	n (%)
EBV IgM & G and EBNA negative	530 (50)
EBV IgM positive, EBV IgG and EBNA negative	9 (0.8)
EBV IgM & G positive and EBNA negative	55 (5.2)
EBV IgM & G and EBNA positive	7 (0.7)
EBV IgM negative, IgG positive, EBNA negative	54 (5.1)
EBV IgM negative, IgG and EBNA positive	392 (37)
EBV IgM & G negative and EBNA positive	13 (1.2)

13 patients with EBNA positive only results did not have follow up EBV testing, however, 5 of the 13 patients had equivocal results for EBV IgG indicating a possible seroconversion to EBV IgG.

Conclusions: EBV immunological panels assist in discerning acute, convalescent, and past infections. 95% of the population have been exposed or had a past infection with EBV, this study shows 530 (50%) of the patients were negative for all three markers. 392 or 37% of the patient population tested demonstrated a convalescent pattern with EBV IgM being negative and EBV IgG and EBNA being positive. 13 (1.2%) of the patient population tested positive only for EBNA with 5 patients testing equivocal for EBV IgG. Based on clinical symptoms, the DiaSorin EBNA and EBV IgG assays may assist in determining late convalescent infection from a past infection.

C-134

A novel assay for the rapid and sensitive detection of precipitating SSA autoantibodies exhibits improved diagnostic specificity

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Background: The detection of autoantibodies to SSA antigen is considered critical for the diagnosis of primary and secondary Sjoegren's syndrome as well as for a number of other rheumatological disorders including subacute cutaneous lupus and neonatal lupus erythematosus/congenital heart block. While current methods (such as ELISA) for the detection of SSA antibodies are highly sensitive, they also exhibit a significant "grey zone" leading to a high incidence of "equivocal" results, many of which constitute genuine false positives that lead to significant diagnostic confusion amongst requesting clinicians. Although the detection of precipitating autoantibodies to SSA is considered by many to have superior diagnostic specificity, traditional methods (such as immunodiffusion and immunoelectrophoresis) are considered to be cumbersome, time consuming and lacking sensitivity. We describe here CIEDia, a rapid, sensitive assay for the detection of precipitating SSA autoantibodies and compare the sensitivity and specificity of this assay with a commercial ELISA assay.

Methods: Fluorescent SSA antigen was synthesised using DyLight 488 or AlexaFluor 488 reactive derivatives and commercially available purified native SSA antigen. In the CIEDia assay precipitating SSA autoantibodies are detected by immunoelectrophoresis on thin agarose gels where the precipitin formed by the interaction of patient autoantibodies with fluorescent SSA antigen can be viewed in real time using a bench top blue light transilluminator with an amber filter. Using this procedure precipitating SSA autoantibodies could be detected in less than 30 minutes with a sensitivity approaching that of ELISA.

Results: To investigate the diagnostic specificity of the new assay SSA autoantibodies in 228 patient samples that were found to be ANA positive as determined by Hep2-IF during routine laboratory testing were further analysed using a commercial ELISA assay (Phadia) and the CIEDia assay. Using the ELISA assay the results revealed 154 samples to be negative, 70 to be "equivocally" positive and 4 to be "unequivocally" positive for SSA autoantibodies. The CIEDia assay also found all of the ELISA-negative samples to be negative for precipitating SSA antibodies whereas all of the ELISA "unequivocally" positive samples were also found to be positive in the CIEDia assay. In contrast, of the 70 patient samples that gave an "equivocally" positive result in ELISA, only 38 were found to have precipitating SSA antibodies as determined by CIEDia. A clinical immunologist blinded to the serological results reviewed patient records for all 228 samples to determine clinical presentation of Sjoegren's syndrome and/or other anti-SSA related diseases using internationally accepted diagnostic criteria. The results indicated that the CIEDia assay for precipitating SSA antibodies has a diagnostic specificity of 91.6%, significantly higher than that of the commercial ELISA assay which was found to be 75.8%.

Conclusions: The CIEDia assay is a rapid, easy to perform method for the detection of precipitating autoantibodies. While the sensitivity of the method for anti-SSA antibodies was found to approach that of ELISA, the diagnostic specificity was found

to be superior. It is envisaged that this novel assay could play a useful role in routine clinical laboratories to further elucidate samples yielding "equivocal" results with alternative autoantibody assays.

C-135

Development of a lateral flow immunoassay for detection of β-adrenergic agonist Ractopamine residues

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Background: Ractopamine (Rac), a β-adrenoceptor agonist, has been used for the treatment of congestive heart failure. At the doses exceeding normally therapeutic usage, Rac would have a strong effect of promoting animal growth with increased accretion of skeletal muscle mass and decreased accretion of body fat. However, Rac is reported to induce unintended side effects on humans, such as increased heart rate, muscular tremors, headache, fever, and chills. Thus, its use has been prohibited in the animal feed over 150 countries, including EU, China and Taiwan.

Methods: BSA-Rac (Clongene, Hangzhou, CHN) and goat anti-mouse IgG (BOLAIRUI, Beijing, CHN) were coated onto the nitrocellulose membrane (Millipore, Billerica, MA) as the test and control lines respectively. Monoclonal anti-Rac antibody (Fapon, Shenzhen, CHN) was labeled with 0.03% colloidal gold solution, and immobilized to a glass fiber (PALL, Port Washington, NY) to make a conjugate pad. A test strip was assembled on a plastic backing plate with absorbent pad (PALL, Port Washington, NY), polyester backed nitrocellulose membrane, detector conjugate pad, and sample pad (PALL, Port Washington, NY). Strips were sealed in an aluminum foil bag in the presence of desiccant gel and stored at 4°C. Rac was diluted in agonist-free swine urine at 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ppb (ng/ml) , other β-adrenergic agonists including Clenbuterol, Salbuterol, Bromobuterol, Cimbuterol, Terbutaline, Epinephine, Norepinephrine and Isoprenaline at 100, 200, 400, 800, 1600 and 3200 ppb. LC-MS verified 216 positive and 178 negative swine urine samples were involved in evaluating the positive and negative coincidence rates.

Results: A semi-quantitative Rac rapid test strip with high sensitivity, high specificity, high stability and high coincidence rate was developed. A clear pink band appears at the test line when the swine urine containing Rac residues below 2 ppb is applied, which shows a negative result. The relative optical density decreases as the Rac concentration in the standard samples increase from 0 to 2 ppb. On the contrary, no pink band could be visualized by naked eyes at the test line when the swine urine contains Rac residues beyond 2 ppb, which shows a positive result. Cross reaction could be detected only if the concentrations of the other β-adrenergic agonists are all greater than 1600 ppb. Strips stored at both 4°C and 30°C were tested and are fairly stable for up to 18 months. The positive and negative coincidence rates were calculated as 100% (216/216) and 99.4% (177/178), respectively according to the outcome data analysis.

Conclusions: We reported in this study that an immunochromatographic test strip detecting β-adrenoceptor agonist Rac was developed successfully. The results demonstrated that the test strip works for Rac residues detection at ppb level in swine urine. Analysis of β-agonists by HPLC, GC-MS or LC-MS requires extensive sample clean-up and professional training. The test strip is more applicable due to its rapid character which makes it a better choice to analyze Rac residues under a testing time limited situation or experimental equipments lacked condition, such as doing a series of Rac testing in a small slaughter house.

C-136

Metalloproteinase-3 Predicts The Response To Anti-Tnfα Iv Patients With Ankylosing Spondylitis.

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Background: To evaluate the disease activity and anti-TNFα response in patients with ankylosing spondylitis (AS) is necessary better objectives measures. In the last years, several biomarkers have emerged, and metalloproteinase-3 (MMP-3) seems to be the most promising although published data are not conclusive. Moreover, there is no evidence that anti-TNFα therapy inhibit radiological progression in the disease. In this sense, it is not clear if inflammation and bone proliferation are or not related processes. The study of biomarkers implicated in these processes such as dickkopf-1 (DKK-1) and sclerostin will help us to understand it.

Methods: From November 2010 to July 2011, patients with AS (New York criteria) who initiated anti-TNFα therapy in a University hospital were included. Socio-demographic and disease characteristics variables were recorded at baseline. Before to initiate the therapy and after 3 months, disease activity was measured, and blood samples for determination of serum levels of MMP-3, DKK-1 and sclerostin by enzyme inmunoassay (ELISA) were collected. Treatment response was defined using BASDAI50 and ASDAS response. The Wilcoxon test was used for evaluations of changes under treatment and the Mann-Whitney U test for between-group comparisons. Receiver operating characteristic analysis to determine the accuracy to predict response and Correlation testing were performed.

Results: A total of 20 AS patients were included, 86% men, 42.4 (31.0-49.0) years old, with disease duration 6.8 (3.0-10.0) years, 83% HLA-B27 positive, from which 80% and 20% received adalimumab and etanercept, respectively. After 3 months of anti-TNFα, all disease activity parameters improved significantly (BASDAI 6.5 vs 3.6; p=0.001, ASDAS 3.6 vs 2.1; p<0.001, CRP 13.5 vs 4.4 mg/L; p=0.001). Serum levels of MMP-3 decreased (100.0 vs 68.1 ng/mL; p<0.05) while levels of DKK-1 and sclerostin did not change significantly (7.07 vs 7.65 pM; p=0.5 and 21.7 vs 22.7 pM, p=0.5, respectively). A significant correlation was only found between baseline levels of MMP-3 and pain VAS. Moreover, we compared biomarker changes based on treatment response, and observed that MMP-3 decreased only in patients who responded to anti-TNFα therapy (122.2 vs 64.1 ng/mL; p=0.01). Baseline serum levels of MMP-3 were significantly higher in patients who had a good response to anti-TNFα vs those who did not respond (122.9 vs 58.9 ng/mL; p<0.05). The area under the curve for MMP-3 to predict BASDAI50 and ASDAS response was 0.73 and 0.78, respectively. The best cut-off was established for levels higher than 59.5 ng/mL, with sensibility of 79-85% and specificity of 50-57%.

Conclusions: Serum levels of MMP-3 decreased after anti-TNFα therapy and were useful to predict response to this therapy in patients with AS. There was no correlation between serum levels of MMP-3, DKK-1 or sclerostin and disease activity parameters.

C-137

Histamine Measurement in Urine using Selective Reaction Monitoring Ion-Pairing LC-MS/MS

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Background: Histamine is a neurotransmitter produced and stored in cytoplasmic granules in mast cells and basophils, and is important in triggering an inflammatory response after exposure to an allergen in immediate hypersensitivity. Elevated levels of histamine can be found following an allergen challenge or in mast-cell proliferative disorders, such as mastocytosis. Histamine is quickly metabolized with a half life of approximately 30 minutes; thus, following a transient allergen challenge, serum histamine levels peak and fall rapidly, resulting in a narrow time window for diagnostic blood/serum histamine testing. Measurement of histamine or its metabolites in urine extends this diagnostic window, making it a useful adjunct in the diagnosis of immediate hypersensitivity and other disorders of mast cell activation. Unfortunately, current urine histamine assays are non-standardized immunoassays that have been plagued by interferences, calibration issues and quality problems.

Objective: To develop and validate a mass spec method for determination of urine histamine levels.

Methods: Urine spiked with stable isotope labeled internal standard (IS) d4-histamine was acidified using 2% H_3PO_4 and applied to a conditioned Bond Elut Plexa PCX polymeric cation exchange solid phase extraction (SPE) cartridge (Agilent Technologies, Santa Clara CA) using a positive pressure manifold. The cartridge was washed with 2% formic acid followed by 50:50 methanol:acetonitrile. Histamine and its IS were eluted with 5% ammonia in methanol:acetonitrile. The eluant was dried down at 45ºC under nitrogen, reconstituted with mobile phase, and subjected to isocratic ion-pairing liquid chromatography on an Agilent Eclipse XDB-C18 analytical column, followed by quantification on an AB Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) in electrospray mode, using selective reaction monitoring (transitions for histamine and IS were *m/z* 112/95, 112/81 and 116/99, 116/85, respectively).

We then determined the critical limit (analyte indistinguishable from blank), limits of detection (analyte detected with >95% confidence) and of quantification (minimal concentration with an inter-assay CV <20%), intra- and inter-assay imprecision across the calibration range, spike recovery and dilution linearity.

Results: No significant interferences were observed. The limit of quantitation was 2.5 ng/mL with a limit of detection at 2.1 ng/mL. The critical limit was 0.4 ng/mL. Intra-assay imprecision (CV) was found to be 3.2%-5.4% for three different histamine levels (2.7-112 ng/mL). Inter-assay imprecision was found to be 8.9%-12.3% for three

different histamine levels (7.7-103 ng/mL). Dilution linearity was shown to be linear up to 279 ng/mL. Spiked recoveries ranged from 80.0-119.0% (average of 103.1%).

Conclusions: We have developed an accurate, simple and rapid method for quantitation of histamine in urine. The combination of SPE, ion-pairing chromatography and tandem mass spectrometry minimizes the risk of analytical interferences. This assay should improve the diagnosis of immediate hypersensitivity or mastocytosis.

C-139

Antibodies to celiac neo-epitopes in comparison to deamidated gliadin antibodies in pediatric and adults celiac disease and dermatitis herpetiformis patients.

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Aim: To compare the performance of two DGP assays with celiac neo-epitope assays, a crosslink of deamidated gliadin peptides with tissue transglutaminase in pediatric and adult population.

Methods: 70 pediatric celiac suspects, 78 adult DH patients and 100 pediatric controls, 50 adult control samples were analyzed on the AESKULISA DGP-IgA, IgG and Check, the AESKULISA tTg New Generation IgA, IgG and CeliCheck, a AESKULISA tTg IgA and INOVA Glia-II-IgA, IgG and Celiac DGP Screen. The samples consisted of 60% female and 40% male; 52% were 1 to 16 years, 33% were between 16 and 46, 15% were above 46 years. A 2x2 comparison as well as a ROC analysis were performed on the different assays.

Results: The assays showed a good correlation to detect celiac disease as well as dermatitis herpetiformis. The diagnostic sensitivity/specificity using the manufacturers cut-off was best with the AESKU tTg Neo-Epitope-IgA assay with 96.6%/100%, over INOVA Celiac DGP Screen 91.7%/98.0%, AESKU DGP-G 89.0%/98.1%, INOVA Glia-II-G 86.9%/98.7%, AESKU DGP Check 84.8%/97.4%, INOVA Glia-II-A 78.6%/99.3%, AESKU DGP-A 76.6%/99.4% and AESKU tTg Neo-Epitope IgG 71.0%/100%. The AESKU tTg-only assay showed results of 91.6%/100%.

Conclusions: All antigens evaluated showed a good performance in the tested population and especially the celiac neo-epitope IgA test showed the highest sensitivity together with a very good specificity. It thus can be used as the only assay to test for gluten sensitive enterophathy.

C-140

MMP-3 Is A Marker For Disease Activity In Rheumatoid Arthritis

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Background: New markers for the monitoring of disease activity and prognosis of progression are urgently needed to optimize individual drug therapy and prevent joint destruction in Rheumatoid Arthritis (RA). Serum MMP-3 has been proposed to be such a marker.

Objective: To investigate the correlation of MMP-3 serum levels in Rheumatoid Arthritis patients with their individual disease activity.

Methods: 64 sera of adult patients (44 female, 20 male) with established RA and 97 control sera of adult healthy donors (48 female, 49 male) were analyzed on the AESKULISA DF MMP-3. Based on clinical data the 64 RA patients were classified as “Active” or “Inactive” according to their individual disease activity. Both RA groups were correlated to their individual MMP-3 serum concentrations (termed as “elevated” and “non-elevated” MMP-3 levels). The normal range of MMP-3 serum concentration was determined by calculating the 95percentile of the measured MMP-3 concentrations for each gender.

Results: 35 of the 64 RA patients were classified as “Active” and 29 as “Inactive” in respect to their individual disease activity. Normal MMP-3 serum concentrations were determined as up to 60 and up to 120 ng/ml for females and males, respectively. 89% (31/35) of “Active” patients had elevated and only 11% (4/35) had normal or borderline MMP-3 levels. 90% (26/29) of “Inactive” patients showed normal and only 10% (3/29) had elevated MMP-3 levels.

Conclusions: MMP-3 serum concentration shows high correlation with RA disease activity. Why this work is novel: AESKULISA DF MMP-3 is the first certified IVD for measurement of MMP-3 and employs two different monoclonal antibodies for maximum specificity.

C-141

Biomarker for spondyloarthritis: Autoantibodies against Class II-associated invariant chain peptide (CLIP

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Background: Spondyloarthritis (SpA) is relatively common inflammatory disorder with a frequency of 1-2% in the European population. Establishing the diagnosis however may be difficult, since abnormalities in conventional X-ray develop with a latency of several years and so far only HLA-B27 has been established as a laboratory marker of the disorders.

The goal of our study therefore was to identify new autoantibodies as markers of SpA.

Methods: As a screening procedure, we used protein array technology for detection of possible new autoantigens in ankylosing spondylitis (AS). Sera of patients with AS without peripheral manifestation (n = 5) and other diseases (n = 45), were studied. In the second step, the results of the protein array were confirmed by ELISA using commercially available recombinant antigens (Abnova, Taiwan and Abcam, UK) and new synthetic derived peptides (Biomatik, USA). Considering the sensitivity and specificity, we developed an ELISA with synthetic peptide CLIP in cooperation with AESKU.Diagnostics (Wendelsheim, Germany). The sera for the ELISA were obtained from 198 axial and peripheral SpA patients visiting the rheumatological outpatients and inpatients clinics of Medical University Hannover and Medical University of Vienna and Department of Gastroenterology, Infectiology and Rheumatology of Charité Berlin, 80 rheumatoid arthritis (RA), 40 systemic lupus erythematosus patients (SLE) and 100 blood donors. All donors provided informed consent for the study which was approved by our local ethical committee (project number 4928).

We correlated the presence of autoantibodies with the disease duration, HLA-B27, BASDAI and therapy in a subset of the spondylarthritis patients.

Results: Using the protein array, we detected IgG antibodies against CD74 in 4/5 SpA sera, but only in 1/45 controls. After we tested different different CD74 proteins as antigens in ELISAs, we chosed a peptide which includes the Class II-associated invariant chain peptide (CLIP) within the CD74 protein. Of the SpA patients with disease onset less than 1/2 year ago 55/57 (96%) were positive for IgG autoantibodies. 141/196 (71%) of all SpA patients showed positivity for IgG autoantibodies against CLIP. In the further control groups, the prevalence of IgG autoantibodies against CLIP was 21/80 (26%) in RA, 5/40 (12.5%) in SLE and 0/100 (0%) in blood donors.

Conclusions: Considering their specifity and sensitivity, antibodies against CLIP will be an useful addition to our diagnostic tools for SpA in the future.

Disclosures: Patent application 10172861.6-2404

C-143

Relationship between peripheral TCRαβ+CD4-CD8-T cells and FAS-mediated apoptosis in patients with Chinese rheumatoid arthritis

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Background: TCRαβ+CD4-CD8-T cells are defined as a kind of novel Treg subsets. Recent advances in the study of TCRαβ+CD4-CD8-T cells have provided new insights into pathogenic mechanisms of autoimmune diseases. FAS, as a receptor, is deemed to be involved in the balance of T cell subsets. The increased expression of FAS receptor on the surface of T cells may lead to high sensitivity of FAS-mediated apoptosis. The main aims of this study are as flowing: (1) to investigate the frequencies of peripheral distinct T cell subsets, including TCRαβ+cells, CD4-CD8-cells and TCRαβ+CD4-CD8-T cells; (2) to detect the expression of FAS receptor on those cell subsets, (3) to explore the relationships between peripheral percentages of TCRαβ+CD4-CD8-T cells and its expression of FAS receptor with disease activity in patients with rheumatoid arthritis (RA).

Methods: 24 RA patients and 24 normal controls were included. Flow cytometry was used to detect the percentages of T cell subsets and the expression of FAS receptor. The relationships between the percentages of T cell subsets and the expression of FAS receptor with RF, WBC, CRP and ESR were analyzed respectively by spearman

relativity analysis.

Results: The percentages of peripheral TCRαβ+cells and CD4-CD8-cells from RA patients were similar to normal controls (P>0.05). Strikingly higher levels of TCRαβ+CD4-CD8-T cells in the peripheral blood of RA patients were detected compared with normal controls (0.82±0.38 vs 0.57±0.26,P=0.013). The expression of FAS receptor on TCRαβ+CD4-CD8-T cells were significantly increased in RA patients as compared to normal controls (1.23±0.69 vs 0.80±0.45,P=0.016). Positively relationship was found between TCRαβ+CD4-CD8-T cells and TCRαβ+CD4-CD8-FAS+cells(r=0.809, P=0.000). There is no significantly relationships between the percentages of TCRαβ+CD4-CD8-T cells, as well as the level of FAS receptor expression, with RF,WBC,CRP and ESR, respectively. There is also no significantly associations between TCRαβ+ cells, as well as CD4-CD8-cells, with RF,WBC,CRP and ESR, respectively.

Conclusions: The significantly upregulated expression of FAS receptor on the surface of TCRαβ+CD4-CD8-T cells may increase the sensitivity to FAS-mediated apoptosis. It may lead to the imbalance of immune respective T cells in RA patients. There are no significant relationships between the percentages of TCRαβ+CD4-CD8-T cells and FAS receptor with the indicators of disease activity, although the percentages of them are markedly increased in the Chinese RA patients. The preliminary results suggest that TCRαβ+CD4-CD8-T cells do not appear to play a critical role in the pathogenic of RA patients.

C-144

Development of a rapid and high-performance chemiluminescence immunoassay based on magnetic particles for PCT in human serum

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Objective: Procalcitonin (PCT) is a new and the most helpful laboratory marker of bacterial infection. Compared to Creactive protein (CRP), PCT is more specific to recognize bacterial infection in different patient groups and conditions. We describe a rapid and high-performance magnetic particle-based chemiluminescence immunoassay (CLIA) technique for assaying PCT in serum.

Methods: Fluorescein isothiocyanate (FITC) and N-(aminobutyl)-N-(ethylisoluminol) (ABEI) were used to label two different monoclonal antibodies of PCT. Both of the labeled antibodies combined with PCT in serum to form a sandwiched immunoreaction. The magnetic particles (MPs) that were coated with anti-FITC antibody served as both the solid phase and the separator. After adding the substrate solution, the relative light unit (RLU) of ABEI was measured and was found to be directly proportional to the concentration of PCT in serum. The relevant variables involved in the CLIA signals were optimized and the parameters of the proposed method were evaluated.

Results: The method was linear to 100 ng/mL PCT with a detection limit of 0.13 ng/mL. The intra-assay imprecision results [mean (CV)] were 0.45 ng/mL (6.81%), 5.68 ng/mL (5.53%) and [20.56 ng/mL (4.24%)]; the total imprecision results were [0.48 ng/mL (7.26%)], [5.49 ng/mL (6.78%)] and [20.61 ng/mL (5.29%)]. The average recoveries were between 90% and 110%. The relationship between the concentration of diluted PCT and the dilution ratios gave a good linear correlation coefficient of 0.9865. The cross-reactivity with calcitonin (CT) was less than 1%.A correlation analysis against an established automated assay generated a slope of 0.9929, an intercept of 0.0039 ng/mL and a correlation coefficient of 0.9905 (0.25~40 ng/mL). An entire test requires less than 30 min, including 20 min incubation time and 5 min for washing and measuring.

Conclusions: The proposed method demonstrates an acceptable performance for quantifying serum PCT and is suitable for the fabrication of a commercial kit with application in the automated chemiluminescence analyzer.

C-145

Complement-dependent cytotoxicity assay using resazurin-based viability test in HLA crossmatching

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Background: Detection of anti-HLA antibodies is important step in organ transplantation because those are associated with an increased risk of rejection. Conventional complement-dependent cytotoxicity (CDC) assay for anti-HLA antibody is insensitive, time-consuming, and labor-intensive and its interpretation is highly subjective. On the other hand, flowcytometry (FCM) detects not only cytotoxic anti-HLA antibodies but also all kinds of lymphocyte binding antibodies. Therefore, to overcome these conventional anti-HLA antibody assay's limitations and monitor anti-HLA antibodies sensitively and quantitatively, we invented new method using resazurin-based viability assay and realtime fluorescence detection system and evaluate its performance and clinical usefulness.

Methods: We isolated the pure population of lymphocytes from whole blood using magnetic beads and antibody cocktails. After complement-dependent cytotoxic reaction and addition of resazurin, the fluorescences of positive, negative control and test reactions were detected using realtime thermocycler. Inverted ratio was calculated to estimate cytotoxicity comparing with negative control. We evaluated its correlation with % PRA and donor specific parameters (maximum chi-square and maximum OD).

Results: In resazurin-based cytotoxicity assay using realtime fluorescence detection, inverted ratio was quantitatively correlated with the quantity of serially diluted positive control (anti-HLA-AB). Inverted ratio was not significantly correlated with % PRA, maximum chi-square but correlated with maximum OD (rho=0.327, p=0.0268).

Conclusions: We invented new method using resazurin-based viability assay and realtime fluorescence detection system and this method could detect complement-dependent cytotoxic antibodies more sensitively and quantitatively. Clinical usefulness of this new method should be studied with comparison of CDC, FCM and solid phase immunoassay.

C-146

A Novel Multiplex Diagnostic Method for Human Immunodeficiency Virus (HIV) 1, Hepatitis C Virus and B Virus Using Nanoporous Sol-gel Based Protein Microarray

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Background: In the past decade, microarray technologies have resulted in a paradigm shift in modern biology. Microarrays enable high throughput screening (HTS) of disease-related molecules, including important signaling proteins/peptides and small molecules in low abundance. Such endeavors require excellent molecular binding performance with high sensitivity, selectivity and low signal to noise ratio. In this study, we applied nanoporous sol-gel based protein microarray to multiplex diagnosis for blood screening. The sol-gel technology has initially been developed to capture enzymes in their active form for longer period. However, this technology has not been widely used to capture antigen markers for antibody detection because of several limitations such as non-specific interaction between smaller immobilized antigen and larger interacting antibody. Kim et al. (*Anal Chem* 2006) has pioneeringly developed sol-gel material screening technology (SG MS) to find novel formulation for optimizing protein capturing capacity, lower background and superior protein array's physical characteristics. Using this optimized sol-gel formulation, we could immobilize any type of molecule, from proteins and antibodies to chemical compounds, without any modification (Kwon et al, *Clinical Chem* 2008; Ahn *et al*., *Anal Chem* 2012). Using this novel formulation, we developed multiplex blood bank screening platform for simultaneous detection of Human Immunodeficiency virus 1 (HIV1), Hepatitis C virus (HCV) and Hepatitis B virus (HBV).

Methods: For manufacturing protein microarray of HIV and HCV detection, 3 different HIV1 antigens and 4 different HCV antigens were individually mixed with sol-gel solution, and spotted onto same well of 96-well plate along with negative and positive control spots by non-contact type piezo arrayer. For HIV1 clinical tests to obtain cut-off value, 143 patient's serum samples which had been previously tested by ELISA (HIV Ag/Ab Combo, Abbott) and western blot (Genelabs diagnostics HIV blot 2.2) (78 HIV1 negative and 65 positive) were used. For HCV tests, 138 patient's serum samples which had been previously tested by HCV antibody screening test and HCV Ab RIBA confirmatory test (83 HCV negative and 55 positive) were used. For multiplex blind screening diagnosis, 62 patients' serum samples (19 HIV1 positive, 21 HCV positive and 22 negative) were tested. For assay platform, secondary antibody conjugated with fluorescence (Cy3) was used, and signals were detected with fluorescence scanner. Cut-off values (signal to control (background) ratio) for each antigen were determined by a statistical method - Particle swarm optimization.

Results: The sensitivity and specificity of multiplex detection platform was 100% for HIV and HCV, which is equal or higher than that of ELISA in HIV1 and/or HCV diagnosis. Our system also showed highly reproducible results (CV value). Additionally, this platform was compatible with the currently employed automated system used for ELISA based blood bank screening.

Conclusions: We have successfully applied novel screening technology to multiplex HIV1 and HCV diagnosis with confirmatory test-level accuracy. We believe that this result will significantly advance the specificity and sensitivity for multiple disease diagnostics in high-throughput blood bank screening field.

C-147

Evaluation of a Ceruloplasmin assay for use on the Binding Site SPA PLUS turbidimetric analyser

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Ceruloplasmin is a 135kDa α-2 serum glycoprotein that is synthesised in the liver. It binds reversibly to copper and has a major role in its metabolism. Elevated serum ceruloplasmin is considered a risk factor for cardiovascular diseases such as atherosclerosis and myocardial infarction. Reduced levels of ceruloplasmin can result in high levels of copper being deposited in the liver and central nervous system causing Wilson's disease. Here we describe the evaluation of a ceruloplasmin assay for use on the SPA PLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random access analyser with host interface capability, primary sample ID and reagent management systems. Precision is promoted through a combination of air-pressure reagent mixing and acid /alkali cuvette washing. The analyser was programmed to construct a calibration curve from a six point, serum based lyophilised calibrator set. The calibrator set was standardised against the Siemens BN™II Ceruloplasmin assay. Standard curves were validated by assay of control fluids supplied with the kit. Samples were initially measured at a 1/10 sample dilution and, if out of range, the instrument automatically re-measured the sample at a higher dilution of 1/20. All dilutions were made with the instrument's on-board pipetting system, which was able to make dilutions between neat and 1/100. The assay time was 10 minutes and was read at end-point. The assay range was 0.03 - 1.00g/L using a 1/10 sample dilution, with a sensitivity of 0.03g/L. Within-run precision studies were performed at three levels - 0.9, 0.23 and 0.05g/L. The coefficients of variation were 2.07% for the high sample, 1.31% for the medium sample and 3.64% for the low sample. To assess assay linearity, a serum sample spiked with purified ceruloplasmin was serially diluted and measured and expected results were compared to actual results. The assay showed a high degree of linearity when expected values were regressed against measured values (y=1.0674x - 0.0094, R^2=0.9943). No significant interference (within 1%) was observed on addition of hemoglobin (500mg/dL), bilirubin (200mg/dL) or Chyle (1500 formazine turbidity units). Comparison was made between this assay and the Siemens Ceruloplasmin assay for the BNII over a range of 0.2g/L - 0.6g/L (CLSI EP09-A2). Good agreement was demonstrated: y=1.0477x - 0.0001 (r=0.9565, n=42). We conclude that this assay measures ceruloplasmin precisely, accurately, and rapidly and may be of use in laboratories where a large instrument is not appropriate.

C-148

Evaluation of a Microalbumin assay for use on the Binding Site SPA PLUS turbidimetric analyser

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Early detection and treatment of nephropathy is important in preventing renal failure in insulin-dependent diabetics. Elevated urinary albumin concentration is a good indicator of glomerular damage in such patients. Increased albumin excretion is also a marker of future cardiovascular problems in non insulin-dependent diabetes mellitus and also occurs in other chronic conditions such as hypertension, malignancy and chronic obstructive airways disease. Here we describe the evaluation of a microalbumin assay for use on the SPA PLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random access analyser with host interface capability, primary sample ID and reagent management systems. Precision is promoted through a combination of air-pressure reagent mixing and acid /alkali cuvette washing. The analyser was programmed to construct a calibration curve from a six point, serum-based calibration set. The calibrator set was standardised against the DA470k international reference material. The standard curves were validated by assay of control fluids supplied with the kit. Samples were initially measured neat and, if out of range, the instrument automatically re-measured the sample at a higher dilution. All dilutions were made with the instrument's on-board pipetting system, which was able to make dilutions between neat and 1/100. The assay time was 10 minutes and was read at end-point. The assay range was 11 - 344 mg/L using a neat sample dilution, with a sensitivity of 11 mg/L when using neat sample. Analytical sensitivity was assessed by running sixty replicates of a blank sample and the lowest calibrator. Two distinct sets of data were generated with a coefficient of variation of 4.88% for the lowest calibrator. Precision studies (CLSI EP5-A2) were performed at three levels in duplicate over 21 working days. Antigen levels of 281.57, 144.18 and 20.14 mg/L were assessed for total, within-run, between-run and between-day precision, using three different reagent lots on three analysers. The coefficients of variation were 8.2%, 2.1%, 1.6% and 7.8% for the high sample, 7.1%, 1.9%, 1.0% and 6.8% for the medium sample and 6.1%, 1.7%, 2.3% and 5.4% for the low sample respectively. To assess assay linearity, serially diluted serum samples were measured and expected results were compared with actual results. The assay showed a high degree of linearity when expected values were regressed against measured values (y=1.012x - 3.818, R^2=0.9999). No significant interference (within ±10%) was observed on addition of bilirubin or ascorbic acid (20mg/dL), Chyle (1500 formazine turbidity units) or total protein (100 mg/dL). Comparison was made between this assay and the Siemens Albumin assay for the BN™II over a range of 13.61mg/L - 1886.27mg/L. Good agreement was demonstrated: y=0.99x + 0.14 (r=1.00, n=79). We conclude that this assay measures microalbumin precisely, accurately, and rapidly and may be of use in laboratories where a large instrument is not appropriate.

C-149

Evaluation of a Transferrin assay for use on the Binding Site SPA PLUS turbidimetric analyser

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Increased serum concentrations of transferrin are associated with iron deficiency, pregnancy and oestrogen administration whereas decreased serum concentrations occur with chronic infection, neoplasia, hepatic and renal disease. Here we describe the evaluation of a transferrin assay for use on the SPA PLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random access analyser with host interface capability, primary sample ID and reagent management systems. Precision is promoted through a combination of air-pressure reagent mixing and acid/alkali cuvette washing. The analyser was programmed to construct a calibration curve from a six point, serum-based calibration set. The calibrator set was standardised against the DA470k international reference material. The standard curves were validated by assay of control fluids supplied with the kit. Samples were initially measured at a 1/10 sample dilution and, if out of range, the instrument automatically re-measured the sample at an alternative dilution. All dilutions were made with the instrument's on-board pipetting system which is able to make dilutions between neat and 1/100. The assay time was 10 minutes and was read at end-point. The assay range was 0.14 - 5.60g/L using the standard 1/10 sample dilution. Precision studies (CLSI EP5-A2) were performed at three levels in duplicate over 21 working days. Antigen levels of 4.40, 3.74 and 0.22g/L were assessed for total, within-run, between-run and between-day precision, using three different reagent lots on three analysers. The coefficients of variation were 2.2%, 4.4%, 2.3% and 5.5% for the high sample, 1.8%, 1.5%, 4.0% and 4.6% for the medium sample and 1.6%, 1.7% 6.5% and 6.9% for the low sample respectively. To assess assay linearity, serially diluted serum samples were measured and expected results were compared with actual results. The assay showed a high degree of linearity when expected values were regressed against measured values (y=9811x + 0.1151, R^2=0.9979). No significant interference (within ±10%) was observed on addition of hemoglobin (500mg/dL), bilirubin (20mg/dL) or Chyle (1500 formazine turbidity units). Comparison was made between this assay and the Siemens Transferrin assay for the BN™II over a range of 0.145g/L - 5.580g/L. Good agreement was demonstrated: y = 0.99x + 0.01 (r = 0.989, n = 57). We conclude that this assay measures transferrin precisely, accurately, and rapidly and may be of use in laboratories where a large instrument is not appropriate.

C-150

Novel Anti-Ganglioside Antibody Elisas With Improved Specificity

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Background: Autoantibodies to ganglioside antigens are used as diagnostic indicators for rare peripheral neuropathies such as Guillian-Barre syndrome and Multifocal Motor Neuropathy. The symptoms of these diseases can be varied, often requiring a physician to rely on ganglioside autoantibody immunoassys for a correct diagnosis. False positive results can lead to improper patient diagnosis, incorrect therapeutic administration and additional reflex testing that may be costly and unnecessary. The most common methods to detect anti-ganglioside antibodies include Western Blot, Line Immunoassay, in-house prepared ELISA and commercially available ELISA. Western Blot and LIA assays tends to have reduced sensitivity, whereas in-house prepared ELISA assays can have consistency issues from batch to batch. Commercially available ELISA assays have shown significant specificity issues, most prevalently with IgM antibodies.

Methods: To reduce these assay related issues, we have developed a series of novel

anti-ganglioside antibody ELISA assays that have excellent specificity. In order to test the specificity of the new assay against a commercially available ELISA, 64 normal and 32 disease control samples (non-ganglioside related autoimmune disorders) were tested on both kits at the same time. Sensitivity was tested with disease associated samples, 9 for IgM and 16 for IgG.

Results: The specificity for IgG was similar between the two kits, with both of them achieving specificity between 93% and 94%. When comparing the IgM specificity there was a more significant difference, with the commerically available kit demonstrating a specificity of 39%, while the newly developed anti-ganglioside ELISA had a specificity of 96%. Sensitivity for GM1 IgG and IgM was 56% and 44%, respectively.

Conclusions: Considering incidence of disease, poor specificity may have a significant adverse impact on the ultimate diagnosis and patient care. These assays limit non-specific reactivity reducing costly and unnecessary testing.

GM1 Clinical Comparison		
	IgG	IgM
Disease Associated	16	9
Disease Control	32	32
Normal	64	64
Total	112	105
Sensitivity	56.3%	44.4%
Specificity	95.8%	96.9%
Overall Agreement	90.2%	92.4%

C-151

A novel chemiluminescent 1,2-dioxetane formulation for application in clinical research platforms: DynaLight™ substrate with RapidGlow™ enhancer.

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Background: Chemiluminescence has been widely adopted in automated platforms for immunoassay detection utilizing either substrates catalyzed by enzyme labels or substrate labels activated by trigger solutions. Substrate label reactions (i.e. acridinium esters or isoluminols) provide fast signal in a ‘flash’ mode - reaching ground state too fast to collect data reliably and are limited to the number of activated labels. Substrate reactions catalyzed by alkaline phosphatase (i.e. our 1,2-dioxetane CDP-*Star*® substrate) provide ultra-sensitive detection primarily due to amplified signal by enzyme turnover in a ‘glow’ mode - reaching maximum signal in minutes and sustaining it for hours. Here we describe the enhanced performance characteristics of a novel 1,2-dioxetane alkaline phosphatase substrate, DynaLight™ Substrate, designed for combination of the greatest characteristics of both substrate and enzyme label detection systems: fast results with amplified signal for ultra-sensitive detection in clinical research platforms.

Methods: DynaLight Substrate was selected for commercialization based upon performance attributes (i.e. fast kinetics, high signal, low background, high sensitivity and thermal stability) obtained from enzyme assays and further validated in microplate- and bead-based immunoassay model assays. For example, DynaLight Substrate when formulated with the new RapidGlow™ Enhancer delivers maximum sustained signal within 2-10 min instead of 30-60 min provided by CDP-Star, meeting or surpassing CDP-Star or competitor’s substrate other performance characteristics. In addition, DynaLight Substrate offers the flexibility of coupling the enzymatic catalysis with a subsequent DynaLight Trigger Solution which raises the pH and velocity of reaction, to provide signal ‘on demand’ - very useful for automated platforms when faster test protocols are employed or when there is a need to switch to new reagents with trigger positions already in place. For this purpose, we studied enzymatic cleavage followed by chemical activation of chemiluminescence under varying buffers to identify the optimal solution for use with DynaLight Substrate.

Results: When combined with DynaLight Trigger solution, DynaLight Substrate with RapidGlow Enhancer delivers results within seconds. The DynaLight Trigger Solution provides fast maximum signal within 1 sec with a half-life to ground state of about 10 sec, allowing ample time to collect data reliably, in addition to being an environmentally friendly reagent. DynaLight Substrate with RapidGlow Enhancer (with or without DynaLight Trigger) can detect attomole amounts of purified alkaline phosphatase in a functional enzyme assay, less than 1 pg/mL of recombinant human IL-6 in a microplate immunoassay model, less than 1 pg/mL of cardiac Troponin I in a bead-based immunoassay model employing Dynabeads® magnetics beads and less than 2 pg/mL of 17β-estradiol using a commercialized microplate immunoassay kit. On-going stress and real-life stability studies for DynaLight Substrate with RapidGlow Enhancer formulation and DynaLight Trigger Solution show shelf-life stability trends of greater than 1 year at 4°C.

Conclusions: We have demonstrated combination of the greatest characteristics of detection systems with DynaLight Substrate and RapidGlow Enhancer formulation by delivering fast results with amplified signal for ultra-sensitive detection in clinical research platforms. For Research Use Only. Not intended for animal or human therapeutic or diagnostic use

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Evaluation of a high sensitivity IgA assay for use on the Binding Site SPA PLUS turbidimetric analyser

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Selective IgA deficiency is the most common form of antibody deficiency, with patients presenting with upper respiratory tract infections, allergies and autoimmune disease. IgA deficiency can be defined as a serum IgA level of less than 0.07g/L in patients greater than 4 years of age (European Society for Immunodeficiencies diagnostic guidelines). It is therefore important that a diagnostic assay is sensitive enough to quantify IgA levels below 0.07g/L. Here we describe the evaluation of a high sensitivity IgA assay for use on the SPA PLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random access analyser with host interface capability, primary sample ID and reagent management systems. Precision is promoted through a combination of air-pressure reagent mixing and acid /alkali cuvette washing. The analyser was programmed to construct a calibration curve from a six point, serum based calibration set. The calibrator set was standardised against the DA470k international reference material. The standard curves were validated by assay of control fluids supplied with the kit. Samples were initially measured at a 1/10 sample dilution and, if out of range, the instrument automatically re-measured the sample without dilution (neat). All dilutions were made with the instrument’s on-board pipetting system, which was able to make dilutions between neat and 1/100. The assay time was 10 minutes and was read at end-point. The assay range was 0.2 - 7.0g/L using a 1/10 sample dilution, with a sensitivity of 0.02g/L when using neat sample. Precision studies (CLSI EP5-A2) were performed at three levels in duplicate over 21 working days. Antigen levels of 3.6, 0.34 and 0.07g/L were assessed for total, within-run, between-run and between-day precision, using three different reagent lots on three analysers. The coefficients of variation were 3.6%, 0.7%, 1.7% and 3.1% for the high sample, 5.1%, 0.9%, 1.0% and 4.9% for the medium sample and 4.3%, 2.0%, 0.5% and 3.8% for the low sample respectively. To assess assay linearity, serially diluted serum samples were measured and expected results were compared with actual results over a range of 0.02 - 7.6g/L. The assay showed a high degree of linearity when expected values were regressed against measured values ($y=1.0x + 0.004$, $R^2=0.999$). No significant interference (within 4%) was observed on addition of hemoglobin (500mg/dL), bilirubin (20mg/dL) or Chyle (1500 formazine turbidity units). Comparison was made between this assay and the Roche IgA assay for the Modular P over a range of 0.046g/L - 2.9g/L, incorporating normal and deficient patients (CLSI EP09-A2). Good agreement was demonstrated: $y=1.03x - 0.01$ ($r=0.996$, $n=84$). We conclude that this assay measures low levels of IgA precisely, accurately, and rapidly and has sufficient sensitivity to determine IgA deficiency.

C-153

Evaluation of a C1 inactivator assay for use on the Binding Site SPA PLUS turbidimetric analyser

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C1 inactivator is an important regulator of the classical complement pathway. It acts by inhibiting the activity of C1s and C1r serine proteases, therefore limiting any potentially harmful effects of over-activation. Measurement of C1 inactivator aids in the diagnosis of hereditary and acquired angioedema. Here we describe the evaluation of a C1 inactivator assay for use on the SPA PLUS analyser, a small bench-top turbidimeter available from The Binding Site Group Ltd. The instrument is an automated, random access analyser with host interface capability, primary sample ID and reagent management systems. Precision is promoted through a combination of air-pressure reagent mixing and acid /alkali cuvette washing. The analyser was programmed to construct a calibration curve from a six point, serum based calibration set. The standard curves were validated by assay of control fluids supplied with the kit. Samples were initially measured at a 1/10 sample dilution and, if out of range,

the instrument automatically re-measured the sample at an alternative dilution. All dilutions were made with the instrument's on-board pipetting system, which was able to make dilutions between neat and 1/100. The assay time was 10 minutes and was read at end-point. The assay range was 0.03 - 0.4g/L using a 1/10 sample dilution. Precision studies (CLSI EP5-A2) were performed at three levels in duplicate over 21 working days. Antigen levels of 0.33, 0.15 and 0.06g/L were assessed for total, within-run, between-run and between-day precision, using three different reagent lots on three analysers. The coefficients of variation were 6.2%, 5.6%, 2.0% and 1.5% for the high sample, 9.3%, 5.1%, 7.4% and 2.3% for the medium sample and 8.8%, 8.0% 2.8% and 2.4% for the low sample respectively. To assess assay linearity, serially diluted serum samples were measured and expected results were compared with actual results. The assay showed a high degree of linearity when expected values were regressed against measured values ($y=0.97x + 0.006$, $R^2=0.9967$). No significant interference (within 5%) was observed on addition of bilirubin (20mg/dL), haemoglobin (500mg/dL) or chyle (1500 formazine turbidity units). Comparison was made between this assay and the Siemens C1 inactivator assay for the BN™II over a range of 0.26g/L - 8.3g/L (CLSI EP09-A2). Good agreement was demonstrated: $y=0.92x + 0.0$ ($R^2=0.95$, n=169). We conclude that this assay measures C1 inactivator precisely, accurately, and rapidly and may be of use in laboratories where a large instrument is not appropriate.

C-154

Evaluation of the positivity of specific IgE to mite, dog and cat dander in a Brazilian sample

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Background: It is known that allergies to dust mites, dog and cat dander are the most common in the general population. Several studies have reported the association between asthma and allergens from dust mites, such as D. farinae and D. pteronyssinus, which are recognized as IgE synthesis inducers. Dogs and cats are the main pets worldwide. The most important dog allergens are the Can f1 and albumin found in fur, saliva and in a less extent in the urine and feces. The cat contribution to allergy is Fel D 1 present in all breeds of cats. In both cases, dander seems to be an important allergen as well. These allergens from pets can be transported and cause sensitization of individuals at a distance without requiring contact with the animal. **Objective:** To evaluate the positivity of specific IgE to mites, dog and cat dander in a population sample from whom allergy testes were requested in Brazil.

Methods: Specific IgE anti- *Dermatophagoides pteronyssinus*, anti- *Dermatophagoides farina*, anti-dog and -cat dander were determined. Results from September to November 2011 were compiled.

Results: During these three months, 4123 measurements of specific IgE to the aforementioned allergens were carried out with a general positivity of 43.9%. Out of these, 51.8% were found among children up to age 16 years old and 48.2% from adults. The frequency of patients with allergy to *D. pteronyssinus* was 32.6%, for *D. farinae* was 37.2%, for dog dander was 17.9% and for cat dander was 9.3%.

Conclusions: These findings are in agreement with those described in the in the literature, demonstrating the high prevalence of mite allergens for the production of specific IgE.

C-155

The Utility of Erythrocyte Sedimentation Rate in Inlammatory Disease Compared with C-Reactive Protein

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Background: Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are among the most frequently routine tests ordered as part of diagnostic workup of inflammatory diseases and certain infections. We compared the clinical sensitivity and specificity of ESR and CRP, and evaluated the sensitivity and specificity of both tests at cut off points used in our institution.

Methods: One hundred (100) consecutive clinical specimens (60 females, 40 males, age range 4 - 90 years) were tested for ESR and CRP. ESR test was performed using modified Westergren method and CRP was measured using a nephelometric assay on Seimens Dimension Vista. Medical history was obtained using our institution's electronic medical record system. From review of the medical history of each patient, we classified each patient as with or without active inflammation at time of ordering the test. Medical histories were available for 89 patients. Different cut offs were assigned for each test (for ESR: 10, 15, 20, 25, and 30 mm/hour, and for CRP 0.3, 0.5, 1, and 1.7 mg/dL). Sensitivity and specificity for each cut off was calculated using a 2x2 table. Sensitivity and specificity at each cut off for each test were later used to generate receiver-operator curves (ROC). The two curves were superimposed to compare sensitivity and specificity of the tests.

Results: ESR and CRP showed comparable sensitivity (89% and 88% at 0.5 mg/dL and 20 mm/hour cut off points, respectively). However, CRP had much higher specificity (82% at 0.5 mg/dL) compared to ESR (42% at 20 mm/hour). Based on the ROC generated, 20 mm/hour, and 0.5 mg/dL were the best cut off values for ESR and CRP tests, respectively.

Conclusions: While of comparable sensitivity, CRP is more specific than ESR in assessing the presence of acute inflammation in clinical practice. We recommend CRP, rather than ESR, should be utilized for this purpose.

C-156

Evaluation of automated digital ANA detection in Brazil by NOVA View

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Backgroung : NOVA View® is a new, automated digital image analysis system, which is used for reading and interpreting anti-nuclear antibody (ANA) testing on HEp-2 cells, based on measured Light Intensity Units (LIU) and pattern recognition. Our goal was to evaluate the performance of a beta version of the NOVA View instrument in comparison with routine indirect immunofluorescent (IFA) ANA detection.

Methods: Method comparison was performed on three sets of samples. The first cohort consisted on 40 pre-screened negative and 40 pre-screened ANA positive samples, provided by INOVA Diagnostics, Inc. as part of the instrument training. The second set contained 40 negative and 40 positive specimens, selected by DASA,core laboratorybased on the routine ANA IFA results. The third cohort consisted of 199 prospective, consecutive samples sent for ANA determination. Samples were processed on NOVA Lite® HEp-2 slides, and scanned by NOVA View. Digital images were verified on the computer screen. Slides were also read under a manual microscope. Positive, negative and total agreement were calculated between manual reading and NOVA View results. Moreover, Spearman correlation coefficient was used to assess the correlation between manual grades and NOVA View LIU results.

Results: NOVA View results are expressed in LIU, and interpreted as negative or positive based on a pre-set cut-off. The automated scan is followed by visual verification of the digital images, allowing for either confirmation or revision of the results by the user. Agreement between NOVA View and manual reading was 95.3% in the first cohort, with excellent correlation between grades and LIU values (Spearman r=0.838). Agreement was 90.8% in the second cohort, with NOVA View producing no false positive, but 7 false negative results. This was reduced to 5 after verifying the digital images on the computer screen. The correlation between grades and LIU was similar to that in the first set (r=0.842). Results in the third cohort (118 negative and 80 positive samples with manual reading) yielded 83.3% total agreement with r=0.768. NOVA View initially reported 1 false positive and 32 false negative results compared to manual IFA. These results suggested that the NOVA View cutoff was set too high, which was confirmed by a validation study performed by the manufacturer. As a result, a new cut-off was set at a different LIU value, and results from the third cohort were re-calculated. The new total agreement was the same (83.8%), but the number of false positive and false negative samples changed to 19 and 14. The number of false negatives was reduced to 4 by user review and revision of the result, improving total agreement to 97.5%.

Conclusions: Our results show good qualitative and quantitative correlation between manual IFA and automated digital ANA detection. The low false negative rate demonstrates the utility of NOVA View as a reliable screening tool. As the output is expressed in LIU, the performance can easily be verified by the user, and optimized by setting a different cut-off value according to the individual expectations and comparison methods of various laboratories.

C-157

Development and evaluation of Analytical Measuring Range validation fluids for the total IgA, IgG and IgM assays for use on the Binding Site SPA PLUS analyser

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Background: Analytical measuring range (AMR) fluids have been designed to assess the linearity of an assay calibration curve in line with the US Food & Drug Administration's Clinical Laboratory Improvement Amendments (CLIA) regulations. These state that linearity tests must cover from 90% of the top of the measuring range

to the bottom of the range at standard sample dilution. In addition composition of those fluids should be matrix matched with routine clinical samples tested in the laboratory against the assay. The validation of the calibration curve of total IgA, IgG and IgM assays available for use on the SPA Plus is required to be performed to this method.

Aim: To develop and evaluate AMR validation fluids for the total IgA, IgG and IgM assays.

Method: Total IgG, IgA and IgM AMR validation fluids were made up with polyclonal IgG, IgA or IgM purified from human serum and spiked into a pool of human sera to achieve suitable analyte concentration for linearity assessment. Polyclonal IgG was supplied from Bio Products Laboratory Ltd. and polyclonal IgA and IgM were purified by immunoaffinity chromatography.

Total IgG, IgA and IgM AMR fluids were assayed at standard sample dilution respectively at 1/10, 1/10 and 1/20. All fluids were first targeted to a concentration value falling between 90-100% of the top of the calibration curve at standard sample dilution making up the 100% fluid. The 100% fluid was then further diluted using the on-board analyser diluent to produce the following dilutions; 75%, 50%, 25%, 12.5%, 6.25% and 3.125%. Each dilution was run in triplicate and a median value was determined. Expected values calculated for each dilution from the 100% fluid concentration were compared with median reported values. Linearity curve fit equation and r^2 were calculated using Microsoft Excel.

Results: Total IgG, IgA and IgM AMR 100% fluids were targeted respectively to 33.716, 7.076 and 6.865 g/L. Differences between the expected values and median reported values for each individual dilution were ranging from -5.5% to 3.5% for total IgG, 0.4% to 5.5% for total IgA and -4.3% to 7.2% for total IgM. All assays showed acceptable linearity over the analytical measuring range tested: IgG: $y=1.0243x - 0.3313$, $r^2=0.9988$; IgA: $y=1.0045x + 0.0239$, $r^2=0.9997$; IgM: $y=0.9962x + 0.011$, $r^2=0.9992$.

Conclusions: We have successfully developed AMR validation fluids for a linearity assessment at standard sample dilution of the total IgG, IgA and IgM assay calibration curve for use on the SPA Plus analyser.

Number of words: 406

C-158

A Comparison of Positivity Rates, Agreement, and Predictive Values of ANA Screening by EIA vs. HEp-2 IFA in a Rheumatologic Referral Population: Is there a Gold Standard? S. Kelsey, D. Mentrikoski, and H. Harrison.

S. Kelsey, D. Mentrikoski, H. H. Harrison. *Geisinger Medical Center, Danville, PA*

Background: The objective of this study was to establish the performance statistics for an enzyme immunoassay (EIA) based, antinuclear antibody (ANA) screening test (Autoimmune EIA, Bio-Rad Laboratories, Hercules, CA, run on DSX) and an indirect immunofluorescence microscopy assay (IFA) for ANA pattern and titer evaluation (ANA HEp-2 Cell Culture, Zeus Scientific, Raritan, NJ, read on a Leitz Laborlux fluorescence microscope) in samples from rheumatology clinic patients. Obtaining such data is one of several recent recommendations made by the American College of Rheumatologists for laboratories that perform ANA testing, with IFA advocated as the "gold standard" method. There are pros and cons to each: EIA is faster, automated, and less expensive; yet is restricted in antigenic content to the cell-based IFA which has a broader antigenic complexity and provides stain pattern detail, but is slower, more subjective and requires five times more technologist labor time for microscopy. In an approach to optimization, our laboratory adopted a two-step algorithm for ANA screening several years ago. When an ANA Screen is requested, we perform EIA first and reflex only positive samples to an IFA exam with pattern and titer. If EIA is negative, IFA is not performed. To assess performance of the two-step approach in rheumatology clinic patients, we compared positivity, agreement, and predictive value rates between methods. Geisinger Health System is rural, with primary care clinics that refer to centralized specialists, and an EHR that can readily identify patients whose testing is requested by primary care physicians versus rheumatologists.

Methods: This pilot cohort included 229 unselected patients tested over two months for the adult rheumatology service. ANA tests were performed according to manufacturers recommendations with EIA positive cutoff >1.0, and IFA positive at 1:40 or greater dilution. Both tests were performed in all cases and results were analyzed with each as the reference method using qualitative method comparison statistics (EP Evaluator, Data Innovations).

Results: There were137 samples with negative EIA, of which 8 had positive IFA slides (FNR=5.8%), with titers of 1:160 (n=7) or 1:320 (n=1). 92 had positive EIA screens, of which 71 were positive by IFA with titers ranging 1:40 to >10,240; with 21 being EIA-positive then IFA-negative. Agreement was 87.3% overall. Positive predictive value (PPV) was 89.9% and negative predictive value (NPV) was 86.0%. Reciprocal analysis with IFA as the reference method yielded values for PPV of 77.2%, and NPV of 94.2%.

Conclusions: Both procedures had false positive and negative results. Screening with EIA (229 tests) led to 92 IFA, of which 21 were FP; testing in the IFA first or only mode would have 229 IFA performed for 79 positives, of which 10% were FP. Thus, screening with EIA has a higher first-positive rate but saves 56% of IFA (129/229=Neg/Neg) at the expense of 3.4% (8/229) false negatives. This amounts to a labor savings of 36%. We are in the process of following up on the patient diagnoses, specific IFA pattern correlations, and extending the study to similar testing of a non-rheumatologic screening cohort.

C-159

New 25-OH Vitamin D total ELISA: a fast and straightforward competitive Elisa for the quantification of 25-OH Vitamin D2/3 (total) in human serum and plasma.

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Vitamin D plays an important role in regulating body levels of calcium and phosphorus and in mineralization of bones. In addition, Vitamin D levels > 30 ng/mL are now discussed to have protective effects on several disease states, such as cancer, cardiovascular diseases, diabetes and autoimmune diseases.

The DRG 25-OH Vitamin D (total) assay is a colorimetric solid phase enzyme-linked immunosorbent assay (ELISA) based on the competitive binding of Vitamin D of the sample and biotinylated Vitamin D to immobilized vitamin D binding protein (VDBG), followed by the detection with a Streptavidin-HRP conjugate.

The Elisa allows the quantitative determination of 25-OH vitamin D (total) covering a measuring range form 4-130 ng/mL. The analytical sensitivity of the assay is 2.3 ng/ml. The test shows good reproducibility with an intra-assay precision of 5.6% (mean of 20 repeated measurements of 3 different samples) and an inter-assay precision of 10.8% (average of 40 repeated measurements of 3 different samples by two observers on 20 days with 2 different lots). The assay shows a good correlation to the RIA (new Roche Cobas total 25-OH Vitamin D; r = 0.948; n=48) and to the Liaison (Diasorin; r=0.86; n=58).

Benefits of the assay are ready-to-use reagents, a total assay time < 2 hours, and a very straight forward procedure for release of vitamin D (no precipitation or centrifugation).

C-160

Analysis of patients with gamma heavy chain disease by heavy/light chain immunoglobulin investigation

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Background: Heavy chain diseases (HCD) are rare lymphoplasmacytic proliferative disorders that are characterized by the production of a monoclonal immunoglobulin that is a truncated heavy chain and lacks a light chain. These are typically diagnosed using serum protein electrophoresis with immunofixation, showing a monoclonal heavy chain with no corresponding light chain. Often the truncated heavy chain is so small that serum concentrations are low due to renal clearance. Because of the low serum concentrations the definitive absence of light chain may be difficult to establish. In addition, some intact monoclonal immunoglobulins have poor reactivity with anti-light chain antisera and show only faint light chain on immunofixation electrophoresis. The Hevylite immunoassay targets the unique junctional epitope between the heavy chain and light chain and therefore requires both chains. A monoclonal gammopathy that is a gamma heavy chain disease will not be recognized by heavy/light chain reagents and will have a low $(G\kappa+G\lambda)/IgG_{total}$ ratio.

Objective: To assess the reactivity of the Hevylite assay to patients with gamma heavy chain disease.

Methods: Serum samples from 15 patients with previously diagnosed gamma HCD were analyzed for IgG kappa and IgG lambda with the Hevylite® immunoassay (The Binding Site Group LTD, Birmingham UK) measured on the BNII nephelometer (Siemens, Tarrytown NY). Total serum IgG was quantitated using Siemens reagent sets. In addition to IgG, Gκ, and Gλ concentrations, the Gκ/Gλ and $(G\kappa+G\lambda)/IgG_{total}$ ratios were calculated. Serum protein electrophoresis was performed with a Helena SPIFE 3000 electrophoresis unit using a SPIFE SPE Vis agarose gel and stained with Acid Blue for visualization (Helena Laboratories, Beaumont TX).

Results: The 15 gamma heavy chain patients had an average M-spike of 1.8 g/dL. Fourteen of the 15 samples had normal Gκ/Gλ ratios, ranging from 1.18-2.76 (reference range: 0.71-3.23), and these Gκ/Gλ ratios presumably represented the

residual polyclonal IgG. The (Gκ+Gλ)/ IgG_{total} ratios ranged from 1.5% to 63.0% in 14/15 patients, with a single patient as high as 79.5% (reference range: 80%-112%) indicating that most of the IgG was not quantitated by the Gκ+Gλ reagents, which was consistent with a diagnosis of gamma-HCD.

Conclusions: This study demonstrated the specificity of the Hevylite reagent set for an epitope requiring the combination of heavy and light chains. The assay may be useful to confirm the absence of cryptic light chains in patients suspected of heavy chain disease.

C-161

Comparison of New IgA and IgG Quantitative Nephlometric Immunoglobulin Kappa and Lamda Assays (Hevylite) with Existing Methods for Monitoring Multiple Myeloma

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Background. The current most sensitive method for detecting immunoglobulins in serum is the non-quantitative immunofixation electrophoresis (IFE). Other laboratory methods used to monitor myeloma patients include protein electrophoresis (SPEP) or M-spike, free light chain assay (sFLC), quantitative IgG, IgA, and IgM immunoglobulins (QIgs), as well as the new serum heavy chain/light chain (HLC) immunoassay. HLC is a quantitative analysis of intact immunoglobulin heavy chain (IgH) and light chain kappa or lambda. This assay provides concentrations of IgH kappa (k) and IgH lambda (λ) as well as the clinically useful IgHk/IgHλ ratio. The primary aim of this study was to evaluate the IgA HLC assay on the Siemens BNII instrument relative to the other laboratory methods.

Methods. The IgA k/λ HLC reagent kits, provided by The Binding Site, Inc, were evaluated on 108 multiple myeloma patient (69 IFE positive and 39 IFE negative) samples with known sFLC (The Binding Site), and QIgs (Siemens) results using the Siemens BNII instrument. A Sebia CAPILLARYS capillary electrophoresis system was utilized to generate SPEP profiles and utilized in tandem with total protein determination (Beckman-Coulter) to calculate monoclonal protein concentrations when possible. This sample set was obtained from specimens of both inpatients and outpatients previously diagnosed with IgA multiple myeloma, and monitored at the University of Arkansas Myeloma Institute.

Results: The published reference intervals for the HLC assay are; IgAκ 0.48-2.82 g/L, IgAλ 0.36-1.98 g/L and IgAκ/IgAλ ratio is 0.8-2.04. The HLC assay (g/L) showed strong correlation versus the quantitative IgA (g/L) assay ($y = 1.173x + 0.109$, $R^2 = 0.96$) and M-spike (g/L) ($y = 0.963x + 0.995$, $R^2 = 0.96$). Of the 69 IFE positive samples, 88% (61/69) were positive (abnormal) by HLC κ/λ ratio, 71% (49/69) were positive by sFLC κ/λ ratio, 58% (40/69) were abnormal for QIgA assay and M-spike was detected in 68% (47/69). Of the 66 samples positive for an abnormal HLC ratio, 88% (60/66) were positive for IFE, 73% were positive for sFLC κ/λ ratio (48/66), and 71% were positive for SPEP (47/66). All samples positive for SPEP (n=47) exhibited an elevated HLC κ/λ ratio.

Conclusions: In this study, the HLC assay appears to be more sensitive than SPEP for quantifying and detecting intact IgA immunoglobulins. Additionally, the HLC assay was strongly predictive of assayed SPEP monoclonal protein concentration determination. HLC assays can be potentially utilized in the monitoring of myeloma patients by providing numerical results for patients that are IFE positive but not readily followed by serum protein electrophoresis, and through calculation of the clinically beneficial IgAk/IgAλ ratio. Biological variations that may impact electrophoretic evaluation of the immunoglobulin protein should not affect this HLC ratio, for example changes in blood volume, hematocrit and metabolism. In this monitoring population, the cohort of specimens of elevated HLC assays did not perfectly overlap the cohort of positive sFLC κ/λ ratios indicating that the HLC assay may offer complementary analytical advantages to serum free light chain assays in some patients. Further clinical studies are required to fully assess the benefit of this assay in monitoring patients.

C-162

Comparison of Luminex 200 (xMAP technology) and Phadia 250 platforms for autoantibody detection in a large scale laboratory

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Background: The request of autoantibodies markers per sample varies between patients. The objective of this study was to evaluate 2 platforms (Luminex and Phadia) that used 2 different approaches: AtheNa Multi-lyte ANA II plus test (Zeus Scientific inc., Raritan, NJ) tested at Luminex platform performs directly and simultaneously 9 markers (SSA, SSB, RNP, Sm, Scl-70, Jo-1, dsDNA, centromere and Histonas) while EliaScreening (Thermo Fisher Scientific IDD, AB Uppsala, Sweden) performs a screening test and opens the test for the markers that were ordered by the physician.

Methods: DASA receives approximately 5000 samples per month. The rate of positive results is 15% and the mean of marker requested per sample is 3.

Results: The table summarizes the comparison between Luminex and Phadia 250 platforms for 5000 samples, assuming a mean of 3 markers/sample and 15% rate of positivity.

	Luminex	Phadia
Tests performed at screening	5000	5000
Number of samples that need to open (15% rate of positivity)	0	750
Number of tests opened (mean of 3 per sample)	0	2,250
Total tests performed	5000	7,250
Number of tests/hour	90	60
Total number of hours	55.5	120.8

Conclusion: Luminex platform showed a better performance for autoantibody large scale screening at DASA laboratory. Phadia, now Thermo Scientific IDD, is launching a new family of high throughput analyzer (Phadia 2500 and 5000) that can change this current scenario.

C-163

Performance analysis of two automated immunoassays for the detection anti-HCV antibodies

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Background: In the routine of a core laboratory in Brazil, an algorithm for the detection of anti-HCV antibodies, two different brands of automated immunoassays are used, names A for screening and B for confirmatory tests. In daily clinical practice, it is known that the methods of immunoassays for anti-HCV possess a sensitivity of about 85% and some results must be confirmed by other methods that are considered more worksome, such as PCR or Immunoblot.Objectives: To clarify the occurrence of discrepancies between the two methodologies evaluating the number of false positives and its influence on the predictive value of positive test and to establish an algorithm based on the results of two tests

Methods: Samples from 404 individuals presenting positive or indeterminate results in the screening immunoassay (method A) were further tested by method B. In addition, 342 other samples with positive or indeterminate results in method A, were also tested by method B and by RIBA.

Results: Considering the total number of positive or indeterminate tests at the screening method (method A), 61% were confirmed by method B, 36% of cases turned out to be negative after being tested by the confirmatory methodology (method B) and 3% were considered really as bearing an indeterminate result. When method A was compared to the immunoblot (RIBA), 59.7% were defined as true positives and 30.5% turned out to be negative in the more specific assay (RIBA). In the table below, the positive predictive value was determined:

CORTE	VP	TP	VPP	SENS	VPN
1	224	242	65,5%	100,0%	100,0%
5	224	280	80,0%	100,0%	100,0%
10	222	265	83,8%	99,1%	97,4%
25	222	251	88,4%	99,1%	97,8%
50	220	237	92,8%	98,2%	96,2%
100	218	227	96,0%	97,3%	94,8%

Conclusion: Following the obtained results, the confirmatory immunoassay (method B) shows similar results to the RIBA, allowing us to perform the confirmation algorithm only with the automated method B. However, in specific clinical indications, confirmatory laboratory diagnosis should be performed by more specific methods, such as RIBA and PCR.

C-164

Evaluation of a Novel tTG/DGP Fusion Immunoassay with Comparable Assays For Screening of Autoantibodies Associated With Celiac Disease

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Objective: We want to determine if a solid phase immunoassay developed with a single recombinant molecule consisting of Tissue Transglutaminase (tTG) and Deamidated Gliadin (DGP) has a higher sensitivity and specificity for Celiac Disease autoantibodies than assays developed with multiple individual antigens. Celiac Disease (CD) is an autoimmune gastrointestinal disorder that may occur in genetically susceptible individuals and is triggered by the ingestion of gluten-containing grains such as wheat, barley and rye. Only a small percentage of patients with CD present with classical symptoms, which include diarrhea, weight loss and malnutrition. Therefore diagnosis based on clinical presentation is unsound, expounding the need for highly sensitive and specific immunoassays to aid in the detection of CD. Our objective was to evaluate the clinical utility of an immunoassay that detects CD at an early onset with a novel recombinant antigen that contains both tTG and DGP expressed in a precise ratio.

Methods: The tTG/DGP fusion immunoassay utilizes recombinant technology to produce an antigen that expresses both tTG and DGP epitopes on a single molecule to reduce epitope masking and non-specific interactions. This new tTG/DGP fusion assay has been evaluated against two commercially available tTG/DGP screen assays that contain a mixture of individual antigens instead of the single molecule recombinant tTG/DGP fusion antigen. Our studies included 119 serum samples from patients with celiac disease submitted for Endomysial Antibody (EMA) tests and healthy normal subjects. In order to stringently test the tTG/DGP fusion assay, many patient sera with low EMA titers (≤ 20) were tested along with samples at higher titers. 65 samples were positive for EMA antibodies. 29 of 65 were low positive (titer less than 20) by IFA. The remaining 54 samples were normal human sera (EMA negative).

Results: The diagnostic performance of the tTG/DGP fusion immunoassay is superior in comparison with two other available screen immunoassays. The tTG/DGP fusion assay achieved a sensitivity of 95% in comparison with EMA while, two other available screen immunoassays yielded sensitivities of 94% and 86%. The specificity of the tTG/DGP fusion antibody assay was 94% compared to other similar assays with specificities of 94% and 79%. The tTG/DGP fusion assay detected 27 of 29 EMA low positive samples including 2 that were not detected by the other assays that utilize antigen mixtures instead of a single recombinant molecule.

Conclusions: The tTG/DGP fusion assay using a novel recombinant antigen containing both tTG and DGP on the same molecule yielded superior sensitivity while maintaining excellent specificity in comparison with other available tTG-DGP screen immunoassays. The tTG/DGP fusion test provides celiac antigen combinations in a novel format for the superior detection of celiac disease.

C-165

Evaluation of Performances of Three enzyme-linked immunosorbent assay (ELISA) for antibodies to native DNA for use as screening in a large scale lab in Brazil

N. Z. Maluf, F. B. Flumian, C. F. Voltarelli, S. A. D. Mendonça, N. Gaburo Jr. *DASA, Barueri, Brazil*

Background: The anti-DNA is an analyte used in clinical practice useful in the evaluation and therapeutic management of Systemic Lupus Erythematosus. Increased levels are found in the disease activity of SLE, including renal involvement, and its major use is as a marker for efficacy of treatment. Currently, the measurement of anti-DNA in a large scale core laboratory in Brazil is carried out by indirect immunofluorescence using *Crithidia luciliae* as substrate. The validation of an ELISA methodology for screening test following with immunofluorescence (IFA) to confirm the positive results would be of benefit for the workflow of such a setting. **Objective:** To evaluate the performance of three brands of ELISA to measure anti-DNA and choose one of them as screening test.

Methods: Ninety samples were firstly tested by immunofluorescence method and fyrther re-tested with three different ELISA brands. Kappa index was used for statistical analysis for validation and the sensitivity and specificity of each ELISA brand was defined based on the comparison with the immunofluorescence methodology.

Results: A kappa index of 0.781 was obtained, with p-value of kappa <0.001 (confidence interval 0.865 to 0.696) for the category of negative results; Kappa index of 0.772, with p-value of kappa <0.001 (confidence interval 0.856 to 0.688) for the category of positive results. The category of indeterminate was not applicable in this study because immunofluorescence does not allow this kind of interpretation. In the analysis of the first brand (Euroimmun) a specificity of 94% and a sensitivity of 88% was evidenced, the second brand (Zeus) had a specificity of 83% and a sensitivity of 83%, the third brand (INOVA) had a specificity of 100% sensitivity of 63%.

Conclusions: The Kappa value can be used for validating the methodology for its use as ELISA screening test. Analyzing the performance of the ELISA methodologies, the first brand showed the best sensitivity value and will be consequently used as a screening test in our routine laboratory.

C-166

Concordance between determination of oligoclonal bands in cerebrospinal fluid, free kappa index and intrathecal synthesis in patients suffering inflammation in the central nervous system

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Introduction: In inflammatory diseases of the central nervous system (CNS), like multiple sclerosis (MS), a highly restricted number of clones of B cells are activated within the CNS in cerebrospinal fluid (CSF) and they are transformed into immunoglobulin-secreting plasma cells. The measurement of oligloclonal bands in cerebrospinal fluid (CSF) is based on that premise because oligoclonal bands are the best biological marker for predicting clinical multiple sclerosis in patients with clinical symptoms. Besides the determination of oligoclonal bands in CSF, other important parameters for a possible diagnosis of inflammatory diseases of the CNS are the Tibbling index, the Tourtellote index and the free kappa index. In this study we relate these indices with the presence or absence of oligoclonal bands and age because these indices are influenced by age.

Methods: We have studied 66 patients from different hospitals in Spain. The study was carried out during the period between November 2011 and February 2012. Each patient who met the study criteria (patient with suspected CNS inflammatory disease) was performed a lumbar puncture and we obtained a sample of CSF. We also obtained a blood sample. With the CSF and serum samples from each patient, we performed in our laboratory of immunology a quantitative analysis of IgG, IgA and IgM by nephelometry (BNII Siemens) and subsequently the qualitative analysis of oligoclonal bands (BOCG) by isoelectric focusing and transfer followed by immunodetection. The FLCs were measured by FREELITE™ (The Binding Site) turbidimetric assay. Tibbling index, Tourtellotte index and K index was determined for every patient. Patients were divided into 2 groups based on the presence (n=35) or absence of oligoclonal bands (n=37). Mann Whitney-U test was used for the comparison of median between the two groups. ROC curve (Receiver Operating Characteristic) was plotted for k index, tibbling index and tourtellotte index. Statistical analyses were performed using IBM SPSS Statistics version 19 for Windows (New York, USA).

Results: K index (111,02 vs. 17,37), tibbling index (0,69 vs.0,52) and tourtellotte index (4,41 vs -1,73) were significantly elevated in multiple sclerosis than in patients with other inflammatory disease of the CNS (figure). AUC was highest for Tibbling index (0,892) followed by Tourtellotte index (0,805) and K index (0,778) respectively.

Conclusions: The study of the cerebrospinal fluid parameters in association with clinical data, help us to diagnose multiple sclerosis. K index, tibbling index and tourtellotte index differ significantly between patients with multiple sclerosis than other inflammatory disease of the CNS. Tibbling index presented the best diagnostic value in the multiple sclerosis than the other indices.

C-167

Validation of a commercial ELISA for CH50 detection and definition of maximum reference value

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Background: The total complement activity is a laboratory parameter used in clinical practice for the evaluation and therapeutic management of diseases that progress with formation and deposition of immune complexes in tissues, with particular attention to the Infectious and non-infectious Acute Diffuse Glomerulonephritis and systemic lupus erythematosus. Increased levels are found in the early stages of infectious and inflammatory diseases in general, as they are considered acute phase proteins. The current routine laboratory methodology used is an in house radial Immune hemolysis. The ELISA method for CH50 detection was evaluated although possessing only the

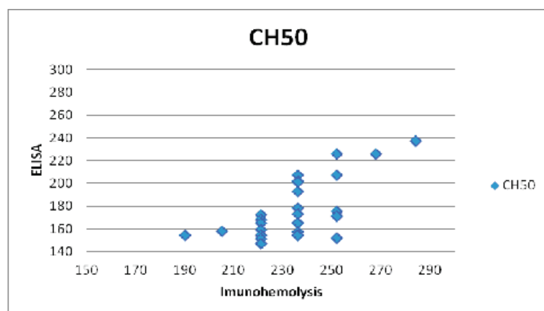
minimum value for well-defined levels of the analyte.

Objective: To evaluate the Complement activation ELISA - CAE (Diasorin Inc, Hillwater, MN) and to define the maximum reference value.

Methods: The results of the ELISA test were compared to the homebrew assay previously established in a core laboratory with 60 patients samples and 40 normal controls. We used the Kappa index for statistical analysis and validation of the FORECAST function and the scatter plot to standardize the maximum range.

Results: A kappa index of 0.926 was obtained, with p-value of < 0.001 (confidence interval - 1.00 to 0.73). The maximum value of the reference range defined by the FORECAST Function (-35) was 265 U / CAE.

Conclusions: An excellent kappa value was found comparing the two methods and the validation of the new method for the routine was considered successful. It was also possible to standardize the maximum value of the reference range, implementing a safe and reliable methodology for the clinical practice.



C-168

Sensitivity of Serum Free Light Chain and M-protein Measurements in Determining Recurrent Disease in Multiple Myeloma after Stem Cell Transplantation

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Objective: To determine the sensitivity of serum free light chain and M-protein measurements in detecting recurrent disease in multiple myeloma patients after stem cell transplantation.

Background: Studies have shown that baseline measurements of serum free light chains (sFLC) have prognostic value in patients with newly diagnosed multiple myeloma (MM). In addition, sFLC have been proven to be more sensitive than serum protein electrophoresis (SPEP) in following disease progression and in indicating whether treatment is effective in MM. We analyzed serial data in 15 patients with MM to determine whether M-protein by SPEP, or serum free light chain (sFLC) concentrations would be more sensitive in diagnosing recurrent disease after undergoing stem cell transplantation (SCT).

Methods: We analyzed 15 MM patients diagnosed at our hospital between 2004 and 2009, who underwent SCT with concomitant follow-up with sFLC and M-protein from 2004 to 2012. Patients to be studied must have a) undergone SCT, b) achieved a complete or partial response to therapy, and c) were routinely monitored by sFLC and SPEP. Serum free light chains were measured by immunoassay on the SPAPLUS analyzer (Binding Sites, UK) and the SPEP was performed using the CAPILLARYS 2 capillary electrophoresis system (Sebia, France). An elevated M-protein was indicated by a level ≥ 0.2 g/dL. An abnormal sFLC was indicated by a κ:λ ratio >1.65 or <0.26.

Results: Among the cohort of 15 patients, there were 9 male patients with a median age of 68 years and 6 female patients with a median age of 70 years. Of the 15 cases, 13 have intact immunoglobulin MM (9 IgG kappa; 2 IgG lambda; 2 IgA kappa) and 2 cases have light chain MM. All 15 cases (100%) achieved a complete response (CR) or partial response (PR) to SCT as defined by the International Myeloma Working Group. After SCT, 4 remained in remission and 11 had a recurrence of MM with a measurable increase in M-protein only (9%), sFLC only (27%) or both M-protein and sFLC (64%). There was no clear pattern observed in the last group, with 3 (27%) patients showing increases in M-protein 6-12 months before sFLC , 3 (27%) patients showing increases in sFLC 3-6 months before M-protein, and 1 (9%) patient showing concurrent increases in both.

Conclusions: Recurrence of MM in SCT patients was detected by either sFLC alone, M-spike alone, or in 64 % of the cases by both of them. Although our study is limited by the sample size, preliminary results indicate that sFLC should be used in conjunction with SPEP for the earliest detection of MM recurrence to ensure that the diagnosis is not missed by using SPEP or sFLC assay alone.

C-169

Measurement of Antinuclear Antibodies by Enzyme Immunoassay with Reflex Confirmation by Multiplexed Flow Immunoassay

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Objective: The purpose of this study was to develop an algorithm that provides enhanced sensitivity and specificity for anti-nuclear antibody testing (ANA) by utilizing multiple methodologies. The premise is to use an enzyme immunoassay (EIA) to determine the presence and semi-quantitative amount of antibody present in the patient sample followed by confirmation testing of positive samples using the multiplex flow immunoassay (MFIA) to determine the presence of specific autoantibodies. Combining the increased screening sensitivity and specificity of the EIA with the diagnostic utility of the MFIA provides a more cost effective alternative to initial screening of patient samples by indirect florescence antibody (IFA) slides. Using this algorithmic approach is in accordance with current practice guidelines and provides clinicians with an alternative option for evaluating patients suspected of having connective tissue disease (CTD).

Methods: Samples from 287 patients submitted for routine ANA testing were evaluated by the Bio-Rad/Helix (96AN) EIA kit, Bioplex 2200 multiplex analyzer and INOVA NOVA lite HEp-2 IFA slides. All three tests were performed on each sample. Due to the highly subjective nature of IFA interpretations, two clinical laboratory scientists read the IFA slides each blinded to the other's readings. The research staff at PAML utilized the Dynex DSX with a modified protocol to perform the EIA assay and the multiplexed results were obtained and run with the routine patient screenings.

Results: From the original 287 samples, 29.3% were found to be positive for ANA by EIA (> 1.0 U); of these patients, 50.0% were positive for one or more nuclear antibodies on the Bioplex. Samples with an EIA value >2.6 U correlated with positive IFA and MFIA interpretations 88.2%. A number of samples demonstrated weakly positive EIA values which may be considered as false positive results and require the clinician to weigh the clinical findings of the patient against the results obtained by the laboratory. A majority of the samples falling into this category, positive by EIA (1.0 - 2.6 U), provided us with ambiguous results by IFA and/or were found to be negative utilizing MFIA as a screening tool. Analyzing all the data collected and reserving the IFA when needed as the referee method, we were able to determine that samples with an EIA unit value > 2.6 were more likely to have the presence of detectable autoantibodies to specific nuclear antigens by multiplex.

Conclusions: By establishing a set point that has a high likelihood of a confirmatory diagnosis, we can offer a screening tool that bridges the gap between MFIA screening and the subjective IFA slide interpretations. By combining the high level of specificity and sensitivity, the capacity for high through-put and the relatively low cost of the EIA screen with the diagnostic utility of the multiplex confirmation testing. We have assembled a valuable screening tool for our clinicians in evaluating patients with CTD.

C-170

Study of Cross-reactive Rosaceae Component Allergens in Japanese School-age Children

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Background: Oral allergy syndrome (OAS) is often associated with extensive IgE cross-reactivity in patients with pollinosis and multiple food allergies. This study evaluated IgE sensitization profiles in a pediatric population using representative *Rosaceae* panallergens from pathogenesis-related protein (PR-10), profilin, and nonspecific lipid-transfer proteins (nsLTP).

Methods: Serum samples were collected from 22 children (aged 6 months to 12 years) with documented histories of fruit-related allergies. The samples were analyzed for specific IgE reactivity to apple, peach, rMal d 1*, rPru av 1*, rBet v 2*, rMal d 4*, nPru p 3*, and rPru av 3* using the IMMULITE® 2000 3gAllergy™ Specific IgE assay (Siemens Healthcare Diagnostics, Tarrytown, NY). Four samples from subjects without any food allergy symptoms were tested as controls. Specific IgE concentration values of ≥0.10 kU/L were considered positive. On selected samples, a basophil activation test (BAT) was performed for a variety of fruits, including peach,

apple, and kiwi.

Results: Out of the 22 samples, 10 (45%) had specific IgE sensitivities to rMal d 4 and rBet v 2. Additionally, specific IgE quantitation for these two allergens demonstrated strong correlation: $r^2 = 0.99$. Eight out of 22 (36%) patients had documented clinical history of allergies to fruits from the *Rosaceae* family. One patient with a history of anaphylaxis specifically to peach was positive to nPru p 3. Two patients with anaphylactic histories to non-*Rosaceae* fruits such as kiwi were confirmed by elevated BAT. Three of the four control samples had no detectable specific IgE for all allergens tested. One control sample had low levels (<0.35 kU/L) of specific IgE to rPru av 1 and rBet v 2.

Conclusions: Known structural similarities between the profilins supports the high specific IgE correlation between rMal d 4 and rBet v 2 and may be useful for screening *Rosaceae* fruit allergens having the homogolous protein.

* Under development. Not available for sale in the U.S.

C-172

Performance Validation of BioPlexTM Anti-CCP Assay against INOVA QUANT LiteTM CCP-3 Assay

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Objective: The 2010 ACR/EULAR Rheumatoid Arthritis Classification Criteria include the laboratory test for antibodies to CCP (Cyclic Citrullinated Peptide). The aim of this study was to evaluate replacing the current INOVA anti-CCP3 enzyme-linked immunosorbent assay (ELISA) using the DXS Automated ELISA System with the BioRad anti-CCP assay run on the BioPlex 2200 automated multiplex flow immunoassay system.

Method: The BioRad anti-CCP IgG assay run on the BioPlex 2200 (Bio-Rad, Hercules, CA) system was evaluated for precision, accuracy, recovery, and linearity. The reference interval of this assay was validated using 40 rheumatoid factor negative patients. A parallel patient comparison of the BioPlex™ 2200 anti-CCP assay and the QUANTA Lite™ CCP3 ELISA (INOVA, San Diego, CA) was performed. The INOVA ELISA assay was performed on the DXS Automated ELISA System (Dynex Technologies, Chantilly, VA).

Results: Precision: The within run %CVs of ten replicates for the low and high controls were 5.6% and 4.6%, respectively. The inter-run precisions (N= 20) of these two levels of controls were 3.7% for the low control and 2.7% for the high control. Five levels of patient samples 1.0, 48.5, 96.0, 143.5, 191 U/mL were run in triplicate to evaluate the assay linearity, accuracy, and recovery. Linearity: Measured (y) = 0.9866Assigned (x) -1.413, $R^2 = 0.9977$. Accuracy and Recovery: The assigned (U/mL)/mean measured (U/mL)/% Recovery for the five level tested were: 1(U/mL)/1.3(U/mL)/113%; 48.5(U/mL)/44.0(U/mL)/90.7%; 96.0(U/mL)/93.47(U/mL)/97.4%; 143.5(U/mL)/140.9(U/mL)/98.2%; 191(U/mL)/187(U/mL)/97.9%. The mean recovery was 99.4%. The claimed assay sensitivity is 0.2 U/mL. To validate the claimed reference interval of 0.5 to 3.0 U/mL, forty rheumatoid factor negative samples were assayed. Sample results reported less than 0.5 U/mL were assigned the value of 0.5 U/mL for calculating the reference interval range. The calculated reference interval was from 0.5 to 1.6 U/mL. Parallel CCP results run by Bio-Rad and INOVA for 58 patients were evaluated. The overall concordance was 91%; negative agreement was 97.4%; positive agreement was 80%. The Cohen's Kappa was at 80% (>75% indicates "high" agreement). Four samples were positive by INOVA but negative by Bio-Rad and one was negative by INOVA but positive by Bio-Rad.

Conclusions: The precision, accuracy, recovery, linearity, and reference interval evaluated for the anti-CCP assay performed on the BioPlex™ 2200 were consistent with results reported in the package insert. Patient comparison indicated that the BioPlex assay was in high agreement with the current INOVA assay. This validation confirmed that Bio-Rad CCP assay by the BioPlex™ 2200 system can replace the current INNOVA CCP assay for clinical use.

Wednesday AM, July 18, 2012

Poster Session: 10:00 AM - 12:30 PM

Pediatric/Fetal Clinical Chemistry

C-174

Iron Deficiency Anemia In Ghanaian Infants.

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Background: Iron-deficiency anaemia (IDA) is a common problem all over the world, which mainly attack infants and children. Infants and toddlers are prone to developing iron deficiency anaemia (IDA). The main objective was to assess the prevalence of IDA in infants at the Korle Bu Teaching Hospital.

Methods: Hematological parameters and the iron status of 100 randomly selected infants who were attending the Child Health Laboratory of the Korle Bu Teaching Hospital were studied. Investigations included estimations of full blood count, (Sysmex KX- 21N Japan), serum iron (Fe), unsaturated iron-binding capacity (UIBC) and total iron-binding Capacity (TIBC). Statistical analysis was done by simple parametric method.

Results: 39% (39) of the infants had PCVs below 0.32, 47%(47) had Hbs below 10 g/dl and 26% (26)had mean corpuscular volume (MCV) less that 70fl. 36% (36)of the children had serum Fe below 3.58 mmol/l, but only 4% had UIBC above 320 mmol/l. 41% (41) had Transferin Saturation Index (TSI) below 10%. 17% (17) had MCV below 70fl associated with TSI below 10% and 67% of these had Hbs below 10 g/dl.

Conclusions: The prevalence of iron deficiency anaemia in infants as shown in this study is very high. The ill effects of iron deficiency in childhood have been well documented. It is suggested that screening for anaemia should be offered at 9 months and that infants found to be anaemic should be treated. However, for cost-effectiveness and taking into consideration the high prevalence rate of iron deficiency in this age group, it might be preferable to give iron routinely to infants aged 9 to 15 months.

C-176

Comparison of parasite lactate dehydrogenase based immunochromatographic antigen detection assay (optimal) with microscopy for detection of malaria parasites in children

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Background: Microscopic examination of blood smears is the widely used routine method for detection of malaria parasite and remains the gold standard for malaria diagnosis. But microscopic examination is laborious and requires considerable expertise for its interpretation. This study was done to compare microscopic examination of blood film with simple dipstick antigen-capture assay which detects the presence of parasite lactate dehydrogenase (PLDH) antigen of malaria parasite in lysed whole blood sample.

Methods: This study included 1603 patients (927 males and 678 females) attending the child Health Laboratory from January 2010 to December 2010. The patients were referred to the lab for investigation of malaria by different clinicians. From each patient 1.0ml of blood was collected into potassium EDTA tube. Thin and thick smear blood films were made, stained with Giemsa stain and examined for malaria parasite by light microscopy. Blood samples were tested with the OPtiMAL test (parasite lactate dehydrogenase based immunochromato-graphic antigen detection assay) according to manufacturer's instructions.

Results: A total of 1603 blood samples were tested for malaria parasites by the OPtiMAL method and the results were compared to those obtained from examination of thin and thick smear blood film. The blood film results indicated that 131 (8.2%) patients were infected with malaria and the rest 1472 (91.8%) were malaria negative. Among the positive patients *P. falciparum* was detected in 119 cases (90.8%), *P.malariae* in 6 cases (4.5%). and *P.ovale* in 3 cases (2.3%) with 3 cases (2.3%) of mixed infections of *P.falciparum* and *P.malariae* . Correspondingly, the OPtiMAL test results indicated that 122 (7.6%) of the patient samples were positive for malaria parasites and 1481 (92.4%) were malaria negative. Infection with *P. falciparum* accounted for 91.8% (112 of 122) while infection with other *Plasmodium species* accounted for 5.7% (7 of 122). Both methods identified 3 patients with mixed infection of *P.falciparum* and other *Plasmodium species*.

The blood film examination identified three *P.ovale* positive samples that were not detected by the OPtiMAL test, however, there was 100% agreement between blood film results and OPtiMAL results for the other 112 samples containing *P. falciparum*. Two cases of *P. falciparum* detected by OptiMAL were not detected by the blood films and three cases of *P. falciparum* detected by blood film were not detected by OPtiMAL method. OPtiMAL had sensitivities of 96.8% and 88.4% when compared to traditional blood films for detection of *P.falciparum* and other *Plasmodium* infections

Conclusions: The antigen detection test identified 7.6% as malaria positive while the blood film identified 8.2% to be malaria positive. Some malaria infections detected by blood film were not detected by the OptiMAL test. Since OPtiMAL detects PLDH which is produced only by living parasites, the blood samples judged negative by OPtiMAL may have been dead parasites and not yet cleared from the host.This evaluation has shown that OPtiMAL is a simple, sensitive and effective diagnostic test for *Plasmodium infections*. The sensitivity of this test is very close to microscopic examination of blood smears but does not require highly skilled personnel to perform or interpret results.

C-177

Study Of Hospital Based Malaria Cases In The Pediatric Department Of Korle Bu Teaching Hospital, Ghana

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Background: Malaria kills about one million children, under five years of age, each year worldwide, with nine out of 10 deaths occurring in sub-Saharan Africa.

Rapid diagnosis is a prerequisite for the initiation of effective treatment and to reduce the mortality and morbidity of malaria. Microscopic examination of blood smears remains the gold standard for the diagnosis of malaria, but it is time-consuming and requires skilled microscopist.The most promising new malaria diagnostics are the serological dipstick tests.

This study was carried out to determine the incidence of malaria in the pediatric department of Korle Bu Teaching Hospital from January 2011 to October 2011, and to compare available diagnostic tests for malaria.

Methods: 978 suspected cases of malaria (507 males and 471 females, aged 1 day - 12 years), attending the Outpatient Department and admitted as inpatients in the Emergency Room of the Pediatric Department of Korle Bu Teaching Hospital were included in this study. 1.0 mls of blood sample was collected into EDTA bottle from all febrile cases clinically suspected of malaria, before starting any treatment. Thick and thin smears were prepared, stained and examined. Subsequently, the blood samples were subjected to antigen detection using the First Response Malaria pLDH/ HRP 2 Combo Test according to the manufacturer's instructions. The results were tabulated and analyzed statistically.

Results: 51 cases out of 978 suspected cases were positive for malaria, with an incidence of 5.2%. Out of these 40 (78.4%) were positive for *Plasmodium falciparum*, 5 (9.8%) were positive for *Plasmodium malariae*, 2 (3.9%) were positive for *Plasmodium ovale*, and 4 (7.8%) were positive for both *Plasmodium falciparum* and *Plasmodium malariae*. The First Response Malaria pLDH/ HRP 2 Combo Test detected 51 positive cases compared with the blood smear study, which detected 41 cases. 36 cases were detected both by the First Response Malaria pLDH/ HRP 2 Combo Test and blood smear study. 15 cases were positive by the First Response Malaria pLDH/ HRP 2 Combo Test, but not by the blood smear study. 5 cases detected to be positive by the blood smear study were found to be negative by the First Response Malaria pLDH/ HRP 2 Combo Test. 937 cases were negative both by the First Response Malaria pLDH/ HRP 2 Combo Test and the blood smear study. Among 51 positive cases, 35 were males with a percentage of 68.6% as compared to females (31.4%).The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency of the First Response Malaria pLDH/ HRP 2 Combo Test when compared to microscopy, were 87.5, 96.8,90, 98.9, and 96%, respectively.

Conclusions: The incidence of malaria in this present study was 5.2%. The sensitivity of First Response Malaria pLDH/ HRP 2 Combo Test is very close to microscopy and it does not require highly skilled personnel to perform or interpret results. Therefore, First Response Malaria pLDH/ HRP 2 Combo Test is a simple, sensitive, and effective diagnostic test for *P. falciparum*, *P. malariae, P. vivax* and *P. ovale* malaria.

C-178

Stability of serum Pregnancy-Associated Plasma Protein-A for screening Down syndrome in a population screening program

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Background: Pregnancy-Associated Plasma Protein-A (PAPPA) has been reported to be a reliable biomarker associated with Down syndrome in high risk pregnancy. The State of California Prenatal Screening Program annually tested over 250,000 cases of pregnancy for Down syndrome in the early stage of pregnancy. The serum samples for Down syndrome screening are collected from pregnant mothers during the first trimester period (gestational age of 10-14 weeks) when the mothers enrolled in the Prenatal Screening Program. The serum samples are then sent by courier to testing laboratories for analysis. From time to time, there is a need for the laboratories to retest the maternal sera.

Methods: The objective of this study is to determine the stability of Pregnancy-Associated Plasma Protein-A (PAPPA) in the original blood collection tubes when the serum samples are stored under refrigeration or stayed frozen at -20 degrees C. The analytical method for testing PAPPA in maternal sera is by means of an automated fluorescent immunoassay (Perkin Elmer AutoDelfia® Immunoassay System) and maternal sera samples are collected in 3.5 mL Becton Dickinson Vacutainer® SST™ tube, containing separator gel. After clot formation, the blood specimens are centrifuged at 1000 x g for 10 minutes at the blood collection station to assure adequate separation of blood clot and serum, before sending the samples to laboratories for testing.

Results: Our data indicated that the PAPPA concentration obtained initially when samples first arrived at the testing laboratory, are statistically the same as PAPPA concentration obtained after storing the serum tubes at refrigeration for 7 days (n= 93, correlation coefficient= 0.999, Student T test= 0.02) or when stored frozen at -20 degrees C for thirty days (n=105, correlation coefficient= 0.999, Student T test=8.3E-13).

Conclusions: We concluded that Pregnancy-Associated Plasma Protein-A (PAPPA) are stable when stored in the original blood collection tubes under the storage conditions cited in this study and are suitable for repeat analysis in the laboratories.

C-179

Analysis of Vanilmandelic Acid (VMA) and Homovanillic Acid (HVA) by LC/MS/MS in serum for diagnostic testing for neuroblastoma

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Background: VMA and HVA are typically measured in urine for aid in diagnosis and monitoring remission and relapse of neuroblastoma, a tumor in children <6 yr. A protocol for evaluation of serum VMA and HVA (as opposed to urine) has been utilized at our institution for approximately 25 years, originally validated by HPLC with an electrochemical detector. In order to improve specificity of analysis and gain efficiency, we validated a serum VMA/HVA method on the Waters Xevo UPLC/MS/MS. Reference range assessment was also performed using samples from 47 pediatric patients without neuroblastoma.

Methods: Extraction was performed on 0.5 mL serum with ethylacetate at acid pH. Samples were dried under nitrogen at 60° C, reconstituted in 10% acetonitrile, and filtered with Millipore Ultrafree centrifugal filters. Stable isotope labelled internal standards (d_3VMA & $^{13}C_6$-^{18}O-HVA) were added prior to extraction. Analysis was performed on a Waters Xevo TQ MS tandem mass spectrometer coupled to Acquity UPLC. Separation was achieved on a Waters Acquity HSS T3 column at 45 °C by an acetonitrile/water (0.1% formic acid) gradient. Total run time was 8min ; 1.3 min for HVA, and 2.7 min for VMA. Analytes were quantified in electrospray negative ionization mode by multiple reaction monitoring (MRM) using two transitions per compound.

Results: VMA and HVA were calibrated with five standards and linearity demonstrated over a range of 2 to 1000 ng/mL. Signal to noise at the lower limit of quantitation was ≥15. Within run and run to run imprecision was < 5% for VMA at levels of 22 ng/mL and 105 ng/mL; < 20% at a level of 5 ng/mL and 6% at a level of 100 ng/mL for HVA. Correlation with HPLC was performed using 45 previously assayed patient samples; HVA (y= 0.636x + 2.346; R^2 = 0.9922) and VMA (y = 0.7731x - 8.696; R^2 = 0.991). Based on linear regression and normal samples assayed, the reference interval for VMA by UPLC/MS/MS was ≤ 20 ng/mL (95% CI 2.9 - 20.3), and HVA ≤30 ng/mL (95% CI = 2.6 - 29.7).

Conclusions: Correlation of VMA and HVA was acceptable, although adjustment of reference intervals was necessary. Of 45 patient samples, seven had elevated VMA and 11 had elevated HVA by both HPLC and LC/MS/MS; there were no discrepancies in classification (normal vs abnormal) after reevaluation of reference intervals. Sensitivity and range for both VMA and HVA was adequate; lowest and highest patient sample was 5 ng/mL and 1289 ng/mL for VMA, and 2.6 ng/mL and 7342 ng/mL for HVA. Previous correlation performed with 24 hour urine and paired serum samples showed no discrepancy in interpretation. Collection of a single serum sample instead of 24 hour urine collection saves time and improves accuracy of measurement due to difficulty of collection of a 24 hour urine sample in a pediatric population.

C-180

Impact of a universal inpatient transcutaneous bilirubin screening program on the distribution of serum bilirubin values among healthy neonates at a single institution

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Objective: To determine whether implementation of a universal inpatient transcutaneous bilirubin screening program changed the distribution of serum bilirubin values obtained on healthy neonates who had bilirubin values measured either in the inpatient (well-baby nursery) environment or after hospital discharge at outpatient follow-up.

Methods: During Period 1 (April 2008-January 2009) serum bilirubin levels were ordered on neonates in the well-baby nursery at the discretion of the healthcare provider. During Period 2 (March 2009-January 2010) all infants in the well-baby nursery had BiliChek (Phillips Healthcare, Andover MA) transcutaneous bilirubin screening performed. Infants with adjusted TcB values in the high-intermediate or high risk category for postnatal age based upon a serum bilirubin nomogram had a confirmatory serum bilirubin performed. Subsequent serum bilirubin measurement was at the discretion of the healthcare provider.

During both periods, infants discharged from the well-baby nursery had serum bilirubin ordered at outpatient follow-up visits at the discretion of the healthcare provider. In addition, during Period 2 infants discharged from the nursery with high-intermediate or high risk serum bilirubin followed a discharge protocol with pre-ordered serum bilirubin levels at outpatient visits 2-5 days after discharge. All serum bilirubin testing was performed on a Roche Modular Analytics system (Roche Diagnostics, Indianapolis IN). For both periods we separately determined median (interquartile range, IQR) serum bilirubin values for inpatients and outpatients; and the number (percent) of serum bilirubin values > 20 mg/dL. The Mann-Whitney test was used to assess for statistically significant differences in median bilirubin values between pre and post- TcB screening periods.

Results: Universal TcB screening reduced the median (IQR) serum bilirubin value among inpatients from 10.2 (8.5-12.2) mg/dL during Period 1 to 9.3 (7.5-11.5) mg/dL during Period 2 (p < 0.0001) (n=670 Period 1 and 805 Period 2). Inpatient TcB screening had no effect on the distribution of serum bilirubin values obtained at outpatient follow-up visits; with median (IQR) outpatient serum bilirubin values of 14.0 (12.1-16.1) mg/dL during Period 1 and 13.9 (11.7-16.1) mg/dL during Period 2 (p = 0.6125) (n= 405 Period 1 and 569 Period 2). The overall (inpatient plus outpatient) median serum bilirubin value decreased slightly from 11.6 (9.3-14.1) mg/dL during Period 1 to 11.1 (8.5-13.8) mg/dL during Period 2 (p=0.0009). No inpatients had serum bilirubin values > 20 mg/dL. Among outpatient values there were 11/405 (3%) during Period 1 vs. 8/569 (1%) during Period 2 that exceeded 20 mg/dL.

Conclusions: Implementation of a universal inpatient TcB screening program decreased the median serum bilirubin value observed on healthy neonates in a well-baby nursery, likely due to earlier intervention in nursery infants with hyperbilirubinemia. TcB screening improved the safety of patient care as measured by median inpatient serum bilirubin values and the number (percent) of infants with high (> 20 mg/dL) serum bilirubin values observed at outpatient follow-up.

C-181

Quantitative Real-Time PCR as a Rapid Prenatal Diagnostic Method for Sex Chromosome Aneuploidies Detection.

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Background: The high prevalence and variable phenotype of sex chromosome aneuploidies, necessitates the development of a robust method allowing rapid prenatal diagnosis. Quantitative real-time PCR , being an accurate and precise tool for the determination of template copy number, represents a potential option for sex chromosome copy number detection in laboratories lacking sequencing facilities. Quantitative real-time PCR is characterized by high throughput , fast turnaround time, accurate quantification over a wide concentration range and low cost. Aim of this work is the development of a quantitative real-time PCR (qPCR)-based method for rapid prenatal detection of chromosome X and Y copy number in normal and sex chromosome aneuploidy cases.

Methods: The analysed prenatal samples included 40 control samples from normal male [n=18] and female [n=22] fetuses and 12 sex chromosome aneuploidies including Turner syndrome [n=6], Klinefelter syndrome (47,XXY) [n=2], 48,XXXY [n=1] and triple X chromosome [n=3]. Sex chromosome copy numbers detection was done through assessment of dosage ratio of the coagulation factor VIII, procoagulant component (F8) gene, mapped to chromosome X and SRY gene mapped to chromosome Y using qPCR-ΔΔCT method for relative quantification. qPCR results were compared with the original conventional cytogenetic results.

Results: The method was able to determine copy numbers of chromosome X and Y correctly in all normal samples as well as sex chromosome aneuploidy samples. The method showed 100% sensitivity and 100% specificity for sex chromosome copy number detection.

Conclusions: qPCR represents a reliable molecular-based rapid prenatal diagnostic method for sexing and sex chromosome aneuploidy detection.

C-182

Evaluation of Carbohydrate-deficient Transferrin (CDT) as Screening Tool for Congenital Disorder of Glycosylation

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Objectives: Carbohydrate-deficient transferrin (CDT) has been proposed as the most specific biomarker of chronic alcohol misuse and can reflect the severity of alcohol dependence. Recently, discussions have been raised to the usage of CDT as a solo biomarker to screen for congenital disorders of glycosylation (CDG) where around 30 different types been reported worldwide. In this study we are evaluating the positive and negative predictive values (PPV, NPV) of CDT in detecting the CDG in our population.

Methods and patients: Blood samples were collected from 111 children who have visited our metabolic clinics at King Fahad National Guard Hospital between September 2006 and December 2009 with a suspected diagnosis of congenital disorders of glycosylation, CDG. Liver function tests were performed locally at our laboratory. The serum samples for measuring CDT were sent to two different laboratories, one of them used the HPLC (26 samples) and the other used the capillary electrophoresis (85 samples). The normal range for CDT is less than 1.7%. Two cut-off points 1.7% and 2.5% were used to evaluate CDT blood level in the suspected patients with CDG.

Results: Age of children ranged from less than one year up to 15 years and they were 62 (57%) female and 49 (43%) male. Ten samples (9%) were found to be abnormal, in which six of them were above the cut-off value 2.5 % and four samples were lying in borderline range. The diagnosis of CDG type Ia and IL has been confirmed in two patients, their CDT values were 35% and 5.8% respectively. Two patients were diagnosed with galactosemia, with CDT values 7.6% and 4.4%. One patient with CDT value of 2% was diagnosed to have glycogen storage disease type IX, and the other patient with CDT value 1.8% was diagnosed to have a progressive familial intra-hepatic hypercholesterolemia. No solid diagnosis has been confirmed in the remaining four patients with abnormal CDT values. The sensitivity, specificity, PPV, and NPV for CDT at cut-off value 1.7% were found to be 100%, 92%, 20% and 100% respectively however, when we increased the cut-off value to 2.5%, only the specificity and PPV have increased to 96% and 33.3% respectively.

Conclusions: CDT can be used as screening tool to diagnose congenital disorders of glycosylation. The false positive cases can be seen in any patient who has a disorder associated with liver dysfunction. Therefore, CDT can be used as a good predicting test in ruling out CDG.

C-183

Validity of Calculating Pediatric Reference Intervals using Hospital Patient Data: A Comparison of the Modified Hoffman Approach to CALIPER Reference Intervals Obtained in Healthy Children

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Objectives: To compare pediatric reference intervals calculated using hospital-based patient data with those calculated using samples collected from healthy children in the community.

Methods: Hospital-based data for 13 analytes (calcium, phosphate, iron, ALP, cholesterol, triglycerides, creatinine, direct bilirubin, total bilirubin, ALT, AST, albumin and magnesium) collected between 2007 and 2011 were obtained. The data for each analyte were partitioned by age and gender as previously defined by the CALIPER study. Outliers in each partition were removed using the Tukey method. The cumulative frequency of each measured value was calculated and plotted. Piece-wise regression determined the linear portion of the resulting graph using the statistical software R. Linear regression determined an equation for the linear portion in each partition and reference intervals were calculated by extrapolating to identify the 2.5th and 97.5th centiles in each partition. Using the reference change value (RCV) as criteria, these calculated reference intervals were compared to those reported previously as part of the CALIPER study.

Results: In general, the reference intervals calculated from hospital-based patient data were similar, however the absolute reference interval values for some analytes were lower than those calculated by CALIPER. Within an analyte, the reference intervals for certain age or gender partitions correlated well with the CALIPER-calculated intervals while for other partitions they did not agree.

Conclusions: These results suggest that calculating pediatric reference intervals from hospital-based data may be useful, as a guide, in some cases but will likely not replace the need to establish reference intervals in healthy pediatric populations.

C-184

Pediatric reference intervals for four serum bone markers on two automated analyzers

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Bone remodeling is a process that occurs throughout ones life. However the rate at which bone formation and resorption occurs varies based upon age and other factors. Our aim was to establish pediatric reference intervals for four different bone makers using two automated platforms. We analyzed three bone formation markers: bone-specific alkaline phosphatase (BSAP), osteocalcin and procollagen type 1 N-terminal propeptide (P1NP), and one resorption marker C-telopeptide (CTx). Osteocalcin, P1NP and CTx were all tested on the Roche Modular Analytics E170 and BSAP was analyzed on the Beckman Coulter UniCel®DxI 800Immunoassay System. The study included 531 females and 546 males from 6 months through 6 years of age. Subjects undergoing an elective surgical procedure at Primary Children's Medical Center were enrolled after obtaining IRB approval and with parental permission. Blood samples were collected, allowed to clot for 30 minutes and centrifuged. Specimens were de-identified and stored in liquid nitrogen prior to testing. Patients were excluded if they were taking any prescription medications. An aliquot was thawed, mixed, and centrifuged prior to analysis. Results were partitioned by age and gender. Non-parametric 95% reference intervals were established when possible. Log-transformed parametric reference intervals were established when insufficient sample size was available ($n<120$). A summary of the reference intervals partitioned by age and gender is provided in the table. Significant differences were observed between boys and girls. Serum BSAP decreased from 1 to 5 years with a spike in the upper reference limit at age 1 for both genders. The median osteocalcin results for girls are slightly higher than median results for boys from ages 2 to 6 years. P1NP serum concentrations decreased until age 3 for both boys and girls. The reference intervals established for children 6 months to 6 years is novel due to the lack of data available for children.

Analyte/Age	Girls				Boys				
	N	Median	2.5th percentile (90% CI)	97.5th percentile (90% CI)	Age	N	Median	2.5th percentile (90% CI)	97.5th percentile (90% CI)
Osteocalcin (ng/mL)									
6 months - 11.9 months	60	69.55	39.84 (34.85 - 45.35)	133.73 (121.19 - 147.22)	6 months- 1 years	146	72.61	45.13 (43.45 - 48.66)	135.29 (111.90 - 142.45)
1 years	65	69.96	37.79 (33.54 - 42.44)	117.85 (107.76 - 128.63)					
2 years	73	72.73	44.00 (40.16 - 48.11)	113.55 (105.69 - 121.85)	2 years	72	67.62	36.45 (32.44 - 40.83)	117.00 (107.27 - 127.38)
3-4 years	143	72.22	44.70 (29.78 - 46.94)	128.27 (112.20 - 140.30)	3-4 years	150	65.44	30.91 (11.06 - 38.74)	115.73 (96.04 - 123.10)
5 years	74	72.50	44.32 (40.41 - 48.50)	116.18 (108.07 - 124.73)	5-6 years	143	69.64	39.55 (37.07 - 48.57)	115.58 (108.60 - 148.90)
6 years	76	77.74	44.92 (40.74 - 49.42)	125.21 (116.11 - 134.83)					
P1NP (ng/mL)									
6 months - 11.9 months	59	1245.00	903.4 (848.4 - 961.9)	1762.8 (1655.5 - 1877.0)	6 months - 11.9 months	73	1269.00	833.4 (778.9 - 891.6)	1856.2 (1735.0 - 1985.9)
1 years	65	968.20	587.1 (539.7 - 638.7)	1504.7 (1383.3 - 1636.8)	1 years	73	1040.00	643.3 (597.2 - 692.9)	1551.9 (1440.8 - 1671.5)
2 years	73	726.30	421.1 (385.8 - 459.5)	1186.6 (1087.3 - 1294.9)	2 years	72	661.55	384.9 (351.0 - 422.0)	1139.6 (1039.3 - 1249.7)
3-4 years	142	573.05	313.9 (289.0 - 365.7)	1020.6 (897.4 - 1090.0)	3 years	73	562.80	302.6 (275.0 - 332.8)	938.4 (853.0 - 1032.3)
5 years	74	505.35	344.7 (321.6 - 369.4)	787.9 (735.2 - 844.4)	4-6 years	220	519.85	303.7 (277.5 - 339.3)	845.2 (785.9 - 965.5)
6 years	76	537.95	324.0 (297.9 - 352.3)	895.2 (823.1 - 973.7)					

C-185

Correlation between cystatin C values and conservative treatment of chronic renal failure (CKD) in pediatric patients.

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Introduction: The prevention of complications of pediatric chronic kidney disease is one of the objectives of the pediatric nephrologist today. The need for a simple marker of GFR, accurate and minimally invasive, remains a limiting factor in clinical practice to assess renal function. Cystatin C (Cys C) is an unglycosylated protein of low molecular weight, synthesized in all nucleated cells in the body, which has a wide tissue distribution, and more accurate in the pediatric population. After correlating the GFR by Cys C and creatinine (Cr) in a pediatric population since two years, we found that the GFR with Cys C is better than GFR with Cr to discriminate the early stages of CKD.

Patients and Methods: Followed 104 children diagnosed with pediatric nephrology chronic kidney disease (stages I, II, III, pre-dialysis), which is determined by comparing creatinine and cystatin C for assessment of GFR. Added to the evaluation values of weight, height, BMI, renal function, microalbuminuria, blood pressure, and the treatment specified: antiproteinuric, antihypertensives, chelators, EPO and iron therapy as well as vitamin D. Cystatin C in serum was determined by particle enhanced immunonephelometry with the BNII (Siemens).

Results: Of the 104 children studied by evaluating the GFR by Cr, found the following **Results:** 54% in the stage of the IRC I, stage II 35% and 7% in stage III.GFR by Cys C, 70% would be in stage I, 16% in stage II and 7% in stage III. If we relate the analytical parameters depending on the clinical severity, the observation of those patients were classified into stages of gravity lower by Cys C, corresponded to patients which requiring minor or no treatment. Although there were children classified as stage I by Cr were classified by cystatin C is stage II and correlating with the clinic, they actually needed more treatment.

Conclusions: Cys C is more effective to determine staging of pediatric chronic renal patients and it can be achieved better clinical correlation with the need for treatment. The glomerular filtration rate with cystatin C in pediatric patients is better indicator the early stages of CKD than GFR using Cr, leading to changes in the monitoring, treatment and prognosis.

C-186

Urinary Cystatin C in patients with Dent disease

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Background: Dent disease is an X-linked proximal tubulopathy with characteristic abnormalities that include low molecular weight proteinuria, hypercalciuria, and nephrolithiasis. In 1996, *CLCN5*, which encodes voltage-dependent chloride channel 5, was identified as a gene responsible for Dent disease. In 2005, Hoopes et al. identified *OCRL1* as a second gene responsible for Dent disease. Dent disease caused by a *CLCN5* mutation is assigned as Dent disease-1, while that caused by an *OCRL1* mutation is assigned as Dent disease-2. We analyzed urinary Cystatin C (Cys-C) in these patients using cellulose acetate electrophoresis (CAE), sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE), and western blotting (WB).

Methods: Spot urine samples were collected from 6 boys with Dent disease-1, 1 boy with Dent disease-2, and 2 boys who were undergoing genetic screening. Urinary Cys-C levels were determined using enzyme linked immunosorbent assay (ELISA). The urine samples were analyzed by CAE and stained with colloidal silver. SDS-PAGE followed by WB was performed to determine the molecular heterogeneity of urinary Cys-C.

Results: CAE of the urinary protein fraction yielded 2 Cys-C bands at the application point for all patients with Dent disease. Cys-C bands corresponding to various molecular sizes were detected by WB for both patients with Dent disease-1 and Dent disease-2. However, the 51.1-kDa band of Cys-C was not present for patients with Dent disease-2.

Conclusions: From the results of this study, we concluded that the presence of 2 Cys-C bands on CAE was characteristic of Dent disease and that the molecular heterogeneity of Cys-C as determined by SDS-PAGE and WB helps in distinguishing Dent disease-1 from Dent disease-2.

C-187

Soluble CD14-Subtype (sCD14-ST) Presepsin in critically ill preterm and term newborns for the early assessment of neonatal sepsis: preliminary results

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Background: Neonatal sepsis continues to be one of the most significant causes of neonatal morbidity and mortality. Early identification of neonatal sepsis is a major diagnostic problem because of the nonspecific clinical signs and limitations of the current diagnostic procedures. Recently, it was reported that the soluble fraction of CD14 may be a very early, specific biomarker of systemic inflammation and sepsis due to bacterial infection. CD14 is a glycoprotein expressed on the surface membrane of monocytes/macrophages (mCD14) and serves as bacterial lipopolysaccharides receptor. The complex LPS-CD14 (-LBP) is released into circulation, where plasma protease activity originates the soluble CD14 subtype (sCD14-ST) or presepsin. The commercial availability of a very rapid and accurate analytical method for measuring sCD14-ST presepsin calls for clinical studies investigating the potential role of this

biomarker in patients with systemic inflammation, sepsis, and severe sepsis. Moreover, there is the need to assess the potential role of sCD14-ST presepsin in predicting outcome in comparison with traditional sepsis and inflammation biomarkers.

Objective: the aim of this study was to evaluate the clinical value of sCD14-ST presepsin in critically ill newborns, admitted in Neonatal Intensive Care Unit (NICU).

Methods: This preliminary study was performed on 30 samples belonging to 13 newborns with gestational age ranging 27 to 36 weeks, admitted on the Pediatric Division, Cagliari. Newborns were divided in two groups: 6 newborns with systemic inflammation/sepsis microbiologically confirmed (gropu A, 15 serum samples) and 7 without sepsis (group B, 15 serum samples). In all the samples we measured C-Reactive Protein (CRP) and sCD14-ST presepsin. CRP was measured by immunonephelometry on the BN II (Siemens Healthcare Diagnostics, Milan, Italy); sCD14-ST was measured by a rapid chemiluminescent enzyme immunoassay on the fully automated PATHFAST® immunoanalyzer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan).

Results: In group A, CRP and sCD14-ST mean values were 50 mg/L and 1578.7 mg/L, respectively (median and interquartile range: CRP 19.5 mg/L, 9.5-72 mg/L; sCD14-ST 1070 mg/L, 880.5-1759 mg/L). In group B, CRP and sCD14-ST mean values were 30.5 mg/L and 638.0 mg/L, respectively (median and i.r.: CRP 14 mg/L, 6.0-22.0 mg/L; sCD14-ST 628 mg/L, 562-736 mg/L). By using the Mann-Witney-U test we found a statistically significance difference between groups for sCD14-ST (p=0.0053) but not for CRP (p=0.327). One baby enrolled in group A died to septic shock 2 days after admission in NICU. In that baby, CRP values were found very increased (up to 65 mg/L) whereas sCD14-ST did not exceed 373 mg/L. However, subsequent investigations together with histology have demonstrated the presence of a disseminate infection from Echo virus 11.

Conclusions: our preliminary results suggest a potential interesting prognostic value for sDC14-ST presepsin. In particular, sCD14-ST strongly correlated with the severity of sepsis in all the babies. More important, sCD14-ST did not significantly increase during a viral infection leading to septic shock and death. This result may support the hypothesis on the high specificity of this new marker in assessing bacterial infections and sepsis.

C-188

Maternal serum human Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Matrix MetaloProteinase-9/NGAL complex levels in pregnancies complicated with pre-eclampsia

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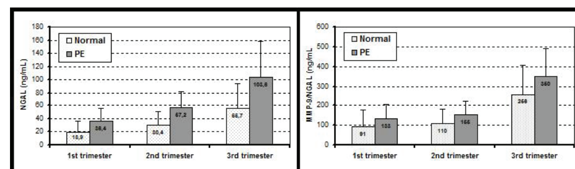
Background: Human Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a low molecular weight protein which belongs to the lipocalin family and it is secreted by various types of human tissues. Although its exact pathophysiological role is not yet fully elucidated, it is considered to bind many other molecules, including matrix metalloproteinase-9 (MMP-9). The MMP-9/NGAL complex protects MMP-9 from autodegradation and increases its activity. In two studies up to now, maternal serum NGAL levels have been reported elevated in pre-eclampsia (PE) compared to normal pregnancies. On the contrary, the complex MMP-9/NGAL has not measured yet in pregnancies with pre-eclampsia.

Methods: In 31 normal pregnancies and 9 pregnancies that developed PE, maternal serum NGAL concentrations were retrospectively determined in the 1st (11-14 weeks), 2nd (20-24 weeks) and 3rd (28-34 weeks) trimester of pregnancy. In 17 of the normal and all the pre-eclamptic pregnancies the complex MMP-9/NGAL was also determined. Both substances were measured with ELISA kits (R&D Systems USA).

Results: Mean (±SD) concentrations of NGAL (ng/mL) in normal pregnancies were: 18.9±16.8; 30.4±20.2; 55.7±38.4 in the three trimesters respectively. In PE pregnancies NGAL concentrations were significantly increased compared to normal pregnancies in all trimesters: 1st (36.4±19.1, P=0.01), 2nd (57.2±24.6, P=0.002) and 3rd (103.6,8±54.3, P=0.02). The mean (±SD) concentrations of MMP-9/NGAL complex (ng/mL) in normal pregnancies were: 91.1±85.4; 110.0±70.9; 256.3±149.3 in three trimesters respectively. In PE pregnancies the concentrations of MMP-9/NGAL complex didn't differ significantly from the concentrations in normal pregnancies in any trimester: 1st (133.3±70.5), 2nd (154.6±66.4), and 3rd (349.5±143.0).

Conclusions: Maternal serum NGAL increases throughout normal pregnancy. In pregnancies that developed PE, NGAL was significantly increased in all trimesters compared to normal pregnancies. In our study, we didn't find significant differences in MMP-9/NGAL complex between normal pregnancies and pregnancies that developed PE, in any trimester.



C-189

An Evaluation of Lamellar Body Count as a Surrogate Assay for the Abbott FLM-II for the Assessment of Fetal Lung Maturity.

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Background: Accurate prediction of fetal lung maturity (FLM) is essential for limiting the risk of respiratory distress syndrome in newborns. Gluck et al. first described the analysis of the lecithin/sphingomylin (L/S) ratio in amniotic fluid which soon became the "gold standard" for the assessment of FLM. However, the L/S ratio assay is time consuming and technically difficult to perform. The Abbott FLM-II assay, which measures the phospholipid-surfactant/albumin ratio in amniotic fluid, has excellent positive predictive value for FLM and has been our laboratory's primary screening assay used in conjunction with the L/S ratio. Accordingly, when Abbott announced they would no longer be supporting the FLM-II assay on their automated TDx fluorescence polarization immunoassay analyzer, we were forced to consider other alternatives which would allow us to continue offering the same level of service to our obstetric clinicians. Currently there is no FDA approved commercial alternative for the FLM-II assay. However, measuring lamellar bodies as a form of stored surfactants has been found to be a reliable predictor of fetal lung maturity, but its clinical correlation with FLM-II and L/S ratio are still unclear. Fortunately, the similarity in size of lamellar bodies to platelets permits the use of automated hematology analyzers to measure their count in amniotic fluid. The present study describes our results for lamellar body count (LBC) performed on our Coulter LH 780 hematology analyzer and correlated with results from the FLM-II and the L/S ratio.

Methods: Our correlation study consisted of fifty-one samples tested by both LBC and FLM-II; seventeen samples were also confirmed by L/S ratio (Helena Fetal Tek 200). Our observed clinical ranges for this study included: 1) LBC - immature < 20 k/mm^3 and mature >= 50 k/mm^3 and 2) FLM-II - immature < 40 mg/g and mature >= 50 mg/g.

Results: Correlation studies yielded the linear regression equation LBC = 0.99 * TDx - 3.0; r^2 = 0.50 and $S_{y,xy}$ = 0.14. While there was less than optimal correlation between the two methods, overall concordance in diagnosis was 80% (41/51). On the seventeen samples on which both LBC and L/S was performed, linear regression yielded r^2 = 0.66 and $S_{y,xy}$ = 2.11, reflecting a concordance level of 94% (16/17). Analytical sensitivity of LBC was found to be 1 k/mm^3. Regarding interferences, meconium and platelets may falsely increase LBC whereas mucous and blood may falsely lower the count.

Conclusions: We observed acceptable linear correlation between LBC and FLM-II values with good diagnostic concordance. Indeed, in no instance was there a diagnostic discrepancy when the indeterminate range was taken into account. Based on these findings, our laboratory has replaced the FLM-II assay with LBC for fetal lung maturity screening whereby we only perform the traditional L/S ratio when indicated by a LBC falling in the indeterminate range of 21 to 49 k/mm^3.

C-190

First Trimester Pregnancy Associated Plasma Protein A As A Marker For Preterm Labor In Patients With Rupture Of Membranes

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Background: Preterm labor is one of the most common reasons for hospitalization of pregnant women, but identifying women with preterm contractions who will deliver preterm is an inexact process. Moreover, infants born between 36 and 38

weeks continue to experience increased morbidity and mortality and thus constitute a population at risk. Induction of labor or cesarean delivery in the absence of labor should be scheduled only after 39-0/7 weeks' gestation because maternal and infant morbidity and mortality are significantly lower after 39-0/7 weeks'.The purpose of this study was to determine whether pregnancy associated plasma protein A (PAPP-A) can be used to identify pregnancies at risk for preterm labor before 39-0/7 weeks' among patients with rupture of membranes.

Methods: The study population that was available for this analysis included 90 singleton pregnancies whom PAPP-A level, free beta subunit human chorionic gonadotropin (fBhCG), nuchal translucency, and pregnancy outcome data were available from combined first trimester screening over a 1-year period. Blood samples were analyzed for PAPP-A level (mUI/mL) with an immunoassay system (Immulite 2000, Siemens, Germany). We excluded pregnancies with aneuploidy, major anomalies, fetal infection, or second trimester premature rupture of membranes (PPROM). Rupture of membranes (ROM) was defined as the rupture of the membranes before the onset of labor between 37 and 38 weeks of pregnancy. We chose the fifth and twenty fifth PAPP-A percentiles as categorical marker for low PAPP-A which correspond to approximately 0.27 and 0.52 MoM, respectively. First, a series of statistical tests were performed to assess relationships with each marker and, in addition, with ROM. Fisher´s exact test and chi-squared test were used for categorical variables and Mann-Whitney test was used for continuous variables.

Results: The mean maternal age at time of blood sampling was 30.9 ± 4.95 years, with a range of 17 to 42 years. All of women were white. Fourteen pregnancies in our study presented ROM between 37 and 38 weeks of pregnancy and thirty after 39-0/7 weeks'.There were no significant relationships between PAPP-A levels and ROM (p=0.790). PAPP-A levels in pregnancies with rupture of membranes before 39-0/7 weeks were lower than pregnancies with rupture of membranes after 39-0/7 weeks, nevertheless this relationship was not significant (p= 0.186).

Conclusions: Low pregnancy associated plasma protein A levels in the first trimester were not associated with rupture of membranes between 37 and 38 weeks of pregnancy in our study. However, we found a trend towards an increased rate of premature rupture membranes in pregnancies with PAPP-A below the twenty fifth percentile.

C-191

Development of selected reaction monitoring assays for quantification of biochemical markers of Down syndrome in amniotic fluid samples

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Background: Down syndrome (DS) is a common birth defect, the most frequent and most recognizable form of mental retardation, appearing in about 1 of every 700 newborns. At present, several screening strategies for the detection of DS are used. These methods are based on the combination of maternal age with several serum and sonographic markers. The calculation of a patient-specific risk allows detecting until 90-95% of DS cases for a 5% false positive rate (FPR). However, since these screening strategies lack diagnostic power, a sizeable number of patients are under go invasive procedures to obtain a diagnostic result. The aim of this study was to discover new biomarkers of DS in amniotic fluid (AF) samples from women carrying fetuses affected by this genetic condition. Thus, mass spectrometry-based selected reaction monitoring (SRM) assays were developed to quantify seven proteins: bile salt-activated lipase (CEL), carboxypeptidase A1 (CPA1), mucin 13 (MUC13), chloride channel accessory 1 (CLCA1), mucin 5AC (MUC5AC), palate, lung and nasal epithelium associated protein (PLUNC) and hyaluronan and proteoglycan link protein 1 (HAPLN1).

Methods: The selection of proteins was based on three criteria: relative abundance in AF, differential expression between DS and chromosomally normal (CN) samples, according to our previous semi-quantitative analysis, and tissue-specificity. Proteins with a moderate or high concentration in blood were avoided. Besides, two more proteins were analyzed: chorionic gonadotropin, beta polypeptide (CGB), as positive control, and transferrin (TF), as negative control. To develop the SRM methods, one unique peptide (proteotypic) for each protein was selected and isotopically labeled peptides were spiked into the AF samples. A 35 min. gradient in an EASY-nLC pump (Proxeon A/S) was used to elute the peptides and quantification was carried out in a TSQ Vantage (Thermo Fisher) by monitoring three transitions for all light/heavy peptides. Fifty-four samples from pregnant women, with gestational ages ranging from 15+0 to 17+5 weeks, were analyzed. AF samples were confirmed as being from women carrying normal (n=37) or DS affected (n=17) fetuses.

Results: The median protein concentrations for DS and CN samples were: 20 and 49 ng/ml (p<0.01) for CEL; 3.7 and 14 ng/ml (p<0.001) for CPA1; 80 and 263 ng/ml (p<0.001) for MUC13; 46 and 135 ng/ml (p<0.001) for CLCA1; 0.65 and 0.93 µg/ml (p0.05) for PLUNC; 144 and 86 ng/ml (p0.05) for TF. The mean gestational age was 114 days in both groups. Moreover, the concentrations were transformed to multiples of the median (MoM) and the ratio DS/CN of median MoM was calculated, resulting: CEL (0.23), CPA1 (0.26), MUC13 (0.30), CLCA1 (0.31), MUC5AC (0.65), PLUNC (0.80), HAPLN1 (1.68), CGB (1.81) and TF (1.00).

Conclusions: Statistically significant differences were found in six of the proteins analyzed, reflecting a different regulation in DS. These proteins could be useful biomarkers in the screening of this pathology in the first and/or second trimesters of gestation. Further analyses with serum samples must be performed to elucidate the real potential of these candidates.

C-192

Increased expression and activation of leptin and insulin receptor signaling in placenta from pregnant women with gestational diabetes mellitus

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Background: Gestational diabetes patients have increased plasma levels of insulin and leptin. Placentas from gestational diabetes suffer from structural and functional changes including overgrowth. Since we have recently found that leptin stimulates protein synthesis in placenta, in a similar way to insulin, by activating protein signaling machinery, we aimed to study the expression of leptin and leptin receptor (LEPR), as well as LEPR and insulin receptor (IR) activation. Thus, we investigated the phosphorylation of down-stream proteins of both the LEPR and IR, including the initiation of translation signaling. We also checked the protein synthesis rate.

Methods: We have studied ten control placentas and ten placentas from patients with GDM. Leptin and LEPR expression were determined by quantitative real time-PCR and immunoblot. Protein phosphorylation was measured by specific immunoblot. The rate of protein synthesis was assessed by [3H]leucine incorporation experiments.

Results: We have found that leptin and LEPR expression are increased in placentas from GDM, and both LEPR and IR are activated as well as the signaling from both receptors in placentas from GDM compared with placentas controls. Finally, the translation machinery activity and protein synthesis rate were also higher in placentas from GDM.

Conclusions: We have found for the first time the activation of leptin and insulin receptors in placenta from GDM and this activity may contribute to the increased protein synthesis rate that we have found. Nevertheless, the specific contribution of each receptor in the increased protein synthesis rate remains to be investigated.

C-193

Candidate Clinical Factors and Biomarkers for Early Prediction of Preeclampsia: Performance of a Multivariate Algorithm in a Low-Risk Population.

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Background: The advent of early preventive measures, such as low-dose aspirin targeting women at high risk of preeclampsia (PE), emphasizes the need for better detection. Despite the emergence of promising biochemical markers linked to the pathophysiological processes, systematic reviews have shown that, until now, no single tests fulfill the criteria set by WHO for biomarkers to screen for a disease. However, recent literature reveals that by combining various clinical, biophysical and biochemical markers into multivariate algorithms, one can envisage to estimate the risk of PE with a performance that would reach clinical utility and cost-effectiveness, but this remains to be demonstrated in various environments and health care settings.

Objectives: To investigate, in a prospective study, the clinical utility of candidate biomarkers and clinical data to detect, early in pregnancy, women at risk to develop PE and to propose a multivariate prediction algorithm combining clinical parameters to biochemical markers.

Population and Methods: 7,929 pregnant women prospectively recruited at the first prenatal visit, provided blood samples, clinical and sociodemographic information. 214 pregnant women developed hypertensive disorders of pregnancy (HDP) of which

88 had PE (1.2%), including 44 with severe PE (0.6%). A nested case-control study was performed including for each case of HDP two normal pregnancies matched for maternal age, gestational age at recruitment, ethnicity, parity, and smoking status. Based on the literature we selected the most promising markers in a multivariate logistic regression model: mean arterial pressure (MAP), BMI, placental growth factor (PlGF), soluble Flt-1, inhibin A and PAPP-A. Biomarker results measured between 10-18 wk's gestation were expressed as multiples of the median. Medians were determined for each gestational week.

Results: When combined with MAP at the time of blood sampling and BMI at the beginning of pregnancy, the four biochemical markers discriminate normal pregnancies from those with HDP. At a 5% false positive rate, 37% of the affected pregnancies would have been detected. However, considering the prevalence of HDP in our population, the positive predictive value would have been only 15%. If all the predicted positive women would have been proposed a preventive intervention, only one out 6.7 women could have potentially benefited. In the case of severe PE, performance was not improved, sensitivity was the same, but the positive predictive value decreased to 3% (lower prevalence of severe PE).

Conclusions: In our low-risk Caucasian population, neither individual candidate markers nor multivariate risk algorithms using an *a priori* combination of selected markers reached a performance justifying implementation. This also emphasizes the necessity to take into consideration characteristics of the population and environment influencing prevalence before promoting wide implementation of such screening strategies. In a perspective of personalized medicine, it appears more than ever mandatory to tailor recommendations for HDP screening according not only to individual but also to population characteristics.

C-194

Performance of Candidate Clinical and Biochemical Markers for Predicting Preeclampsia at the End of Second Trimester in a Low-Risk Population.

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Background: Early identification during pregnancy of women at risk of developing preeclampsia (PE) is the most promising approach to implement preventive measures such as low-dose aspirin. However, it is still relevant to evaluate pregnant women with the objective of detecting PE before occurrence of clinical symptoms and/or to have better tools to assist in its differential diagnosis. Recently, measurements of biomarkers such as soluble fms-like tyrosine kinase-1(sFlt-1) and placental growth factor (PlGF) have been proposed and some manufacturers are already marketing reagents for this purpose.

Objective: To examine in a prospective study the performance of selected clinical and biochemical markers for identifying during late mid-term pregnancy women who are at risk of developing PE within a few weeks.

Population and Methods: 7,929 pregnant women prospectively recruited at the first routine prenatal visit, provided blood samples, clinical and sociodemographic information. After stabilization, samples were stored at -80°C until further use. 214 pregnant women developed hypertensive disorders of pregnancy (HDP) of which 88 had PE (1.2%), including 44 who presented severe PE (0.6%). We performed a nested case-control study from the whole cohort including cases of HDP and two pregnancies with normal outcome after matching for maternal age, gestational age at blood sampling, ethnicity, parity, and smoking status. Based on a recent review of literature we selected the most promising clinical and biological markers and included them in a multivariate logistic regression model: mean arterial pressure and body mass index (BMI), PlGF, sFlt-1, inhibin A, PAPP-A,. All markers were measured between 20 and 32 week's gestation except for BMI (early pregnancy). All biological marker results were transformed in multiples of median for each gestational week. Multivariate logistic regression analyses were then performed in order to develop a prediction algorithm.

Results: The resulting regression models discriminated the affected and normal pregnancies as indicated an area under the receiver operating characteristics (ROC) curve of 0.8. But at a 5% false positive rate, only 28% of the women who have developed HDP would have been detected. Even when the statistical analyses were limited to severe PE, the performance was poor: sensitivity 30%, positive predictive value 2.7%.

Conclusions: In our low-risk Caucasian population, neither individual candidate markers nor multivariate risk algorithms using an *a priori* combination of selected clinical and biochemical markers reached a performance justifying implementation as a screening procedure. These results emphasize the necessity to take into consideration environment, population and health care settings influencing prevalence and characteristics of HDP before promoting wide implementation of such screening strategies. In a perspective of personalized medicine, it appears more than ever mandatory to tailor recommendations for HDP screening according not only to individual but also to population characteristics if clinical utility has to be reached.

C-195

Increased expression and phosphorylation of the RNA binding protein Sam68 in the placenta from women with gestational diabetes mellitus

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Background: Gestational diabetes mellitus (GDM) is a common pathology of pregnancy. Patients with GDM have an increased levels of insulin and leptin in plasma and increased expression of leptin in placenta. Leptin plays a physiological role in placenta trophoblast cell survival, proliferation and cell growth, acting as an autocrine hormone. Sam68 is a member of STAR family of RNA-binding proteins with a role as an adaptor molecule in signal transduction of insulin and leptin receptor. We have previously found that Sam68 participates in the main pathways activated by leptin in trophoblastic JEG-3 cells, where leptin stimulation also promotes, in a dose dependent manner, sobreexpression and Tyr-phosphorylation of Sam68. Those results suggested a role of this protein in leptin physiological action in placenta, raising the question of a possible altered pattern of Sam68 expression and phosphorylation in placentas from women wit GDM. Thus, we aimed to study the expression of Sam68 and its phosphorylation level in the placenta from GDM compared with the placenta from normal pregancy.

Methods: We have studied 10 term placentas for normal pregnancies and 10 placentas for GDM pregnancies after cesarean section delivery. The GDM pregnancies were defined under biochemical criterion of an altered oral glucose tolerance test. Placental explants were obteined and Sam68 expression were determined using RT-PCR and protein level using immunoblot analysis. Sam68 phosphorylation level was assesed by immunoprecipitation and immunoblot analysis. Statistical analysis was carried out by comparison of the means using the Student´s T test.

Results: Sam68 protein quantity and gene expression were found increased over 80% in placenta from GDM, compared with the control placenta, showing a similar level to that observed in vitro, upon leptin stimulation of normal placenta explants. In addition, we have found that placentas from GDM pregnant women also have increased tyrosine phosphorylation level of Sam68 as compared with control normal placentas.

Conclusions: Placenta from GDM has increased Sam68 expression and phosphorylation level, suggesting a role for Sam68 in leptin receptor signaling and its trophic effect on placenta in this pathology of pregnancy, and pointing to a new molecular target in GDM.

C-196

An Atypical Case of 2-Methylacetoacetyl-CoA-Thiolase Deficiency

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2-Methylacetoacetyl-CoA-thiolase deficiency, also known as ketothiolase deficiency, oxothiolase deficiency or 2-methylacetoacetic aciduria is a rare inborn error of isoleucine catabolism (<1:1000,000). It is characterized by urinary excretion of 2-methyl-3-hydroxybutyric acid, 2-methylacetoacetic acid and tiglylglycine. Affected patients experience episodes of vomiting, dehydration and hypoglycemia with severe ketoacidosis. The typical age of presentation is 6-24 months.

We report a case of a 2 year old previously healthy male, who presented with severe vomiting (>10 times/day), severe dehydration, Kussmaul respirations and obtundation, after an upper respiratory infection with a high fever. Significant laboratory findings at the time of admission included a blood pH of 6.94, bicarbonate of <5 mmoles/L and base deficit of -28.8 mmoles/L on blood gas analysis, glucose of 65 mg/dL, calculated anion gap of >18 mmoles/L, high ketones on urine analysis, highly elevated β-hydroxybutyrate of 8957 µmoles/L (reference range <270), normal lactic acid and negative urine drug screen for >200 drugs. An organic acidemia or an inherited defect of ketone utilization was suspected. Urine organic acid, plasma

acylcarnitine and amino acid profiles were ordered.

Urine organic acid profile showed massive acetoacetic and 3-hydroxybutyric acids indicating severe ketosis. 2-methyl-3-hydroxybutyric and 2-methylacetoacetic acids were significantly elevated, and tiglylglycine was also elevated (Table). These findings pointed toward the diagnosis of 2-methylacetoacetyl-CoA-thiolase deficiency. Interestingly, 3-hydroxyisovaleric and glutaric acids were also significantly elevated. These findings are not typical of 2-methylacetoacetyl-CoA-thiolase deficiency. We hypothesize that increased 3-hydroxyisovaleric and glutaric acids may be due to overwhelming sickness and generalized mitochondrial dysfunction. This is supported by the fact that lactic acid and several 2-keto and 2-hydroxy acids were elevated on urine organic acid profile. Plasma acylcarnitine showed significant elevation of C4OH and plasma amino acid profile showed elevated branched chain amino acids. In conclusion we present an atypical case of possible 2-methylacetoacetyl-CoA-thiolase deficiency. We are in the process of gene sequencing for confirmation.

Abnormal findings on urine organic acid, plasma acylcarnitine and plasma amino acid profiles. Refer

Urine Organic Acids (mmoles/mole creatinine)	**Plasma Acylcarnitine (µmoles/L)**	**Plasma Amino Acids (µmoles/L)**
2-Methyl-3-hydroxybutyric – 194 (<14)	C4OH – 2.72 (<0.3)	Leucine – 478 (49-216)
2-Methyl-acetoacetic – 28 (<1)	C2 – 44.4 (2.6-39.2)	Isoleucine – 245 (22-107)
Tiglylglycine – 3 (<1)	C5 – 0.4 (<0.3)	Valine – 717 (74-321)
3-Hydroxybutyric – 89074 (<7)		Alloisoleucine – Not detected
Acetoacetic – 91745 (<10)		
3-Hydroxyisovaleric – 1717 (<37)		
Glutaric – 171 (<9)		

C-198

Pediatric Reference Intervals for Thyroglobulin

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Background: Thyroglobulin (TG) is an iodoglycoprotein synthesized in the follicular cells of the thyroid and acts as the precursor to thyroxine and triiodothyronine. TG is useful in monitoring patients with differentiated thyroid carcinoma. It has utility in diagnosis of thyrotoxicosis factitia, determining etiology of congenital hypothyroidism, and assessing activity of inflammatory thyroiditis. A pediatric reference interval study was conducted for TG using the Access 2 (Beckman Coulter) immunoassay.

Methods: Children from 6 months to 6 years of age were screened before undergoing elective surgery at Primary Children's Medical Center, Salt Lake City, UT. Eligible children were enrolled after obtaining parental consent. Blood was collected via intravenous catheter by the anesthesiologist prior to administration of general anesthesia. Children from 7 to 17 years of age were recruited via public posters and/or community magazines. They were enrolled after receiving parental consent. Physical exams were performed by one Physician Assistant and blood collection by one phlebotomist to ensure standardization. In addition to exclusion for known medical conditions or medication use, subjects were excluded if positive for TG antibody and/or thyroid peroxidase antibody. The subjects included 629 boys and 632 girls. Testing for TG was conducted using the Access 2 TG immunoassay following manufacturer's instructions. Results were analyzed and partitioned by age and gender. When partitioning tests indicated no significant differences, groups (gender and/or age) were combined. EP Evaluator release 8 software was used for partitioning tests and non-parametric reference interval analysis.

Results: No significant difference was observed between boys and girls and their results were combined. Based on age group partitioning tests the following proposed reference intervals (Table 1.) were determined.

Conclusions: TG reference intervals decreased with increasing age and reached adult levels (1.3 - 31.8) by 8 years age. Establishment of pediatric TG reference intervals should be of value in clinical practice.

Table 1. Pediatric TG Reference Intervals for Access 2 Immunoassay

Age	n	Lower Reference Limit	90% CI* (Lower Reference Limit)	Upper Reference Limit	90% CI* (Upper Reference Limit)
		(ng/mL)		(ng/mL)	
6 months to 3 years	279	7.4	6.2 - 8.6	48.7	44.0 - 62.1
4 to 7 years	284	4.1	2.5 - 5.5	40.5	34.0 - 43.6
8 to 17 years	698	0.8	0.3 - 1.3	29.4	27.2 - 33.8

*CI = confidence interval

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Pediatric Reference Intervals For Thyroid And Parathyroid Hormones: A Caliper Study Of Healthy Community Children

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Background: In recent years immunoassay-based measurement of several biomarkers has improved significantly. However, current reference intervals used by clinical laboratories are based on older methods and small groups of reference children. There is a critical need for a new and comprehensive age- and gender-specific database of pediatric reference intervals for many endocrine biomarkers. As part of the CALIPER study we have determined reference intervals for TSH, FT3, FT4, TT3, TT4 and iPTH on a large cohort of healthy community children from birth to 18 years of age.

Methods: This study followed the CLSI C28-A3 guidelines. Samples were collected and analyzed from children, ages birth to 18 years. A minimum of 120 samples were collected for each age/sex partition. Thyroid and parathyroid function hormones were measured on the Abbott ARCHITECT i2000. Outliers were excluded prior to further analyses. Final age- and sex-specific partitions were determined for each analyte using parametric and non-parametric methods. The 2.5^{th} and 97.5^{th} percentiles were determined for each partition and the 90% confidence interval for the limits was calculated.

Results: There were no sex-specific differences observed in any of the hormones analyzed in this study. TSH TT3, TT4 and iPTH concentrations remained relatively the same throughout all ages but a small decrease was noted in the concentrations of fT3 and fT4. Detailed calculated reference intervals for each analyte and scatter plots will be presented.

Conclusions: This study provides detailed pediatric reference intervals for thyroid and parathyroid function hormones filling a critical gap in this field. Availability of these new reference intervals, established based on a large cohort of healthy children, will allow for a better assessment and monitoring of children with thyroid and parathyroid endocrine diseases.

C-200

Pediatric Reference Intervals for 7 Special Chemistries and Immunoassays in Healthy Community Children: a CALIPER Study

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Objectives: Many special chemistry analytes measured in the clinical laboratory change throughout childhood development; these changes should be reflected in paediatric reference intervals. However, reference intervals based on a healthy paediatric population are either not available or need updating. We present results from the CALIPER study showing age- and sex -specific paediatric reference intervals for 7 common special chemistry analytes and immunoassays.

Methods: This study followed the CLSI C28-A3 guidelines. Samples were collected and analyzed from ethnically diverse healthy children ages birth to 18 years. A minimum of 120 samples were collected for each age/sex partition investigated. B12, folate, total homocystine, AFP, Ferritin, insulin and vitamin D were measured on the Abbott ARCHITECT i2000 system. Outliers and results affected by common interferences were removed. Age- and sex-specific partitioning was determined for each analyte individually. Parametric and non-parametric methods were used to establish the 2.5th and 97.5th percentiles for the reference intervals. The 90% confidence interval for the limits was calculated.

Results: Differences between age groups were observed for AFP, B12 and insulin whereas sex related differences were observed for ferritin and homocystine. Scatter

and box-plots along with the calculated reference intervals for each analyte were generated to assess alterations in analyte concentration throughout the paediatric age (0-18 years).

Conclusions: These data demonstrate the need for updated pediatric reference intervals based on a large diverse healthy cohort that are age and sex specific. These results will contribute to improved assessment and management of children with medical concerns.

C-201

The Value of the Serum Dipeptidyl Peptidase-IV and Adenosine Deaminase Activity in the Diagnosis of Mucopolysaccharidosis

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Background: The mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders that result from the absence or malfunctioning of enzymes required for the breakdown of glycosaminoglycans (GAGs). The clinical features of the MPSs result from lysosomal accumulation of partially degraded or undegraded GAGs and typically include coarse facies, corneal clouding, organomegaly and mental retardation. Determination of biomarkers that will enable the early diagnosis of MPS is important for successful treatment. At present, urinary GAG measurement and measurement of the activity of missing enzyme is being used for the clinical diagnosis of MPS. In this study, it is aimed to investigate the value of serum Dipeptidyl Peptidase-IV (DPP-IV, also known as the leucocyte antigen CD26), Total Adenosine Deaminase (ADA) and ADA-1 isoenzyme activity , and urinary GAG/creatinine concentrations in the diagnosis of MPS.

Methods: The present study included 20 controls and 20 MPS patients which were previously diagnosed by clinical and enzymatic analysis. Serum DPP-IV activity was measured according to Beesley et al and reported as nmol/min/ml. Serum total ADA and ADA-1 isoenzyme activity was measured according to Guisty G, Galanti B. colorimetric method and reported as U/L. Urinary GAG concentration was measured with dimethylmethylene blue method , creatinine was measured by the modified Jaffe method and results were reported as mg GAG/mmol creatinine.

Results: The results of serum DPP-IV activity for the MPS patients (median=124 nmol/min/ml, range 79-230 nmol/min/ml) were approximately 3-fold greater than the controls (median=45 nmol/min/ml, range 35-52 nmol/min/ml). ADA1 activity and ADA1/Total ADA ratios were approximately 2-fold higher in patients (ADA1 for patients: median=8 U/L, range 2-18 U/L, for controls: median=3 U/L, range 1-6 U/L; and ADA1/Total ADA ratio for patients: median=32 %, range 10-60 %, for controls: median=13 %, range 5-26 % respectively) where the total ADA results were similar (Total ADA for patients: median=22 U/L, range 8-43 U/L, and for controls: median=21 U/L, range 12-39 U/L). In addition, urinary GAG/creatinine levels were found approximately 5-fold higher in patients than controls (For patients: median=23 mg/mmol creatinine range 15-52 mg/mmol creatinine, for controls: median=4 mg/mmol creatinine, range 2-11 mg/mmol creatinine)

Conclusions: The relationship between the abnormal turnover of GAGs in MPS and the increase in DPP-IV activity and ratio of ADA 1/ Total ADA is interesting and may throw some light on the pathogenesis of MPS. Serum DPP IV activity can be used as a complementary to urinary GAG/creatinine for MPS screening. Further studies in larger patient population are required to understand the effectiveness of DPP-IV in the diagnosis of MPS.

C-202

Reference values for Cystatin C in newborns at the Hospital Virgen Macarena in Seville.

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Introduction: Cystatin C is a single chain unglycosylated protein of low molecular weight. It´s 13.360 kD, 120 aminoacids and two disulfide bridges. This protein is a good marker of renal function in newborn infants because of its independence from the weight, muscle mass and sex. Until today we have no knowledge of the existence of reference values for cystatin C in these children.

Methods: Blood samples of 90 children were collected at birth (from umbilical cord), at 72 hours and seven days of life. The period of study was two years. Cystatin C was measured by nephelometry (BNII Siemens). Children were divided into three groups according to gestational age: group A (24-27 weeks), group B (28-33 weeks) and group C (34-37 weeks). The Kruskal-Wallis Test was used to relate cystatin C values according to gestational age. The statistical analysis was performed with the IBM SPSS Statistics 19 (New York, USA) for Windows with a statistical significance of $p<0.05$.

Results: The range for Cystatin C in blood from umbilical cord at birth was from 1.45 to 1.64 mg /L; at 72 hours was from 1.27 to 1.48 mg /L and at seven days of live was from 1.42 to 1.63 mg /L. By gestational age groups; the ranges of Cystatin C in blood from umbilical cord were: group A from 1.24 to 1.65 mg /L, group B from 1.42 to 1.56 mg /L and group C from 1.52 to 1.91 mg/L with significant differences between groups B and C (p=0.034). The ranges at 72 h were: group A from 1.00 to 1.26 mg /L, group B from 1.29 to 1.45 mg/L and group C from 1.22 to 1.67 mg /L with significant difference between groups B and C again (p=0.030). The ranges at seven days were: group A: from 1.20 to 54 mg /L, group B from 1.43 to 1.62 mg /L and group C from 1.34 to 1.81 mg /L without significant difference between groups (p=0.329).

Conclusions: We found that the reference values of Cystatin C in newborns are higher than in children over one year. The values at 72 hours are lower than in the other two measurements for all groups. There are significant differences between the values in blood from umbilical cord at birth and blood at 72 hours between term infants and post-term infants with a higher value of Cystatin C in the last group.

C-203

Association between Hypoalbuminemia and Clinically Significant Discrepancies in Whole Blood versus Serum Sodium Results in Critically Ill Pediatric Patients

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Background: Rapid turn-around-time and low sample volume requirements have led to increased use of POC testing for electrolytes in hospitalized patients. Sodium concentration measured by direct ion selective electrode (ISE) is not considered susceptible to aberrant results in the setting of altered plasma protein concentration compared to results measured by indirect ISE.

Objective: To determine the relationship between hypoalbuminemia and sodium result differences in samples measured by direct versus indirect ISE in Pediatric Intensive Care Unit (PICU) patients.

Methods: All sodium results between 11/1/11 and 1/31/12 from PICU patients were evaluated. Whole blood sodium (NaPOC) was measured by direct ISE (GEM Premier 4000 analyzer, Instrumentation Laboratories, Bedford, MA) and serum sodium (NaCORE) by indirect ISE (Siemens Dimension Vista analyzer, Siemens Diagnostics, Deerfield, IL). Patients having NaCORE and NaPOC resulted within 30 minutes of each other and with concurrent serum albumin level (ALB, g/dL) were included for analysis. NaCORE minus NaPOC was considered significantly different if the results were > 4 mmol/L different and additionally clinically discrepant if the result was classified differently based on age-specific reference ranges. Results were stratified by ALB level and average difference determined for each ALB level group. Statistical significance of the differences was determined by student t-test for each ALB group compared to ALB group >3.3.

Results: 570 time-paired NaCORE and NaPOC results were identified. Detailed results are described in Table 1. In the clinically discrepant pairs with higher NaCORE, NaCORE result was hypernatremic in 87 pairs and NaCORE was normal in 25 pairs (15.3% and 4.4% of all pairs).

Conclusions: Clinically significant differences in sodium results in PICU patients using direct vs indirect ISE increased significantly with decreasing serum albumin. The resulting pseudohypernatremia or pseudonormonatremia with serum measurement can have significant consequences for patient management and the impact of sodium measurement method in this population should be emphasized.

Table 1

Albumin Level (g/dL)	Number of Pairs	NaCORE - NaPOC (mmol/L) (SD)	p value	Pairs with Difference >4 mmol/L	Clinically Discrepant Pairs
	110	6.8 (2.4)	<0.001*	95 (86%)	28 (25%)
2.4 to 2.8	174	5.4 (2.1)	<0.001*	125 (72%)	46 (26%)
2.9 to 3.3	161	4.2 (3.0)	0.064	86 (53%)	25 (16%)
>/=3.4	125	3.6 (2.2)	N/A	44 (35%)	13 (10%)

C-204

Identifying pre-analytic variables that contribute to hemolysis in neonatal blood specimens: sample collection site, transportation method and lipid emulsion infusion status.

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Objective: A quality improvement project was initiated to identify major pre-analytic variables associated with specimen hemolysis in the Neonatal Intensive Care Unit (NICU) with the goal of reducing the rate of hemolysis in neonatal specimens. In this study, the effects of sample collection site, specimen transportation method and lipid emulsion infusion status were systematically investigated.

Methodology: Over a period of two months, specimen collection site information and lipid emulsion infusion status were recorded for patients in the NICU at Saint Marys Hospital (Rochester, MN) who had orders for routine chemistry tests (direct and total bilirubin, CRP, Mg, Phosphorus) performed in the Central Clinical Laboratory (CCL), located 1.5 miles from the NICU. All samples were collected in microtainer serum separator tubes. Chemistry analytes and H-index, to quantitate hemolysis, were measured on Roche Modular analytics (Roche Diagnostics, Indianopolis, IN). In addition, specimen transport from NICU to CCL was alternated between being hand-carried or transported by the pneumatic tube system. The data were analyzed as a binary function of hemolyzed or non-hemolyzed using the most stringent H index cut-off for the tests included (direct bilirubin).

Results: Thirty-nine unique patients (20 males and 19 females) ranging in age from 28 to 68 days old (median = 46 days) were included in the study. A total of 137 samples were collected through either arterial/venous line (66%) or venipuncture (34%). The percentage of hemolyzed specimens for each collection site was 41% (21/51), 15% (7/46) and 13% (5/40) for arterial line, venipuncture and venous line, respectively. In this study 67% of patients were prescribed lipid emulsion infusion. Rates of hemolysis were 38% (8/21) for infants receiving lipid infusion at the time of sample collection, 24% (13/54) for infants prescribed lipid emulsion but for whom the infusion was paused at the time of sample collection, and 24% (11/45) for infants without a prescription for lipid emulsion therapy. Specimens that were hand-carried to the laboratory had a hemolysis rate of 9% (3/32) compared to 20% (11/54) for samples sent through the pneumatic tube. Among samples sent through the pneumatic tube, the percentage of hemolyzed samples was greatest when the patient was receiving lipids at the time of phlebotomy, 50% (6/12); compared to when the lipids were paused, 30% (10/33) and when no lipids were prescribed, 33% (8/24).

Conclusions: In our study, the largest contributor to serum sample hemolysis in NICU patients was the method of sample acquisition (arterial line collection), followed by lipid infusion status at time of collection and the transport method. The data also suggest that there is an additive effect of lipid infusion at the time of sample acquisition and transport through the pneumatic tube system. The trends identified provide a starting point for practice improvements that may reduce hemolysis rates in the NICU patient population including reducing arterial line collections when possible and changes in lipid emulsion infusion protocols.-

Wednesday PM, July 18, 2012

Poster Session: 2:00 PM - 4:30 PM

Nutrition/Trace Metals/Vitamins

D-01

Prevalence of Iron Deficiency Anemia among Children attending Korle Bu Teaching Hospital.

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Background: Iron deficiency anemia (IDA) is the most prevalent micronutrient deficiencies on a worldwide basis, especially in developing countries. It is the commonest cause of nutritional anaemia in children particularly in developing countries. The impact of severe IDA can have mortal consequences, since without adequate hemoglobin, the brain and body become deprived of oxygen and, if allowed to continue, death may ensue. Infants and toddlers are prone to developing iron deficiency anaemia (IDA). This study was carried out to determine the prevalence of IDA among children attending Korle Bu Teaching Hospital, Accra Ghana.

Methods: 142 apparently healthy children (72 males and 70 females) aged 6-24 months were recruited for this study after an informed consent. Full Blood Counts estimations were carried out using Sysmex KX- 21N automated analyzer, Japan. Estimations of serum iron (SI), total iron binding capacity (TIBC), serum ferritin (SF) and transferrin saturation (TS) were also determined in children with anaemia (Hb concentration < 11.0 g/dl). Statistical analysis was done using simple parametric method.

Results: Fifty five (78.1%) children had anaemia. The mean Hb concentrations of all the age groups were less than 11.0 g/dl. Twenty one (14.9%) children had IDA (defined as aneamia plus 2 or more of the following--MCV < 70fl, Ts < 10% or SF < 10 ug/dL). The mean age of children with IDA (8.96 +/- 2.54 months) was statistically lower than for those without the condition 10.94 +/- 4.55 months (p = 0.016).

Conclusions: The prevalence of IDA in this study is high especially before the age of 12 months and an average weekly intake less than 3 times a week of iron rich foods like animal protein and vegetables was significantly associated with IDA. Emphasis should be on the inclusion of iron rich foods in the diet following exclusive breastfeeding to reduce the prevalence of IDA in these children.

D-05

Comparison of three different methods for 25-hydroxyvitamin D determination

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Background. Determination of 25-hydroxyvitamin D [25(OH)D] represents a unique challenge, considering its lipophilic nature. Considering the widespread prevalence of vitamin D deficiency, which leads to increasing number of requests for 25(OH) D determination, immunoassay measurements adjusted to automated analyzers are being developed. Because of the variability among assays, it is often difficult to monitor vitamin D status and supplementation. The aim of this study was to compare results of two immunoassays with high performance liquid chromatography using ultraviolet detection (HPLC-UV).

Methods. We have evaluated analytical characteristics of electrochemiluminescent immunoassay, Elecsys® Vitamin D total (Roche Diagnostics GmbH, Mannheim, Germany) performed on Cobas® e601 analyzer, and of chemiluminescent microparticle immunoassay, ARCHITECT 25-(OH) vitamin D (Abbott Diagnostics, Wiesbaden, Germany) performed on Architect® *ci*8200 analyzer. For comparison studies we used HPLC analysis of 25-(OH)-Vitamin D_3/D_2 (Chromsystems Instruments & Chemicals GmbH, Munich, Germany) using Waters isocratic HPLC-UV system, because it uses pretreatment of samples which minimizes the influence of interferences.

Results. For imprecision assessment, four serum pools were prepared with following 25(OH)D concentrations: ~10 µg/L, ~20 µg/L, ~30 µg/L and ~50 µg/L. Obtained CVs for Roche method were 1.5-2.8% for within-run and 4.0-6.7% for between-run imprecision. For Abbott method, CVs were 0.7-4.4% for within-run and 3.8-7.2% for between-run imprecision. Inaccuracy was analyzed with commercial control sera. Obtained deviations from target value were 2.1% for Roche assay and 1.3-1.5% for Abbott method, and were not statistically significant ($P>0.05$). Comparison of Roche and HPLC-UV methods using Passing-Bablok regression analysis gave the following equation for the regression line y=0.94x+3.76 ($S_{y/x}$=6.33 µg/L; r=0.739; n=97) and the regression line equation from the comparison of Abbott and HPLC-UV methods was y=0.74x+4.12 ($S_{y/x}$=5.07 µg/L; r=0.793; n=97). Mean difference and SD for Bland-Altman plot were -1.8 µg/L and 17.4 µg/L, respectively for Roche method and 2.5 µg/L and 15.1 µg/L, respectively for Abbott. Statistical analysis (Chi-square test) of frequency distribution among different vitamin D status categories (<10 µg/L severe deficiency, 10-20 µg/L deficiency, 20-30 µg/L insufficiency and >30 µg/L sufficiency) showed that the frequency distribution obtained with Abbott method was significantly different from the distribution of the HPLC results, in contrast to Roche results frequency distribution which did not differ significantly Also, statistical analysis of the agreement between the three methods for each vitamin D status category showed that results of both Roche and Abbott methods were significantly higher than HPLC ($P = 0.005$ for Roche, $P = 0.0407$ for Abbott), and in the sufficiency category Abbott method significantly underestimated concentration of 25(OH)D compared to HPLC results ($P < 0.0001$).

Conclusion. Despite acceptable imprecision and inaccuracy of both methods, results obtained with them did not correlate well with HPLC-UV (r<0.9), which was used as a reference. Despite this fact, methods showed satisfactory ability to classify patients into vitamin D status categories, which is important for diagnosis of vitamin D deficiency and therapy follow-up.

D-06

Effect of Vitamin E on the hypercholesterolemia-induced oxidative stress.

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Background: Hypercholesterolemia is known to produce oxidative stress in aorta of rabbit. It is not known if it also induces oxidative stress in heart, kidney and liver. It is also not known if Vitamin E, an antioxidant would reduce hypercholesterolemia-induced oxidative stress in heart, liver and kidney. The main objectives of this study were to investigate: a) if long term hypercholesterolemia induces oxidative stress to the same extent in heart, liver and kidney. b) If oxidative stress is dependent upon the duration of hypercholesterolemia. c) If Vitamin E reduces the oxidative stress in these organs.

Materials and Methods: The studies carried out in rabbits assigned to the following groups: Group 1,regular diet; Group II, 0.25% cholesterol diet for 2 months; Group III, 0.25% cholesterol diet for 4 months; Group IV, 0.25% cholesterol diet for 2 months followed by 0.25% cholesterol diet with Vitamin E (40mg/kg body wt) for an additional 2 months. At the end of the protocol, the heart, kidney and liver were removed under anesthesia for measurement of lipid peroxidation product, malondialdehyde (MDA), an index of oxidative stress. The MDA was measured as thiobarbituric acid reactive substances and expressed as nmol/mg proteins.

Results: The levels of MDA in the heart, liver and kidney of rabbits in group I were 0.074 ±0.015, 0.094±0.03 and 0.068±0.022 nmol/mg protein. The MDA content of liver was higher than those of heart and kidney. The cardiac MDA content of group.II, group.III, and group IV were 3 folds, 14.36 folds and 2.36 folds higher respectively than that of group I. Vitamin E reduced the cardiac MDA significantly. The MDA content of liver in the 4 groups were not significantly different from each other. The MDA content of kidney in group II, III and IV were respectively 5.5 folds, 10.25 folds and 3.75 folds higher than in group I. Vitamin E significantly reduced the MDA content of kidney.

Conclusions: It is concluded that

1. Hypercholesterolemia induces oxidative stress both in the heart and kidney but not in liver.

2. The MDA contents of the heart and kidney were positively associated with the duration of hypercholesterolemia.

3. Vitamin E reduces the oxidative stress both in the heart and kidney.

D-07

Association of Vitamin D Status with Acute Lower Respiratory Infection in Children

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Background: Acute lower respiratory tract infection (ALRI) is a common disease in children. Micronutrient deficiencies may increase the risk of ALRI. The aim of this study is to determine the association of vitamin D status and susceptibility to acute lower respiratory infection by comparing serum 25 hydroxy-vitamin D [25(OH)D] levels in a group of young children under 5 years old with acute lower respiratory infection to healthy children.

Methods: Serum 25(OH)D concentrations were measured in patients under 5 years old admitted to hospital with a diagnosis of pneumonia or bronchiolitis (n=40 or 34, respectively) as well as in health, similar aged patients without respiratory symptoms (n=54). Serum 25(OH)D concentrations were measured with electrochemileuminescence immunoassay.

Results: The mean serum 25(OH)D levels in acute lower respiratory infection group were similar among case and control groups (28.4±9.0 versus 29.1±7.1 ng/mL). And there was no case-control difference in the prevalence of 25(OH)D insufficiency using two thresholds(<15 ng/mL: 8.1 versus 5.6%, P = 0.75 , <30 ng/mL: 59.5 versus 64.8%, P = 0.75)

Conclusions: In our study no difference was observed in vitamin D levels between the acute lower respiratory infection group and control group. Therefore Vitamin D status was not associated with the risk of hospitalization for acute lower respiratory infection in children.

D-09

Glycine restores the decreased serum 25-hydorxyvitamin D in bile duct-ligated guinea pigs

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Background: Patients with chronic disorders of liver function had lower serum concentrations 25-hydroxyvitamin D (25-OHD). Glycine, as a nonessential amino acid, exerts anti-inflammatory, cytoprotective, and immunomodulatory properties. Many reports have been shown that glycine would improve survival in rat liver transplantation, protect hepatocytes against hypoxia. However, the mechanism of glycine protective effects remains unclear. This study aimed to establish a method for serum 25-OHD measurement using liquid chromatography-tandem mass spectrometry, and to investigate whether glycine would restore the decrease of 25-OHD caused by bile duct ligation in guinea pigs.

Methods: Bile duct ligation was performed on male guinea pigs at 300-400 g, sham-operated as the control group. Glycine was given by intraperitoneal injection daily. The animals were sacrificed and examined at 7 and 14 days after bile duct ligation. Serum concentrations of total bilirubin and enzyme activities of AST, ALT, ALP, and GGT were measured. Serum 25-OHD2 and D3 were extracted with methanol-isopropanol mixture containing the internal standard (hexadeuterated 25-OHD3). The supernatant was injected into a C18 column, and 25-OHD was quantitated by API 5000 mass spectrometer using the positive ionization mode.

Results: The intra-assay imprecision (CV%) for 25OHD2 and 25OHD3 was 6.4% and 5.2%, respectively, and inter-assay imprecision 10.2% and 3.7%, respectively. The average recoveries were 100% for 25-OHD3, and 103% for 25-OHD2 using NIST standard SRM 972. Serum 25-OHD concentrations were decreased in bile duct ligation animals at 14-day with mean(SD) 31.9(12.5) ng/mL, compared to sham 65.3(10.6) ng/mL ($p<0.005$). Glycine treatment prevented the reduction of serum 25-OHD (52.6(10.7) ng/mL, $p<0.05$) caused by bile duct ligation. Glycine treatment also prevented the liver damage indicated by the levels of serum AST ($p<0.005$) and ALT ($p<0.05$).

Conclusions: The established LC-MS/MS method is accurate and precise for serum 25-OHD measurement. Glycine may provide a protective effect in bile duct ligation-associated liver injury through the vitamin D metabolism.

D-10

Comparison of the Siemens Centaur Vitamin D Assay to the DiaSorin Liaison

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Background: Over the last five years there has been a dramatic increase in Vitamin D testing volume prompted by the potential association of Vitamin D with various disease states. The increasing Vitamin D testing volume has resulted in a number of manufacturers producing immunoassays, however, the reference method remains LC/MS/MS. Vitamin D testing by immunoassay presents a number of challenges since Vitamin D occurs in two primary forms, D2 and D3, bound and unbound. This study compares the DiaSorin chemiluminescence Vitamin D immunoassay to the Siemens Centaur monoclonal immunoassay.

Methods: The primary method used by the laboratory is the DiaSorin assay. 100 patient samples were chosen for the comparison study. To insure an adequate concentration distribution, patient samples were grouped as follows: less than 20ng/mL, 20 to 40ng/mL, 40 to 60ng/mL, and greater than 60ng/mL. After determination on the DiaSorin Liaison the grouped samples were refrigerated immediately and transported to a secondary testing facility performing the Siemens Centaur assay.

Results: Linear regression analysis between the Siemens Centaur and the DiaSorin Liaison demonstrated a poor relationship with a correlation coefficient of 0.6063. The patient subgroups were divided and analyzed separately with the first group of patients (n =16) having values of less than 20ng/mL. The correlation coefficient for the less than 20 ng/mL group was 0.4376. The second patient group with results between 20 and 40 ng/mL (n=44) had a correlation coefficient of 0.2798. The last patient group with values greater than 40 ng/mL (n=40) had a correlation of 0.1599. Bland-Altman plots demonstrate Vitamin D testing results varying between -18 ng/mL to 80 ng/mL with per cent differences ranging from -47% to 131%. The most striking differences between the two assays occur for Vitamin D levels that are greater than 40 ng/mL. The level of 40ng/mL presents problems for result interpretation since 32 ng/mL is generally considered the acceptable lower level of “normal.”

Conclusions: The Centers for Disease Control and Prevention (CDC) in conjunction with the National Institute of Standards (NIST) have a calendar 2012 initiative to provide a Vitamin D reference material to standardize commercial assays. This study demonstrates significant differences between the DiaSorin Liaison and the Siemens Centaur. Both methods are immunoassay based and are required to measure equimolar concentrations of D2 and D3. Vitamin D is bound and transported by the Vitamin D Binding Protein (VDBP) and immunoassays must be able to account for the different forms and possible heterophile antibody interference problems. The Siemens Centaur assay appears to have a low bias compared to Diasorin at Vitamin D levels less than 40ng/mL. Above 40ng/mL the assay does not compare well to the DiaSorin Liaison having differences ranging from -18ng/mL to 80ng/mL. This study highlights the necessity to standardize all Vitamin D assays.

D-11

Measurement of pyridoxal 5-phosphate and pyridoxic acid in human plasma by liquid chromatography-tandem mass spectrometry

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Background: Vitamin B6 is a complex of several vitamers: pyridoxine (also known as pyridoxol), pyridoxal, pyridoxamine and their 5’-phosphate esters. Pyridoxal 5-phosphate (P5P or PLP) is considered to be the biologically active form of vitamin B6 and acts as a cofactor in numerous enzymatic reactions. Therefore monitoring of pyridoxal 5-phosphate levels provides information on the state of vitamin B6 sufficiency. Symptoms associated with deficiencies of vitamin B6 include dermatitis, anemia, peripheral neuropathy and seizures. Deficiencies of vitamin B6 have been postulated as a factor in the development of carpal tunnel syndrome. Individuals presenting with chronic, progressive nerve compression disorders should also be evaluated for vitamin B6 deficiency.

The measurement of pyridoxic acid (PA), the major vitamin B6 catabolite, in conjunction with pyridoxal 5-phosphate levels, can assist in the evaluation of hypophosphatasia, a disorder characterized by elevated levels of pyridoxal 5-phosphate, low levels of pyridoxic acid and a range of accompanying skeletal abnormalities.

Methods: Deuterated stable isotopes of pyridoxal 5-phosphate-d2 and pyridoxic

acid-d2 were synthesized from pyridoxine-d2 dimethyl acetal by the Chemical Synthesis Core Facility (Mayo Clinic Jacksonville, FL). Twenty-five microliters of internal standard (pyridoxal 5-phosphate-d2 or pyridoxic acid-d2) was added to heparinized plasma, followed by protein precipitation via the addition of 10% meta-phosphoric acid. The sample was centrifuged for 10 minutes at 1500 xg. Pyridoxal 5-phosphate, pyridoxic acid and internal standards were then separated by liquid chromatography (TLX4, Thermo Fisher Scientific, Waltham, MA) followed by analysis on a tandem mass spectrometer (API 5000, AB SCIEX, Foster City, CA) equipped with an electrospray ionization source in positive mode. Ion transitions monitored in the multiple reaction monitoring (MRM) mode were m/z 248.1 → m/z 150.2 for pyridoxal 5-phosphate, m/z 250.1 → m/z 152.2 for pyridoxal 5-phosphate-d2, m/z 184.1 → m/z 148.1 for pyridoxic acid and m/z 186.1 → m/z 150.1 for pyridoxic acid-d2. Calibrators consisted of six standard solutions of pyridoxal 5-phosphate and pyridoxic acid ranging from 0 to 400 ug/L.

Results: Lithium and sodium heparinized plasma fortified with pyridoxal 5-phosphate and pyridoxic acid were assessed for precision, linearity, recovery and specimen stability. Intra-run precision coefficients of variation (CVs) ranged from 2.6% to 5.9% for pyridoxal 5-phosphate and 2.3% to 7.5% for pyridoxic acid. Pyridoxal 5-phosphate inter-run precision CVs ranged from 3.7% to 9.9% and pyridoxic acid inter-run precision CVs ranged from 4.9% to 11.3%. Linearity was demonstrated over each assay range (2 to 400 ug/L) with an R^2 value of 0.9995 for pyridoxal 5-phosphate and an R^2 value of 0.9990 for pyridoxic acid. Pyridoxal 5-phosphate recovery averaged 100% and 98% for lithium and sodium heparin specimens, respectively. Pyridoxic acid recovery averaged 101% and 98% for lithium and sodium heparin specimens, respectively. A stability study demonstrated that plasma specimens are stable when stored in amber tubes refrigerated (2-8ºC) for up to 7 days and frozen (-20ºC or lower) for up to 14 days.

Conclusions: This method provides for the simultaneous and reliable high throughput analysis of pyridoxal 5-phosphate and pyridoxic acid in heparinized plasma.

D-12

Development of a total vitamin D Evidence biochip based immunoassay for the equimolar determination of 25(OH)D2 and 25(OH)D3

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Introduction Vitamin D is a fat soluble vitamin that exists in two forms: Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol). Both forms of vitamin D must undergo two hydroxylations in the body for activation. The physiologically active 1α,25-dihydroxyvitamin D [1α,25(OH)$_2$D] is tightly regulated therefore measurements do not give an accurate reflection of vitamin D storage levels in the body. The most widely used indicator of vitamin D status is the measurement of 25-hydroxyvitamin D [25(OH)D] in either serum or plasma. Low serum concentrations of 25(OH)D result in the debilitating bone condition, rickets, and recent epidemiological studies implicate 25(OH)D with an increased risk of various cancers, type 1 diabetes, multiple sclerosis, depression, Alzheimer's, deficiencies in the immune system and increased risk of cardiovascular events.

Relevance With the increasing awareness that 25(OH)D deficiency is associated with multiple clinical outcomes there has been a rapid increase in the number of tests requested. Therefore within a clinical laboratory setting a high throughput assay which can accurately measure total 25(OH)D [i.e. both 25(OH)D2 and 25(OH)D3] and does not require high levels of technical expertise is imperative. This study reports the development of a biochip based immunoassay for the measurement of both forms of vitamin D: 25(OH)D2 and 25(OH)D3 in serum which is of value for applications in clinical test settings.

Methods This biochip based assay utilises a competitive chemiluminescent format. Following displacement of serum binding proteins, endogenous 25(OH)D in the sample competes with a 25(OH)D conjugate for binding to antibody immobilised on the biochip surface. Following removal of unbound material, chemiluminescent substrate is added and the resulting signal is measured. The intensity of the signal is inversely proportional to the 25(OH)D concentration in the original sample. The immunoassay was applied to the Evidence Investigator analyser (Randox Laboratories Ltd., Crumlin, UK).

Results Calibrator values for this total 25(OH)D assay are traceable to LC-MS/MS with an assay range up to 128 ng/ml. Assessment of percent cross reactivity was estimated by comparison of the concentration yielding 50% inhibition in human 25(OH)D depleted serum spiked with either 25(OH)D2 or 25(OH)D3 and values of 100% and over 90% respectively were achieved. 39 serum samples, which encompassed a clinically relevant range of 25(OH)D concentrations, were assessed using the biochip assay and LC-MS/MS and the results were compared. There was good agreement between the two methods with a slope of 0.9824 and regression coefficient of 0.91.

Conclusions This analytical evaluation indicates that the developed biochip based immunoassay exhibits equimolar recognition of the 25(OH)D2 and 25(OH)D3 forms and compares favourably with LC-MS/MS when serum samples were assessed. The total 25(OH)D assay will be available on the fully automated random access immunoassay analyser, Evidence Evolution. This will result in a straightforward high throughput assay for the quantitative determination of 25-OH vitamin D in human serum which is of interest in clinical settings.

D-13

Determination of whole blood chromium reference range by a novel inductively coupled plasma mass spectrometry method with high sensitivity

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Background Chromium (Cr), a trace metal element has been shown important for diabetes and cardiovascular disease. A hypochromic state has been associated with poor blood glucose control and unfavorable lipid metabolism. Sensitive and accurate measurement of blood chromium is very important to assess the chromium nutritional status. However, interferents in biological matrices and inappropriate sample handling make the sensitive analysis challenging. The primary goal of this study was to develop a high-sensitivity inductively coupled plasma mass spectrometry (ICP-MS) method for quantification of Cr in whole blood and to establish a reference range in a local healthy population.

Methods This method was developed on a Thermo Fisher X Series2 ICP-MS with a collision/reaction cell controlled by PlasmaLab (ver. 2.6.2.337). Interference was minimized through the use of both kinetic energy discrimination between the quadrapole and hexapole and a selective collision gas (helium). Collection of blood samples for reference range determination was approved by the institutional review board. In brief, whole blood samples (n = 51) were collected in EDTA tubes free of trace elements from healthy adults (12 males), aged 19-64 y (38.8 ± 12.6), after a minimum of 8 h fasting. Patient exclusion criteria were: pregnancy; body mass index (BMI) below 15 or above 30; having a cold, flu, virus or other infection in the past two weeks; a diagnosis of diabetes, malabsorption syndrome or Crohn's disease; gastric or intestinal surgery, or frequent diarrhea; chemotherapy in the past year, current immunosuppressant therapy. Blood samples were aliquoted into cryogenic vials and stored at -70°C until analysis. All the samples were analyzed with controls and calibrators in a single batch. Statistics were calculated using Excel (Microsoft, Redmond WA, USA) and EP Evaluator Release 9 (Data Innovations, South Burlington, VT, USA).

Results Full technical validation was performed and the assay met the institutional requirements. The linearity of the assay was 0.2 to 75.1 µg/L with recovery from 88.3 to 99.6%. The high sensitivity was achieved by minimization of interferents through the use of selective kinetic energy discrimination and selective collision using helium. Data analysis revealed a slight skew in the distribution of whole blood Cr in the reference population. The reference interval for Cr using a parametric method was determined to be 0.2 µg/L to 0.6 µg/L with no gender difference.

Conclusion This ICP-MS methodology was highly sensitive and the determined reference range was 0.2 - 0.6 µg/L in a local reference population.

D-14

Evaluation of the ARCHITECT® 25-Hydroxy Vitamin D Assay and Comparison with LC-MS/MS

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Vitamin D is involved in calcium and phosphate homeostasis. The majority of vitamin D circulating in the body is obtained from exposure to sunlight, with a small portion resulting from dietary intake. Vitamin D measurement has dramatically increased in recent years, thus rapid test methods are useful for the clinical laboratory. The objective of this study was to evaluate the ARCHITECT $i2000_{SR}$ 25-hydroxy (OH) vitamin D assay by analyzing imprecision and comparing patient results to an in-house liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. Imprecision studies were performed by measuring manufacturer's quality control materials in duplicate twice a day for 5 days. Total coefficients of variation were

5.8% (20 ng/ml), 3.2% (40 ng/mL) and 2.5% (75 ng/mL), which was comparable to manufacturer's claims. Method comparison testing was performed on 200 serum specimens previously tested by the in-house LC-MS/MS. The majority of samples contained D3 only (n=134), the remaining 66 contained both D2 and D3. A summary of performance characteristics for all 200 samples, samples with D2 and D3, and samples containing D3 only is provided below. Mean recovery was approximately 12% higher in samples containing D3 only. Additionally, 5 samples from DEQAS (Vitamin D External Quality Assessment Scheme) were analyzed on the ARCHITECT and compared to both the mean of all methods and all LC-MS/MS assays included in the DEQAS survey. Percent recoveries in comparison with the mean of all DEQAS methods ranged from 100.9% to 114.4% and from 92.0% to 98.3% for all LC-MS/MS methods. In conclusion, the ARCHITECT 25-OH vitamin D assay compared well with our LC-MS/MS method. However, samples containing D3 only may have elevated values in comparison to LC-MS/MS.

	Slope	Intercept	Correlation Coefficient	Mean Recovery (%)
All samples (n=200)	1.04	0.20	0.86	106.8
D2 and D3 samples (n=66)	0.97	-1.79	0.82	96.4
D3 only samples (n=134)	1.09	0.56	0.90	111.9

D-15

The effect of omega-3 fatty acid supplementation in type 2 diabetic patients on the levels of advanced glycation end products (AGEs) and receptor (RAGE).

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Background: In diabetes mellitus, chronic hyperglycemia leads to non-enzymatic modification of proteins with formation of advanced glycation end products (AGEs). Binding of AGEs to receptors of AGE (RAGE) causes in deleterious effects. RAGE is known to mediate cellular effects through the activation of the transcription factor nuclear factor kappa B (NF-kappaB). Soluble RAGE (sRAGE) which is one of RAGE successfuly binds to AGEs in sera and, due to this ability, it prevents the activation of RAGE, thereby protecting cells from AGE-induced injury. On the other hand, in populations with a high consumption of omega-3 fatty acids, a lower prevalence of diabetes mellitus has been reported. The purpose of our research was to investigate the effects of omega-3 fatty acids (EPA and DHA) supplementation in type 2 diabetes patients on the levels of AGEs (carboxymethyl lysine (CML) and pentosidine), sRAGE and NF-kappaB and the relations between them.

Methods: Type 2 diabetic patients aged 45-75 years (n=38), with a mean age of diabetes 6,00±5,63 years were treated with oral hypoglycemic agents, without insulin. All diabetic patients were supplemented with omega-3 fatty acids (1.2 g/day) for 2 months. Both before and after omega 3 fatty acids supplementation CML, pentosidine, sRAGE and NF-kappaB levels were measured by ELISA.

Results: Omega-3 fatty acid supplementation significantly reduced glucose (p<0,01) , HbA1c (p<0,05) and pentosidine (p<0,05) levels. HbA1c and pentosidine levels were positively correlated in diabetic patients supplemented with omega-3 fatty acid (r=0,373, p=0,035). Systolic and diastolic pressures were significantly decreased due to omega-3 supplementation (p<0,01).

Conclusions: Our results show that supplementation with omega-3 fatty acids at a dose of 1.2 g/day has beneficial effects on both systolic and diastolic blood pressures and the levels of glucose, HbA1c and pentosidine. However the supplementation failed to decrease these parameters to the reference ranges for healthy subjects. Taking into consideration the low consumption of fish in the Turkish population, we suggest the supplementation of omega-3 fatty acids in type 2 diabetic patients.

D-16

Vitamin D Deficiency in Seropositive Rheumatoid Arthritis, Infection and Healthy Individuals

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Background: Vitamin D plays a regulatory role in calcium homeostasis and bone mineralization, whilst its deficiency being highly prevalent among general population, is also known to be associated with various clinical conditions. Recent studies support the pleiotropic role of vitamin D in immunity, systemic inflammatory response and metabolic dysfunction. To support the potential of vitamin D utilized as a biomarker or an acute phase reactant, we evaluated vitamin D deficiency in rheumatoid arthritis, infection and healthy individuals.

Methods: For the evaluation of vitamin D deficiency among general population in comparison with disease groups, groups of healthy individuals, sero-positive rheumatoid arthritis patients and patients with infection (pneumonia, post-operation wound infection) were selected at Kyung Hee University Hospital at Gangdong. Laboratory findings of CRP, ESR and vitamin D were measured by Hitachi 7600-110 automated chemistry analyzer using Sekisui CRP reagent, Test1 Capillary Micro-photometer for ESR (Alifax S.P.A.) and ADVIA Centaur XP immunoassay system (SIEMENS) using total 25(OH) vitamin D assay reagent, respectively. Statistical analyses were performed by correlation analysis, t-test, ANOVA with multiple comparison and chi square test. SPSS 15.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis and P value of <0.05 was considered statistically significant.

Results: The study group consisted of 211 males and 113 females, age ranging from 15 to 85 (mean 50.4 ± 14.2). Mean values of vitamin D in each category were 9.20 ± 3.87 ng/mL in healthy individuals, 8.40 ± 4.18 ng/mL in RA patients and 7.80 ± 3.12 ng/mL in infection patients. Mean values of CRP were 0.1 ± 0.1 mg/dL in healthy individuals, 0.8 ± 1.4 mg/dL in RA patients and 6.7 ± 5.2 mg/dL in infection patients. Mean ESR were 9.4 ± 6.7 in healthy individuals, 25.6 ± 19.3 in RA patients and 28.9 ± 19.6 in infection patients. The cut-off value for determining vitamin D deficiency defining lower 5 percentile in this study was 4.85 ng/mL. The percentage of individuals with vitamin deficiency was higher in disease groups of both RA (15/106, 14.2%) and infection (9/45, 20%) than healthy individuals (11/173, 6.4%). However, the level of total (OH) vitamin D did not correlate with ESR or CRP (P = 0.12, P = 0.49).

Conclusions: Statistically meaningful finding in this study was that vitamin D deficiency was more prevalent in disease groups compared to healthy individuals and correlated with the presence of disease. Although the vitamin D level did not correlate to ESR or CRP which implies that vitamin D deficiency may not represent the current disease activity, it still is a supportive finding for vitamin D playing a pleiotropic role other than calcium homeostasis and bone mineralization. The finding from this comparison study supports preexisting evidence that vitamin D deficiency is more prevalent in groups with illness, and further prospective studies are required to evaluate the relationship between deficiency and severity of the illness if any.

D-17

Performance Evaluation of Siemens ADVIA Centaur and Roche MODULAR Analytics E170 Total 25-OH Vitamin D Assays*

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Background: Vitamin D testing has increased 20-fold in our laboratory over the past 5 years. Laboratories are challenged to provide reliable total vitamin D results in a timely, cost-effective manner. Our goal was to choose an automated total 25-OH vitamin D assay based on a performance evaluation that compared the Roche MODULAR Analytics E170 Total Vitamin D (E170) assay and the Siemens ADVIA Centaur® Vitamin D Total (ADVIA Centaur) assay to LC-MS/MS. Both assays were also compared to the DiaSorin LIAISON 25 OH Vitamin D TOTAL (DiaSorin LIAISON) assay.

Methods: The ADVIA Centaur and the E170 vitamin D assays were evaluated using CLSI protocols for imprecision, linearity, interference, and method comparison. The method comparison study also included the DiaSorin LIAISON and LC-MS/MS (reference method) assays, and 79 samples across the assays' measuring ranges, among them 15 with detectable 25-OH vitamin D2 (endogenous). Method comparison data were evaluated by regression and Bland-Altman analyses. In addition, 10 DEQAS samples were used to evaluate ADVIA Centaur and Roche E170 assay accuracy.

Results: The ADVIA Centaur assay demonstrated total CVs of 14.1% at 52.6 nmol/L and 7.4% at 225 nmol/L, and the E170 assay total CVs of 5.9% at 41.3 nmol/L, 1.9% at 129.5 nmol/L, and 2.8% at 204.6 nmol/L. Both the ADVIA Centaur and E170 assays demonstrated good linearity, with R2 values of 0.9945 and 0.9966, respectively. The ADVIA Centaur assay demonstrated interference with bilirubin at 800 μmol/L, hemolysis at 1.25 g/L, and triglycerides at 2.8 mmol/L. The E170 assay did not demonstrate interferences at the tested concentrations. In comparison to LC-MS/MS, the ADVIA Centaur assay demonstrated an R2 value of 0.893, with an average bias of −8.8%, and the E170 assay an R2 value of 0.872, with an average bias of 14.3%. Regression and Bland-Altman analyses demonstrated underestimation of 25-OH vitamin D2-containing samples for the E170 assay. Compared to the DiaSorin LIAISON assay, the ADVIA Centaur assay demonstrated an R2 value of 0.781, with

an average bias of −17.3%, and the E170 assay an $R2$ value of 0.823, with an average bias of 11.4%. The ADVIA Centaur results differed from the assigned DEQAS sample values by 18.5% to −13.7%, and the E170 assay by 43.4% to −1.6%: the ADVIA Centaur assay demonstrated a bias of less that 20% in 10/10 samples versus the E170 assay in 8/10 samples.

Conclusions: The Siemens ADVIA Centaur and Roche E170 vitamin D assays demonstrated excellent linearity (~0.99) and acceptable imprecision. Using LC-MS/MS as the reference method, the ADVIA Centaur assay demonstrated a higher R2 value (0.893) and a smaller average bias (-8.8%) than the E170 assay (R2 = 0.872, average bias = 14.3%). The E170 assay demonstrated consistent underestimation of 25-OH vitamin D2 levels. The ADVIA Centaur and E170 assays both met the DEQAS accuracy criteria.

* The authors conducted this study, and Siemens and Roche provided assay kits. Tricia Bal, provided writing support.

D-20

Vitamin D insufficiency among healthy children in Bangkok, Thailand

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Background: Recent studies have demonstrated that hypovitaminosis D in children is widespread. However, vitamin D status in children of Thailand has not been evaluated. The objective of this study is to survey the plasma levels of 25-hydroxyvitamin D [25(OH)D] in children aged 6 to 12 years in Bangkok (latitude: 13.45^{O}N), the capital of Thailand.

Methods: Cross-sectional study included 79 healthy children aged 6 to 12 years from one primary school in Bangkok were studied. EDTA blood samples were collected in the fasting condition and plasma parathyroid hormone (PTH) and total 25(OH)D were analyzed using electrochemiluminescence immunoassay on Elecsys 2010 analyzers (Roche diagnostics, Mannheim, Germany). 25(OH)D deficiency was indicated by plasma concentration below 10.0 ng/mL, values between 10.0 and 30.0 ng/mL were considered insufficiency.

Results: No children had 25(OH)D deficiency (<10.0 ng/mL). However, 65 (82.28%) children demonstrated vitamin D insufficiency (< 30 ng/mL). Plasma PTH level in vitamin D insufficiency children was slightly higher than children with normal vitamin D level (37.99 $\pm$ 12.72 vs 31.47 $\pm$ 12.74, P = 0.862).

Conclusions: The prevalence of vitamin D insufficiency in Thai healthy children is very high despite the exposure to sunlight. Recommendation for vitamin D supplement in Thai children should be considered.

D-21

Seasonal Variation of Vitamin D Levels in Outpatient and Inpatient Populations in the Long Island, NY Area

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Introduction: While there is controversy regarding the reference range for vitamin D, there is general consensus that levels between 11 - 20 ng/mL indicate insufficiency and levels of 10 and below indicate deficiency. Vitamin D levels of 21 - 30 may indicate possible risk for insufficiency but this is controversial.

Objective: This study was undertaken to investigate the distribution of vitamin D levels in outpatients and inpatients in central Long Island, NY and to compare relative percentages of insufficiency & deficiency in winter and summer months.

Methods: Results of all physician-ordered serum vitamin D tests over two fifteen day periods in January and July 2011 were retrieved from the laboratory computer system for outpatients within the North Shore-LIJ Health System. This data was de-identified and the frequency distribution of vitamin D levels determined in bins of 10 percentiles. Data was collected similarly for physician-ordered vitamin D tests on inpatients at North Shore University Hospital for the months of Jan. and July 2011. Since the monthly number of inpatient vitamin D tests was low, additional data was collected which included all inpatient vitamin D tests ordered from Jan. through Aug., 2011. Vitamin D measurements include both 25-hydroxyvitamin D2 & D3 and were performed at the NS-LIJ Core Laboratory using a chemiluminescent immunoassay on the DiaSorin Liaison instrument.

Results: The study focused on the distribution of vitamin D results below 30 ng/mL to determine relative percentages of patients classified as having deficiency (0 - 10), insufficiency (11 - 20) and possible insufficiency (21 - 30 ng/mL). Comparing the relative percentage of outpatients in these three categories, deficiency/insufficiency/possible insufficiency, the distribution in Jan. (n = 4,197) was 6.9/27.3/36.4% and in July (n = 6,301) was 1.7/14.0/33.5% indicating increased percentages of patients in the deficiency and insufficiency category in Jan.. There was little difference in the percent of patients in the possible insufficiency category. In a similar comparison of the inpatient data the distribution in Jan. (n = 67) was 20.9/29.9/26.9% and in July (n = 53) was 7.5/35.8/30.2% showing increased percentage of patients in the deficiency category but somewhat less in the insufficiency/possible insufficiency category in Jan.. The second inpatient study including all vitamin D results from Jan. through August (n = 495) showed an intermediate distribution of 17.0/34.1/26.5%.

Conclusions: For the outpatient population there were increased percentages of patients in the deficiency and insufficiency categories in Jan. vs. July indicating increased risk during winter. For the inpatient population there was an increased percentage of patients in the deficiency category only in Jan.. The inpatient group had a larger percentage of patients in the deficiency and insufficiency category compared to the outpatient group in both Jan. and July and this probably reflects some bias in physician ordering criteria, e.g., screening for outpatients but clinical indications for inpatients. The percentage of patients in the possible insufficiency category showed little seasonal or population change.

D-22

Assessment of Vitamin D status among pregnant woman in central Thailand

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Background: Vitamin D is an important nutritional factor in maternal and infant health. Vitamin D insufficiency in utero or early life has been associated with a number of diseases such as increased risk of childhood wheezing, respiratory infection, type 1 diabetes, multiple sclerosis and schizophrenia. Vitamin D deficiency is unexpected in a tropical country such as Thailand, where there is abundant sunshine for most of the year. Currently, vitamin D supplement has not been routinely prescribed for pregnant woman in Thailand.

Objective: To determine the prevalence of vitamin D insufficiency and deficiency in pregnant women in Bangkok, Thailand (13.45°N).

Methods: 107 subjects were enrolled at the delivery room of the Department of OBGYN, Siriraj Hospital, Mahidol University, Bangkok, Thailand from September 2011 to December 2011. Inclusion criteria included Thai singleton pregnant women, ages 18-45 year-old and signed informed consent. Exclusion criteria included presence of diseases or treatment with medications known to affect vitamin D status. Blood samples were collected in the nonfasting condition before labor and were stored at -20°C for future analysis. EDTA plasma parathyroid hormone (PTH) and total 25(OH)D were analyzed using electrochemiluminescence immunoassay on Elecsys 2010 analyzers (Roche diagnostics, Mannheim, Germany). 25(OH)D deficiency was defined as serum concentration below 10.0 ng/mL, values between 10.0 and 20.0 ng/mL were considered insufficiency.

Results: A total of 107 women were 18-43 year old, mean±SD 27.83±6.48. The concentration of 25-hydroxyvitamin D was 24.97±7.72 ng/mL .No pregnant woman had 25(OH)D concentration indicative of vitamin D deficiency (<10 ng/mL); whereas, 33 (30.8%) pregnant women had vitamin D insufficiency (<20 ng/mL). Plasma PTH in vitamin D insufficient pregnancy was slightly higher than in women with optimal vitamin D level. (31.57±15.03 vs 27.47±10.00, P =0.10). Maternal plasma 25(OH)D showed a negative correlation with maternal plasma PTH (r = -0.21). 73 samples were collected during rainy season while 34 were collected during winter. The mean 25(OH)D concentration between samples collected from rainy season and winter were not different (25.57±6.90 vs 23.69±9.22, P = 0.29).

Conclusions: Despite the abundant sunshine in Thailand, about one-third of pregnant women still had vitamin D insufficiency. The paradox may be explained by several factors such as low sunlight exposure due to contemporary life, use of sunscreen and low intake of vitamin D-rich diet. Pregnant women should be investigated for vitamin D insufficiency or deficiency and correction of 25(OH)D are indicated to prevent the implication of the deficiency for skeletal and extraskeletal health in both pregnant women and their children.

D-23

Vitamin D2 concentration impacts on total vitamin D measurement: comparison of three commercial total 25-OH-vitamin D chemiluminescent immunoassays (CIAs) to liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay

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Background: 25-Hydroxy-vitamin D (25-OH-D) has two major molecular forms, D2 and D3, with similar biological activity. Serum concentration of 25-OH-D reflects vitamin D storage status in the human body. Recognition of concentrations <20 ng/mL has relevant nutritional and metabolic repercussions, increasing its testing demand. Automated CIAs can measure both D2 and D3 molecular forms, but not individually. In contrast, LC-MS/MS can measure both by detecting their unique masses and fragmentations. We investigated the impact of vitamin D2 content on total 25-OH-D measurements of three commercially available CIAs when comparing to LC-MS/MS assay.

Methods: LC-MS/MS method was validated in-house to quantify D2 and D3 concentrations and served as a standard for comparison of three CIAs (DiaSorin Liaison -performed at a referral laboratory-, Abbott Architect and Siemens Centaur assays). Assay validation studies of each method except for the Liaison assay were performed. Fifty-eight patient serum samples were collected and divided into four aliquots for each analytical method. Samples were tested for total 25-OH-D levels by three CIAs, and compared with those obtained by LC-MS/MS.

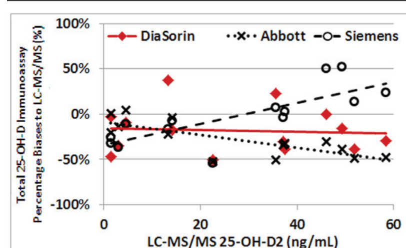
Results: Precision and linearity for Abbott, Siemens and LC-MS/MS are shown below in the Table. A total of 15 samples (26%) contained D2. Concentrations of LC-MS/MS 25-OH-D, D2 and D3 spanned 4.8−90.7, 1.4−58.4 and 3.4−90.7 ng/mL, respectively, all within linearity range and acceptable reproducibility. Comparison of CIAs to LC-MS/MS was evaluated by mean percentage bias (Table), in three scenarios: all samples, and samples with and without D2. Mean bias of the DiaSorin assay was not significantly influenced by the presence of D2, whereas those of the other two CIAs were significantly affected (Table and Figure).

Conclusions: D2 concentrations affect the CIA measurements of total vitamin D for the Abbott and Siemens assays. One potential cause of such phenomenon might be the preferential antibody reactivity towards D2 or D3 in the two CIA assays.

Total Precision	Control 1[a] (ng/mL, CV)	Control 2[a] (ng/mL, CV)	Linearity range (ng/mL)
LC-MS/MS D total	21.1, 7.5%	154, 6.8%	4.0 – 350
LC-MS/MS D3	12.2, 9.3%	84.9, 8.4%	3.0 – 200
LC-MS/MS D2	8.9, 12.5%	68.9, 15.4%	1.0 – 150
Abbott Architect	6.9, 6.2%	76.9, 3.8%	0.0 – 96.0
Siemens Centaur	8.5, 11.2%	86.8, 4.1%	3.7 – 150

Mean bias ± SD to LC-MS/MS (%)[b]	All samples (58 samples)	With D2 (15 samples)	Without D2 (43 samples)
DiaSorin Liaison	-17.6 ± 21.1	-16.4 ± 25.8	-18.1 ± 19.3
Abbott Architect	-4.1 ± 41.5	**-21.8 ± 25.0**	2.6 ± 44.7
Siemens Centaur	-21.7 ± 27.0	**-5.1 ± 29.8**	-28.0 ± 23.2

[a]Control materials used for evaluating precisions of each assay listed are from different sources. Control 1 and Control 2 serum samples at low and high total concentrations, <20 ng/mL and >70 ng/mL, respectively, were chosen.
[b]Percentage bias = (CIA – LC-MS/MS)/(LC-MS/MS) × 100%



D-24

Comparison of a New Binding Protein Based 25-Hydroxy Vitamin D Assay with a Liquid Chromatography -Tandem Mass Spectrometric Method

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Background: 25-hydroxy vitamin D (25-OHD), is commonly used to evaluate vitamin D status. Vitamin D exists in 2 forms: Vitamin D3 and D2. Various methods have been developed to detect 25-OHD and a substantial inter-method variability has been reported. Currently, four methodologies for 25-OHD testing are available: 1) vitamin D binding protein (DBP) based assay; 2) Immunoassay; 3) High performance liquid chromatography; 4) liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS). Usually, the first two methods only report total 25OHD including 25-OHD2 and 25-OHD3. Although most manufacturers claim that their methods can recognize 25-OHD2 and 25-OHD3 equally, some biases have been reported. LC-MS/MS separates 25-OHD2 and 25-OHD3 based on their structures and thus the two forms can be reported individually. Here we compared a new DBP based 25-OHD assay to LC-MS/MS to determine if the DBP assay measures 25-OHD2 and 25-OHD3 equally.

Methods: Seventy-six serum samples were collected and divided into three groups according to 25-OHD2 and 25-OHD3 concentrations determined by LC-MS/MS: group A containing only 25-OHD3 (25-OHD2 < 3 ng/mL, total 25-OHD range: 6.7-92.7 ng/mL), group B containing both vitamin 25-OHD2 and 25-OHD3 (25-OHD2>3ng/mL and 25-OHD3>10 ng/mL, total 25-OHD range: 17.7-111.8 ng/mL), and group C containing mainly 25-OHD2 (25-OHD3 < 10 ng/mL, total 25-OHD range:22.1-96.7 ng/mL). 25-OHD2 and 25-OHD3 were separated in a SunFire C8 column on Alliance HPLC 2795 XE Separation module (Waters) and 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). The DBP assay (Diazyme) is based on the principle of α-complementation of β-galactosidase and the competition between an enzyme donor 25-OHD conjugate and 25-OHD in serum for DBP binding. The assay consists of a 25-OHD extraction step during which 25-OHD is irreversibly dissociated from the endogenous DBP. The 25-OHD concentration is proportional to the β-galactosidase activity. The correlations between LC-MS/MS and DBP assay were analyzed with linear regression analysis and the biases between the two methods were also calculated.

Results: The correlation analysis between LC-MS/MS (x) and DBP assay (y) in the 3 groups showed significant positive associations: y=0.509x+18.033 (R^2=0.70, n=28) in group A; y=1.099x-6.439 (R^2=0.76, n=27) in group B, and y=0.546x+31.244 (R^2=0.46, n=21) in group C. On the other hand, the biases in total 25-OHD concentrations between LC-MS/MS and DBP assay were 18.74% for group A, -16.93% for group B, and -28.54% for group C.

Conclusions: The correlations between LC-MS/MS and DBP assay varies among the 3 groups. The DBP assay shows a better association with LC-MS/MS for samples containing both 25-OHD2 and D3 as compared to samples with 25-OHD2 or 25-OHD3 only. Based on LC-MS/MS, the DBP method overestimates 25-OHD3 but underestimates 25-OHD2. The bias is reduced in samples containing both 25-OHD2 and 25-OHD3.

*We appreciate Gold Standard Diagnostics providing Diazyme total 25-OHD kits.

D-25

Evaluation of the Multigent Iron Assay (6K95) for Serum and Plasma Specimens on the Abbott Architect c8000 Chemistry System

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Objective: To evaluate the analytical performance of the MULTIGENT Iron assay (Fe-Pl, List #6K95) for the measurement of iron in human serum and plasma.

Methods: Precision, linearity, method comparison, reference interval verification, and interference studies were performed as per CLSI protocols. Precision was evaluated at high and low concentrations using commercial quality control material. Measurements from 102 plasma samples were compared to those obtained with the Abbott ARCHITECT Iron assay (List #7D68) using matched serum samples. Interference due to ascorbate, unconjugated bilirubin, and hemoglobin was assessed using patient pools with iron concentrations of 9 and 31 µmol/L, respectively.

Results: Total imprecision equaled 3.2% and 1.5%, at 11.8 µmol/L and 45.2 µmol/L, respectively, over 20 days (single reagent lot and 2 calibrations). Linearity was observed between 1-183 µmol/L. Comparison of the current ARCHITECT Iron assay (x) versus Fe-Pl (y) gave y = 1.059x - 1.1; R=0.9813. The current reference interval (9-32 µmol/L) was verified in healthy subjects (n=40). The Fe-Pl assay showed <10% error in the presence of up to 1026 µmol/L bilirubin or 680 µmol/L ascorbate. However, significant positive interference occurred at hemoglobin concentrations above 0.1 g/L and 0.7 g/L in the low and high iron patient pools, respectively.

Conclusions: The MULTIGENT Iron (Fe-Pl) assay demonstrated acceptable precision and correlation with the current ARCHITECT Iron assay. The linear measurable range met the manufacturer's stated claim. Not surprisingly, hemoglobin caused significant interference. The ability to test plasma specimens should prove useful to laboratories seeking to consolidate chemistry testing in plasma collection tubes.

D-26

Performance Characteristics of the Siemens Centaur XP for the Determination of total 25 OH Vitamin D.

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Background: Being nicknamed the “Sunshine Vitamin” was not the only reason that made vitamin D a hot topic. Although it has been well known for its role in bone metabolism, the important role of vitamin D beyond that was the discovery of the 1,25 dihydroxyvitamin D nuclear receptor in most tissue. This increased interest in vitamin D has come about with recently introduced evidence that vitamin D can regulate the immune system and thereby it is implicated in several immune-mediated disease states. Several studies have linked vitamin D levels with different clinical outcomes like incidence of cancer, diabetes, autoimmune disorders and may play an important role in keeping the brain in good working order in later life.

Methodology: The ADVIA Centaur VitD assay is a one-pass, 18-minute antibody competitive immunoassay that uses an anti-fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles, an anti-25(OH) vitamin D monoclonal mouse antibody labeled with acridinium ester, and a vitamin D analog labeled with fluorescein. An inverse relationship exists between the amount of vitamin D present in the patient sample and the amount of relative light units (RLU) detected by the system. The assay is fully automated. We evaluated the assay sensitivity based minimum detectable concentration (MDC), we took 20 blank samples RLU, add 2*SD and back calculate the dose; in addition, we calculated the sensitivity based on limit of blank (LoB) which is the 95% tile of the data set observed when running a blank sample. We checked linearity, precision/accuracy (20 replicates) , reportable range, and 418 samples correlation with Liaison assay, discordant samples were sent for verification using LC-MS/MS. Statistical analysis were done using Analyse-it.

Results: the sensitivity was 2.52 ng/mL based on MDC and 2.39 ng/mL based on LoB, within assay coefficient variation were 8.2% and 3.3% for a concentration of 22.2 ng/mL and 92.8 ng/mL respectively. Analytical range was verified from 4.3-142.6 ng/mL. Regression analysis between Centaur and Liaison gave a slope of 1.18 and intercept -7.15 and correlation coefficient of 0.7986. Agreement between Centaur and Liaison was: low agreement 91.91%, normal agreement 56.67%, high agreement 100% and total agreement was 76.98%. The LC/MS/MS results were closer to the Centaur XP assay than to the Liaison.

Conclusions: Centaur vitamin D assay gave the benefit of a fully automated, high throughput, high precision and acceptable sensitivity assay. The Centaur XP assay requires less steps and reagent preparation than the Liaison assay which gives it the advantage of saving technician time and making it a more user friendly assay. The agreement between LC/MS/MS and Centaur make this assay a better choice to assess vitamin D status.

D-27

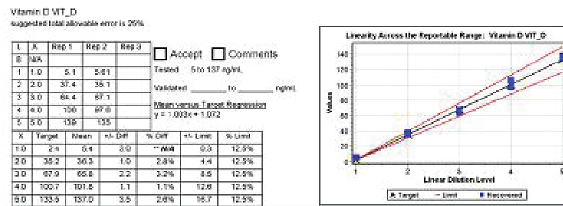
New Calibration Verification / Linearity Test Kit for 25-Hydroxyvitamin D

K. Strait, D. Kelsey. *Maine Standards, Windham, ME*

Objective: To provide a calibration verification / linearity test kit for Vitamin D in a human serum protein based matrix. Relevance: In the last decade, there has been an increased interest in the role of Vitamin D in human health. Studies have revealed that most people do not have sufficient circulating level of Vitamin D. These results have led to a great increase in the amount of Vitamin D testing, with many labs experiencing a 50 - 100% increase in testing year after year. Unlike many commercial calibration verification materials that are manufactured in animal protein matrices, the VALIDATE® Vitamin D linearity set is made using a human serum protein matrix and should better reflect performance of native samples.

Methodology: VALIDATE Vitamin D linearity set was assayed for 25-hydroxyvitamin D using DiaSorin’s Liaison instrument. Samples were analyzed in triplicate and results were processed using MSDRx® Calibration Verification software (Maine Standards Company). Validation: VALIDATE Vitamin D was manufactured in a manner to produce an equal delta’s between consecutive levels, allowing users to determine linearity according to Clinical and Laboratory Standards Institute EP06-A guideline for linearity testing. Data regression statistics, shown in Figure 1, represent observed values regressed against software-calculated target values.

Conclusions: The VALIDATE Vitamin D linearity set, prepared in a human serum protein matrix, displays linearity with an excellent coverage of the claimed range for 25-hydroxyvitamin D.



Vitamin D VIT_D
suggested total allowable error is 25%

L	X	Rep 1	Rep 2	Rep 3
0	N/A			
1	1.0	5.1	5.61	
2	2.0	37.4	35.1	
3	3.0	64.4	67.1	
4	4.0	100	97.8	
5	5.0	139	135	

Accept Comments
Tested 5 to 137 ng/mL
Validated ______ to ______ ng/mL
Mean versus Target Regression
y = 1.003x + 1.072

X	Target	Mean	+/- Diff	% Diff	+/- Limit	% Limit
1.0	2.4	5.4	3.0	** N/A	0.3	12.5%
2.0	35.2	36.3	1.0	2.8%	4.4	12.5%
3.0	67.9	65.8	2.2	3.2%	8.5	12.5%
4.0	100.7	101.8	1.1	1.1%	12.6	12.5%
5.0	133.5	137.0	3.5	2.6%	16.7	12.5%

D-30

Comparison of 25-hydroxyvitamin D assay on Abbott Architect and Siemens Advia Centaur against liquid chromatography-tandem mass spectrometry.

Y. Lee, K. Sobhani, H. Sadrzadeh. *Cedars-Sinai Medical Center, Los Angeles, CA*

Objective: The objective of this study is to evaluate the performance of Abbott Architect 25-hydroxyvitamin D assay and of Siemens Advia Centaur against liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Background: The role of vitamin D in calcium and phosphorus homeostasis and related disorders has been established. Also, vitamin D has been associated with disorders such as cardiovascular, neurodegenerative, neurological disorders, and cancers. An ongoing issue in laboratory medicine is the accurate measurement of 25(OH)D2, 25(OH)D3, and 1,25-(OH)2D. Although LC-MS/MS has been shown to be the “gold standard” in measuring vitamin D, there is still a lack of method/calibration standardization between different laboratories. Currently, several 25(OH)D immunoassays have been introduced to measure total vitamin D. The purpose of this study was to evaluate 25(OH)D immunoassays by Abbott Architect and Siemens Centaur against LC-MS/MS.

Methods: 209 blood specimens were tested for total 25(OH)D by LC-MS/MS, Architect and Advia Centaur. Frozen human serum samples from External Quality Assessment Scheme (DEQAS, a proficiency survey) and 1,25(OH)2D, were also run on both platforms for comparison. Abbott Architect 25(OH)D assay is a delayed, one-step chemiluminescent microparticle immunoassay and the Centaur 25(OH)D assay is a one-pass, antibody competitive immunoassay.

Results: Within-run precision for Architect 25 (OH)D assay was 1.9% (at 75 ng/mL) and 3.7% (at 20 ng/mL), and for the Centaur assay was 7.2% (at 20 ng/mL) and 2.4% (at 90 ng/mL). Linearity was from 5 ng/mL to 121 ng/mL for Architect assay and 4 ng/mL to 160 ng/mL for Centaur method. There was no carry-over in either assay. Results generated by the Centaur correlated well with those from LC/MS/MS (r^2= 0.86, slope of 1.19 and an intercept of -7.36, for the total 25 (OH)D values ranging from 8.3-96.0 ng/mL). The correlation between Architect and LC/MS/MS was also acceptable (r^2= 0.89, slope of 1.13 and an intercept of -11.4, for the total 25 (OH)D concentrations of 8.3-96.0 ng/mL). Both immunoassays detected over 80% of 25 (OH) D2 (estimated by calculation)

Conclusions: Our results show that both Abbott Architect and Siemens Advia Centaur 25(OH)D assays are accurate, precise, and easy to run. Both immunoassays detected greater 80% of 25(OH)D3 and correlated well with the LC-MS/MS.

Wednesday PM, July 18, 2012

Poster Session: 2:00 PM - 4:30 PM

Point-of-Care Testing

D-31

Comparison of dried blood spot to venous methods for hemoglobin A1c, glucose, total cholesterol, high-density lipoprotein cholesterol, and C-reactive protein

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Background: Dried blood spots (DBS) have been used in newborn screening and increasingly in large population-based studies. Compared to venipuncture, DBS can be collected by non-phlebotomists in non-clinical settings, and are relatively inexpensive, more easily transported and can be stored more conveniently. Disadvantages of DBS include difficult assay development and validation when compared with venipuncture.

Objective: To compare dried blood spot methods to venous methods for hemoglobin A1c, glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and C-reactive protein (CRP) as part of the National Health and Nutrition Examination Survey (NHANES).

Methods: Collection of dried blood spots and venipuncture were performed together on 401 participants ages 20 and above in the NHANES mobile examination center from late 2009 through early 2010. The participant's finger was pricked by a phlebotomist using a Becton Dickinson contact-activated lancet and drops of blood were collected on Whatman 903 protein saver card to fill five 1/2" discs. The cards were allowed to dry at least 4 hours at room temperature. DBS cards were put in plastic bags with desiccant packs and stored in a refrigerator for up to 7 days. The DBS cards were shipped on dry ice to the University of Washington Department of Laboratory Medicine for analysis. Upon receipt of the DBS cards, the laboratory inspected the cards for quality and stored the cards in sealed bags at -70C. For analysis, the DBS cards were thawed to room temperature, and 3.2 mm blood punches were produced by a semi-automatic puncher into a 96-well microtiter plate for elution. The eluent was then analyzed. The CRP was measured by sandwich ELISA, hemoglobin A1c by ion-exchange HPLC, and the glucose, total and HDL cholesterol were measured using fluorescent assays. The DBS data for each participant were compared to the following venous **Methods:** CRP (nephelometry), hemoglobin A1c (HPLC), glucose, total and HDL cholesterol (photometric).

Results: The Pearson correlation coefficients were high for hemoglobin A1c (0.96), CRP (0.92) and glucose (0.90). The Pearson r was low for total cholesterol (0.58) and HDL cholesterol (0.55). Deming regressions were developed to predict the DBS from venous concentrations. Sensitivity and specificity were calculated at selected clinical cut-points using venous methods as the reference methods. Respectively, sensitivity and specificity was relatively high for CRP (84.7%, 95.1% at a cut-point of 3 mg/L), glucose (85.7%, 90.5% at 126 mg/dL), and hemoglobin A1c (85.2%, 97.6% at 6.5%). Low sensitivity and relatively high specificity were seen for HDL cholesterol (42.9%, 86.2% at 40 mg/dL) and total cholesterol (31.0%, 92.6% at 240 mg/dL), respectively.

Conclusions: Dried blood spot methods were compared to venous methods for NHANES participants. The hemoglobin A1c, glucose and CRP correlated well between DBS and venous methods (Pearson $r > 0.90$), but there was a poor correlation for total and HDL cholesterol ($r < 0.58$). This resulted in low sensitivity (< 43%) of DBS methods for total and HDL cholesterol.

D-32

The Nova Stat Strip Glucose Meter Evaluation in Intensive Care Unit

A. Ivanov. *Tartu University Hospitals, Tartu, Estonia*

Background: The ISO 15197 guideline states that meter measurements should be within 0,83 mmol/L of the result for glucose <4,2 mmol/L and within 20% for glucose ≥4,2 mmol/L.

We assessed the performance of a point-of-care (POC) glucose meter by using the spreadsheet program is designed for estimating the bias between two methods using patient samples. In this case the imprecision was estimated by using control samples.

Methods: The study was performed over a one week period using samples obtained from the intensive care unit of the Anesthesiology and Intensive Care Clinic of Tartu University Hospital. Method correlation was perfomed by analyzing 41 whole blood specimens on the Nova Stat Strip glucose meter compared to ABL 825 blood gas analyzer (Radiometer). Patient samples were measured in duplicates, 3 levels of quality control samples were measured daily in triplicate on the three glucose meters. The reference method measurement was performed on 20 plasma specimens using the Cobas 6000 (Roche) using the hexokinase method. Whole blood was collected into sodium heparin BD Vacutainer for plasma glucose testing and heparinised Safe Pico (Radiometer) syringe for glucose testing on the glucose meter and on the blood gas analyzer. Specimen collection was perfomed by umbilical arterial catheter, plasma glucose samples put immediately in the +4C° container to prevent subsequent artifacts from glycolysis. Mean glucose concentration was 7,62 mmol/L (range =3,1-32 mmol/L).

Results: The percent coefficient of variance (% CV) for glucose meter across the 3 levels (3,3/6,4/16,1 mmol/L) was 6,1/3,4/5,4 %. The Stat Strip glucose meter results corresponded to a confidence level of 95% was 3,4±0,42 mmol/L (3,4 mmol/L ±12,2%), 6,0 ± 0,42 mmol/L (6,0 mmol/L±6,8%) and 15,7 ±1,68 mmol/L(15,7 ±10,8%).

The linear regression analysis demonstrated a slope 1,18 and glucose meter had the lowest mean biases (-0,160 mmol/L) compared with laboratory hexokinase method. The mean difference glucose meter compared with ABL 825 was -0,037 and a slope 1,01. The results demonstrate that Stat Strip and laboratory methods had not significant bias (*p*-0,545 for hexokinase and 0,723 for ABL).

Conclusions: Stat Strip glucose meter met ISO 15197 perfomance criteria and demonstrated a close correlation to the laboratory methods.

D-33

Screen-positive rates and predicted sensitivities for elevated blood lead (EBL), defining EBL as either >=5 µg/dL or >=10 µg/dL, based on simulated sampling of a pediatric population distribution for [Pb] using point-of-care testing

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Background: There has been advocacy for a change in definition of pediatric EBL from >=10 µg/dL (cutoff *A*) to >=5 µg/dL (cutoff *B*). Using a known current pediatric patient population distribution for [Pb] as a basis dataset, we determined the effects of this change on screen-positive rate and on predicted sensitivity of point-of-care testing (POCT) for detection of EBL. Predicted POCT sensitivity was evaluated by simulated sampling of the basis distribution, using [Pb]-dependent measurement precision characteristic of POCT analyzers, and tracking the resulting rates of misclassifications of EBL using cutoffs *A* or *B*.

Methods: A basis current pediatric patient population distribution for [Pb] was that of first-or-only Pb measurements for pediatric subjects (<18 y) during a 1-year interval (2011) at the Nebraska Medical Center (n=10,333). For the basis dataset, screen positive rates for *A* and *B* were compared. For sake of argument in assessing predicted POCT sensitivity, the basis dataset was taken as a "true" [Pb] distribution. Predicted sensitivities for detection of EBL for *A* and *B* were determined by simulated sampling of the original distribution with [Pb]-dependent precision. As government agencies responsible for followup of lead testing results typically receive data from multiple sites, and because the POCT LeadCare II analyzer (LCII) is the predominant method among all participating sites in the national Pb proficiency testing program (Wisconsin State Laboratory of Hygiene), we utilized interlaboratory precision data for LCII as the basis for assigning [Pb]-dependent POCT precision. For 2011 surveys, the interlaboratory standard deviations (s) for [Pb] measurement using LCII were linear with [Pb] (x): $s=0.074x+0.502$ µg/dL ($r^2=0.969$, $n=18$). For example, at $x=5$ µg/dL, $s=0.87$ µg/dL (CV=17.4%); at $x=10$ µg/dL, $s=1.24$ µg/dL (CV=12.4%). By simulation, the original patient population distribution was repetitively sampled for x using a random number generator and according to dataset probabilities for x. A new distribution of results was formed by replacing each sampled x with a new value x', where x' was determined using a random number generator according to probabilities of a normal distribution centered on x with standard deviation $s(x)$. Simulation additionally tracked the number of results on either side of the cutoffs *A* or *B* for which x' was misclassified relative to the original classification for x; for example, for x>A or $x>B$, simulation tracked whether x' was a true positive (TP) or a false negative (FN). Whereas sensitivity (S=TP/(TP+FN)) is the greatest programmatic concern for EBL screening, we compared simulation results for S between cutoffs *A* and *B*.

Results: For the basis dataset, screen-positive rates (P) were 1.02% for *A* and 4.69% for *B*; $P(B)/P(A) = 4.6$. Results for S by simulated sampling of the original distribution (1,000 passes x 10,000 samples/pass) were $S(A)$=94.2±2.4% (CV=2.5%),

S(*B*)=89.3±1.5% (CV=1.7%).

Conclusions: For the given current pediatric population distribution for [Pb], change of EBL cutoff from *A* to *B* would greatly increase (>4-fold) the EBL screen-positive rate. By simulation, POCT using cutoffs *A* or *B* had sensitivity for EBL of less than 95%; for proposed cutoff *B*, approximately 1 in 10 EBLs were misclassified by POCT.

D-34

Evaluation of the NexScreen and DRUGCHECK Waive urine drug detection cups

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Background: Urine drug testing is commonly performed for clinical, workplace and criminal applications. Immunoassay is the most common method used for initial screening. On-site (point of collection) immunoassay devices are now widely marketed. The main advantage of these devices is timely availability of laboratory test results, within a few minutes. Concerns include poor sensitivity and specificity. We evaluated performance of two CLIA waived immunoassay based urine drug screen cups.

Methods: The NexScreen cup and the DRUGCHECK Waive cup were purchased from Amedica Biotech Inc. (Hayward, CA) and Express Diagnostics Int'l Inc. (Blue Earth, MN), respectively. Both cups claim to detect amphetamine, barbiturates, benzodiazepines, cocaine, marijuana, methadone, methamphetamine, oxycodone, opiates, and phencyclidine. The NexScreen cup claimed to also detect ecstasy (MDMA) and tricyclic antidepressants. This study included a sensitivity and precision challenge with 4-6 replicates at concentrations 0-150% of the manufacture's claimed cutoff, by using drug-free urine spiked with Cerilliant purified reference standards (Round Rock,TX). Stability of test results was evaluated by reading the results at 5, 10, 15, 30, 60, 120 and 1440 minutes. The specificity of the test was evaluated by parallel comparison of pooled patients' specimens, representing 56 patients and 37 known drug compounds.

Results: The Table shows the detection limit and stability of the results. When comparing results to validated liquid chromatography mass spectrometry results, false positives were observed in the NexScreen cups for benzodiazepine, methamphetamine, methadone, opiates, and tricyclic antidepressant tests, but no false negatives. DRUGCHECK Waive cups showed false negative results for barbiturates and opiates but no false positives.

Conclusions: Overall the NexScreen cup demonstrated sensitivity better than claimed, whereas the sensitivity of DRUGCHECK Waive cups did not meet claims. Specificity was a concern with both cups. Results of immunoassays are always considered presumptive until confirmed.

Detection limit and stability of urine drug detection cups

	Nex Screen cup(required 30 mL, read at 10 min.)				DRUGCHECK Waive cup (required 75 mL, read at 5 min.)			
	Claimed detection limit (ng/mL)	Observed detection limit (ng/mL)	Sensitivity based on claimed conc.(%)	Stability (min.)	Claimed detection limit (ng/mL)	Observed detection limit (ng/mL)	Sensitivity based on claimed conc. (%)	Stability (min.)
Amphetamine	1000	520	≥100	60	1000	>1562	75	15
Phenobarbital	300	150	≥100	120	450	150	≥100	60
Oxazepam	300	<82	≥100	≥1440	300	>495	50	15
Benzoylecgonine	300	<42	≥100	≥1440	300	167	≥100	10
11-Nor-d9-THC-COOH	50	15	≥100	≥1440	50	>45	Not Determined	-
Methadone	300	<80	≥100	10	300	>450	0	-
Methamphetamine	1000	500	≥100	≥1440	1000	>1500	0	-
Oxycodone	100	<19	≥100	≥1440	100	114	75	5
Codeine	300	<73	≥100	≥1440	300	145	≥100	120
Morphine	300	<71	≥100	≥1440	300	284	≥100	10
Phencyclidine (PCP)	25	12	≥100	≥1440	25	>35	25	5
MDMA (Ecstasy)	500	592	≥100	30				
Nortriptyline	1000	500	≥100	≥1440				

D-35

Proficiency testing (PT) for Pb point-of-care testing (POCT): predicted effect of change of PT acceptance criterion, from ±4 µg/dL to ±2 µg/dL, on PT pass rates for [Pb] <10 µg/dL

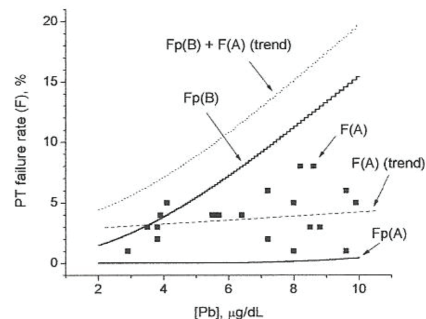
F. R. Bordash[1], L. J. McCloskey[1], J. D. Landmark[2], D. F. Stickle[1]. [1]*Jefferson University Hospitals, Philadelphia, PA,* [2]*University of Nebraska Medical Center, Omaha, NE*

Background: The current acceptable Pb PT performance criterion is ±4µg/dL in the range of [Pb]=0-40µg/dL. CDC recommends utilization of methods with performance of ±2µg/dL for [Pb]<10µg/dL. As the majority of PT participants utilize POCT, we examined the potential effect of a change in PT acceptance criterion to ±2µg/dL on POCT PT pass rates for [Pb]<10µg/dL.

Methods: National Pb PT survey data for LeadCareII POCT Pb analyzers were collected from the summary data for samples having more than 100 respondents (Wisconsin State Laboratory of Hygiene; 111 samples, 2008-2011). Data comprised sample average [Pb] (x), interlaboratory standard deviation for x ($s(x)$), and survey failure rates (F) for each sample. Predicted random-error-only survey failure rates (F_p) as a function of x were calculated for two assumed performance criterion, either ±4µg/dL (A) or ±2µg/dL (B): F_p(A), F_p(B) = percentage of results from a normal distribution for x characterized by $s(x)$ that fall outside of boundary A or boundary B, respectively.

Results: For (A), PT failure rates F(A) were 3.7±2.0% for [Pb]<10µg/dL (n=27) (Figure). Across all survey results, $s(x)$ was a linear function of x: s=0.0731x+0.674µg/dL (r^2=0.879, n=111); for example, s([Pb]=10µg/dL)=1.41µg/dL. Based on $s(x)$, calculated F_p(A) was well below the observed F(A) for [Pb] in this range (Figure), meaning that most results in F(A) were outliers with respect to the reported $s(x)$, representing a background rate of systematic rather than random errors. Calculated F_p(B) is dramatically different, however, ranging from 1.6-15% for this range of [Pb] for random errors alone (Figure).

Conclusions: Based on interlaboratory POCT precision data, change of Pb PT acceptance criterion to ±2µg/dL for [Pb]<10µg/dL would substantially increase POCT Pb PT failure rates (e.g., F>15% at [Pb]=10µg/dL), due to the extent to which random error alone would exceed those limits.



D-36

Effects of Environmental Stress on Point-of-care Cardiac Biomarker Test Results During Simulated Emergency/Disaster Rescue

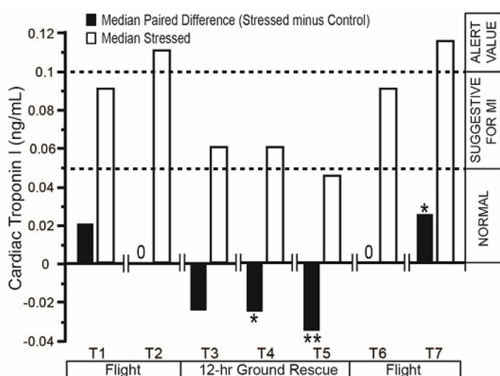
W. J. Ferguson, R. F. Louie, C. M. Curtis, J. H. Vy, C. S. Tang, G. J. Kost. *UC Davis-LLNL Point-of-Care Technologies Center, Davis, CA*

Objective: Our objective is to characterize the effect of environmental stress on point-of-care (POC) cardiac biomarker test results during simulated rescue from Hawaii to the Marshall Islands and back.

Methods: Test cards, which use a fluorescence immunoassay to measure troponin I (cTnI), BNP, CK-MB, myoglobin, and D-Dimer, were placed inside a Tenney chamber simulating a 24-hour rescue with flight conditions, 20ºC and 10% relative humidity, and ground conditions, 22.3 - 33.9ºC and 73 - 77%. Paired measurements were obtained with test cards stressed inside the chamber and controls at room temperature during seven time points (T_{1-7}): $T_{1,2}$, 6-hr outbound flight; $T_{3,4,5}$, 12-hr ground operation; and $T_{6,7}$, 6-hr return flight. Wilcoxon signed rank test was used to analyze paired differences (stressed minus control). We assessed decision making using key thresholds: 95th percentile of normal individuals (cTnI < 0.05 ng/mL), suggestive of myocardial infarction (cTnI ≥ 0.05 ng/mL), and an alert value associated with high morbidity and mortality (cTnI ≥ 0.1 ng/mL). Recommended test card operating temperature is 20-24ºC.

Results: The Figure shows cTnI median paired differences and median stressed results for T_{1-7}. cTnI results (n=10) at T_4 (p<0.05), T_5 (p<0.01), and T_7 (p<0.05) differed significantly from control. During ground rescue, 36.7% (11/30) of stressed cTnI measurements falsely generated normal levels. At T_5, 20% (2/10) of cTnI results were highly discrepant_stressed test cards produced results interpreted as normal, while control results were > 0.10 ng/mL. Despite short term (4-12 hrs) temperature elevation (33.9ºC), simulated rescue conditions led to a decreasing trend in cTnI that could mislead diagnosis and affect outcomes adversely.

Conclusions: We recommend that first responders store POC reagents at manufacturer specified conditions and be aware that even short exposure to environmental stress should be avoided by using appropriate thermomodulation containers.



D-39

Analytical and operational evaluation of the ROM-Plus test for rupture of fetal membranes

P. Gong, F. R. Bordash, L. J. McCloskey, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: ROM-Plus (Clinical Innovations) is a recently approved test for rupture of fetal membranes, intended for use at point-of-care. The test is a lateral flow sandwich immunoassay for detection of either placental protein 12 (PP12, or insulin-like growth factor binding protein-1, IGFBP-1) or alpha-fetoprotein (AFP) in vaginal pool fluid, as markers for presence of amniotic fluid. We investigated analytical and operational characteristics of the assay: sensitivity for detection of controls, stability of controls, dilution factor of swab samples, and titre of near-term amniotic fluid and of biological fluids other than amniotic fluid.

Methods: Operation of the assay was according to manufacturer's instructions, using either direct application (DA) of fluids to the application point (as for controls) or by application after swab transfer (ST) to diluent (as for samples), as noted. Controls are provided in sealed glass ampules within pliable plastic holders. For positive control, release of solvent by breakage of the ampule subsequently dissolves lyophilized protein within the holder. The holder is also a dropper device for direct application of fluid to the application point. For vaginal pool samples, a plastic holder with diluent is provided wherein a swab sample is placed for elution of sample from the swab. After score-point breakage of the tip which then remains in the diluent, the holder has an attached dropper cap wherewith sample is applied. A dye-diffusion timer on the device is activated by finger. Samples are to be read as positive or negative by appearance of a line at the test position not more than 20 min after sample application.

Results: Mass-carrying capability of swab for 7 g/dL albumin solution was on average 79±13 µL (n = 6); given diluent volume of 380 µL, this indicated an average minimum dilution for ST samples of 18%. Positive control (stated concentrations [AFP] = 600 ng/mL, PP12 = 20 ng/mL) was positive (DA) to 1:30 dilution, consistent with stated device analytical sensitivity ([AFP] = 150 ng/mL, PP12 = 5 ng/mL) for ST when accounting for ST dilution. Control 1:8 titre remained positive (DA) after 10 days storage either refrigerated or frozen. By ST, near-term pregnancy pooled, previously frozen amniotic fluid (submitted for fetal lung maturity testing) was positive to titre less than 1:3000. EDTA-whole blood samples from males, non-pregnant females (<36 years of age), and near-term pregnant females were all positives by ST. Pooled plasma specimens for near-term pregnant females were positive by ST to titre between 1:3 and 1:10. Urine from near-term pregnancy was negative (ST).

Conclusions: Positive ROM-Plus ST results for samples other than amniotic fluid are likely due to high test sensitivity for IGFBP-1. Compared to amniotic fluid, near-term pregnancy plasma samples were positive by ST application only at low titre (>1:10). Thus, barring bloody samples, a test positive by ST is likely the result of the presence of amniotic fluid, in accordance with premise and intent of the assay. Analytical performance results verified manufacturer's FDA-approval studies. The operational design of the ROM-Plus assay was judged to be highly suitable for use as point-of-care testing.

D-40

Method comparison and bias estimation at clinical decision levels for electrolytes and hemoglobin results measured with point-of-care blood gas and laboratory analyzers

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Background: Point-of-Care Testing (POCT) is widely used in clinical practice based on medical benefits. Patient samples could be analyzed with different devices within an institution. According to specific guidelines, the comparability of POCT and core laboratory results must be verified before using at any POCT setting.

Objective: Estimate bias at clinical decision levels in order to establish whether electrolytes and hemoglobin results measured with a point-of-care blood gas and laboratory analyzers are interchangeable.

Material and Methods: The blood gas analyzer involved in the study was ABL90® (Radiometer). Dimension Vista® (Siemens) and Cell Dyn Sapphire® (Abbott) were considered as reference measurement procedures for electrolytes (sodium, potassium and chloride) and hemoglobin respectively. According to CLSI protocol EP-9, 40 patient samples were performed with 2 replicates per analyzer. Whole blood samples were used for ABL90 and Cell Dyn Sapphire and lithium heparin plasma for Dimension Vista obtained from the same sample collection. Linear regression with slope and intercept was calculated for each parameter in order to estimate bias at the medical decision levels (provided by www.westgard.com). The allowable bias was established by laboratory according to biological variation criteria. Statistical analyses was performed using StatisPro™ software (Clinical and Laboratory Standards Institute).

Results

Parameter	Clinical decision levels	Estimated bias	95% Confidence interval	Allowable bias	Allowable bias (%)
Sodium (mmol/L)	115	0.52	-0.87 to 1.92	1.61	1.4%
	135	0.40	-1.00 to 1.79	1.89	
	150	0.30	-1.06 to 1.66	2.10	
Potasium (mmol/L)	3.0	-0.19	-0.27 to -0.11	0.26	8.7%
	5.8	-0.36	-0.48 to -0.23	0.50	
	7.5	-0.46	-0.69 to -0.22	0.65	
Chloride (mmol/L)	90	1.7	1.0 to 2.3	2.0	2.2%
	112	2.5	1.6 to 3.3	2.5	
Hemoglobin (g/dL)	4.5	-0.21	-0.57 to 0.15	0.28	6.2%
	10.5	0.09	-0.27 to 0.44	0.65	
	17.0	0.41	-0.24 to 1.05	1.05	
	23.0	0.70	0.05 to 1.35	1.43	

Conclusions: The allowable bias was greater than estimated for sodium, potassium, chloride and hemoglobin at stated clinical decision levels. Therefore, ABL90 is equivalent to Dimension Vista and Cell Dyn Sapphire for these parameters, of which are interchangeable. If more than one system is used to follow patients, it is important to consider not only the method comparison but also the relative bias that could be safely tolerated between methods at medical decision points.

D-41

A High Specificity Point of Care Assay for Low Density Lipoprotein to Assess Cardiovascular Disease Risk

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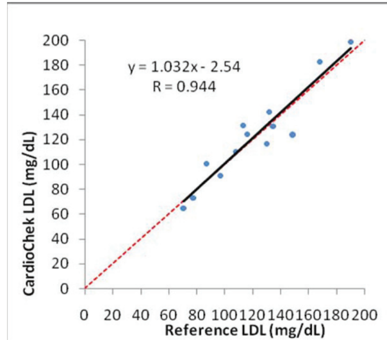
Background: Total Cholesterol (TC) level as well as cholesterol fraction levels, e.g., low density (LDL), high density (HDL) and triglyceride (Trig) are critical measurements for assessment of cardiovascular disease (CVD) risk. While TC and the TC/HDL ratio is a common screening tool, NCEP-ATPIII guidelines define preferred LDL targets for assessing CVD risk and management of therapy. In health screening settings where POCT provides effective counseling of patients, POCT-LDL is not often used. A major drawback of current POCT-LDL assays is interference of non-LDL fractions. We have developed a novel POCT-LDL test to quantify the levels of LDL cholesterol in serum, plasma and whole blood. The test uses Trinder chemistry methodology and the principles of reflectometry in a CardioChek® system.

Methods: The POCT-LDL assay achieved reduction of non-LDL interference through

use of novel surfactants systems selected based on specificity for LDL and non-LDL (for e.g, HDL and VLDL & Chylomicrons). These surfactants along with specific cholesterol enzymes are impregnated on membranes and assembled into a test strip. LDL is quantified based on the degree of reflectance reading using a CardioChek which is correlated to a reference laboratory standard (Roche, COBAS Integra+).

Results: Initial studies using serum samples quantified LDL component. The within run LDL precision (CV%) was 5.28 at 75 mg/dL, 7.50 at 131mg/dL and 9.08 at 178 mg/dL. Correlation studies vrs COBAS yielded excellent accuracy (y = 0.967x + 3.32) and correlation (R= 0.98); total analytical error of 18.27%. Excellent accuracy and correlation was also achievable using donor whole blood (graph). Specificity of the surfactant system for LDL was demonstrated in comparative analyte recovery of spiked samples (TC, LDL and HDL); confirming the cholesterol fraction specificity.

Conclusions: The described POCT-LDL assay unaffected by non-LDL factions offers potential value for CVD risk stratification in health screening applications.



D-43

Evaluation of the Siemens RAPIDPoint 500 Blood Gas Analyzer

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Background: This study evaluated the analytical performance of the new RAPIDPoint 500® (RP500) Blood Gas System* (Siemens Healthcare Diagnostics, Tarrytown, NY) using the RAPIDPoint® 405 (RP405) Measurement Cartridge. The new system utilizes the existing RP405 cartridge, RAPIDPoint waste wash cartridges, and Automatic QC cartridges. The RP500 analyzer has an updated operating system as well as other new features, including a USB port and an integrated barcode reader. The RP500 system will support measurement of pH, pCO_2, pO_2, Na^+, K^+, Cl^-, Ca^{++}, Glu, tHb and co-ox fractions, as well as neonatal bilirubin.

Methods: Method comparison testing was based on Clinical Laboratory Standards Institute (CLSI) EP09-A2-IR, and precision testing was based on CLSI EP05-A2.

Results: The table shows Deming regression analysis statistics for the method comparisons, in which the RP500 was compared to the RP405 across each analyte range. The slopes for each analyte fell between 0.985 and 1.011, with $R^2 > 0.992$.

Conclusions: On the basis of the data provided, the RAPIDPoint 500 Blood Gas System reads accurately across all claims and levels for each analyte when compared to the RAPIDPoint 405 system. The RAPIDPoint 500 system provides reliable clinical blood gas results.

Analyte	n (pairs)	Slope	Intercept	R	R^2	Range	Sy.x	Mean x	Mean y
Ca	987	1.009	-0.012	0.9984	0.9968	0.27 - 4.98mmol/L	0.04	1.262	1.26
Cl	971	1.011	-1.1	0.9959	0.9918	71 - 139 mmol/L	1.00	100.3	100.2
Glu	659	0.998	0.2	0.9989	0.9978	26 - 745 mg/dL	7.90	144.3	144.3
K	617	1.008	-0.031	0.999	0.9980	1.02 - 14.76 mmol/L	0.11	4.223	4.227
Na	984	1.008	-1.27	0.9967	0.9934	104 - 174.2 mmol/L	0.93	137.1	136.97
pCO_2	759	0.995	0.85	0.9929	0.9859	6.9 - 158.6 mmHg	3.96	59.42	59.97
pH	627	1.000	-0.0014	0.9986	0.9972	6.950 - 7.644	0.01	7.2975	7.2973
pO_2	753	0.985	1.02	0.9997	0.9994	21.5 - 667.8 mmHg	3.45	121.69	120.92
FCOHb	735	1.000	-0.43	0.9998	0.9996	0.1 - 81.6 %	0.36	8.61	8.18
FHHb	737	1.001	0.13	0.9995	0.9990	0.3 - 65.8%	0.62	12.73	12.87
FMetHb	735	0.996	0.02	0.9997	0.9994	0.0 - 53.6%	0.23	3.9	3.9
FO_2Hb	720	0.997	0.44	0.9995	0.9990	17.4 - 98.6%	0.65	75.51	75.75
nBili	355	1.010	0.03	0.9924	0.9849	2.1 - 29.4 mg/dL	0.74	11.6	11.7
tHb	725	0.996	0.2	0.998	0.9960	2.8 - 24.0 g/dL	0.24	14.31	14.44

D-44

Glass Chip Development for Microarray Measurements of Antiphospholipid Antibodies Using Reflectometric Interference Spectroscopy (RIfS)

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Background: Antiphospholipid syndrome (APS) is an autoimmune disease that results in thrombophilia and causes recurrent fetal loss. Antiphospholipid antibodies (aPL) target anionic phospholipids, mainly cardiolipin, but also phospholipid-binding plasma proteins, such as β2-glycoprotein I (β2-GPI) or prothrombin (PT) and phospholipid-protein complexes. Using reflectometric interference spectroscopy (RIfS) a novel, label-free microarray shall be developed to record the interactions of aPL with APS antigens in serum. This requires immobilization strategies and processing conditions suitable for all antigenic structures of interest. The solid-state surface for reflectometric interference spectroscopy consists of glass carrier chips that allow for a series of bioconjugate chemistries. This increases the probability to find an immobilization strategy compatible for a variety of chemically completely different antigens and a surface with attenuated unspecific binding characteristics.

Methods: The APS antigen β2-GPI was immobilized by applying six different strategies. In a first step glass chips were coated with 11-aminoundecyltrimethoxysilane (11-AUTMS), polyethylene glycol (PEG) and amino dextran (AMD) respectively. Subsequently, the antigen was immobilized in two different ways. Either directly to the modified surfaces or as biotinylated variant to a covalently bound streptavidin moiety. To compare selectivity and sensitivity of those diverse surfaces, sera of APS patients and healthy controls were analyzed.

Results: We designed a chip surface that allows immobilization of β2-GPI without compromising its antigenicity. Measurements with APS patient sera showed a good sensitivity and selectivity for the detection of anti-β2-GPI antibodies with the β2-GPI directly coupled to 11-AUTMS. In contrast, the PEG-β2-GPI surface provided only for moderate discrimination of APS patients and healthy controls. All three streptavidin-biotin immobilization variants did not allow the detection of aPL.

Conclusions: β2-GPI immobilized on 11-AUTMS allowed for a clear differentiation between aPL positive sera and healthy controls. Thus, microarray chips with PT, annexin V, cardiolipin, phosphatidylserine and phosphatidylethanolamine are now under development.

D-45

Evaluation of the MindRay BC3200 Cell Counter in an Emergency Department Setting

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Background: To evaluate the MindRay BC-3200 auto hematology analyzer with three part differential for use in an Emergency Department Point of Care (POC) setting.

Methods: Split sample comparisons were performed over a 4 week time period using the Main Laboratory cell counters (Beckman Coulter LH750) as the predicate device. Over the same time period three levels of control material were run twice daily on each day that sample analysis was performed. Linearity, carryover and reproducibility studies were also performed.

Results: The LH750 produces a 5-part differential while the BC-3200 produces a 3-part differential. MindRay indicates the mapping between the sub-populations is as follows: lymphocytes to lymphocytes; mid range to the sum of monocytes, basophils and eosinophils, granulocytes to granulocytes. The correlation between the LH750 and the BC-3200 for the analytically derived analytes and the differential, as well as the range of CVs obtained on control materials, are listed in the following table:

Analyte	Slope	Intercept	R2	N	Range of CVs (%) on Control Material
WBC	0.8575	0.988	0.9910	106	1.56 - 2.92
RBC	1.0169	-0.005	0.9836	106	0.85 - 1.45
Hemoglobin	1.0080	-0.229	0.9927	106	1.56 - 2.48
MCV	1.0193	-3.6	0.9643	106	0.40 -0.47
PLT	1.0257	5.6	0.9898	106	2.23 - 11.97
Lymphocytes %	0.8515	1.8	0.9312	41	0.15 - 1.39
Mid Range %	0.3414	4.4	0.1420	40	1.07 - 3.77
Granulocytes %	0.8149	16.1	0.8077	40	0.79 - 1.39

During linearity, carryover and reproducibility studies the BC-3200 matched or exceeded the manufacturer's stated performance specifications for all channels.

Conclusions: In this study the BC-3200 meet the manufacturer's stated performance targets. However, in order to be used in our Emergency Department POC Laboratory, correlation with results from the Main laboratory is crucial. Although acceptable correlation was seen for the complete blood count, the differential results could not be used interchangeably with results obtained from the LH750 analyzer.

D-46

Paired t-Test for Detecting Differences Between Lots of Reagents. A Practical Example with i-STAT® cTnI cartridges.

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Background: To ensure continuity of patient care, different lots of reagent should result, for the same patient specimen, values that are within a specified interval of maximum allowable difference. We employed the paired t-Test and its graphic representation to detect differences between lots of i-STAT cTnI cartridges used in the Emergency Department of our hospital for screening patients with suspected myocardial infarction (MI).

Methods: Patient specimens were obtained by venipuncture and assayed in parallel, within five minutes, with i-STAT cTnI cartridges (Abbott) from new and old lots. The observations were accessioned in Minitab® (Version 7, Minitab, Inc.) statistical software.

Results: The observed differences (N=29) for six pairs of lots (S10927, S10330F, T11043, T11107, R12008, U11199, U11265) showed mean: -0.03 ng/mL (95% CI: -0.1,0.05), s:0.19 ng/mL (95%CI: 0.15,0.26), quasi-normal distribution, Cp:0.7, Cpk:0.64, Pp:0.69, Ppk:0.63 for specifications limits -0.4, 0.4 ng/mL. Regression analysis showed that the observations were highly correlated (new lot = -0.01 + 0.96 old lot, r = 0.98). Consequently the paired t-Test was appropiate for testing for equality of means. Power analysis for the paired t-Test showed that for alpha = 0.05, difference = +/- 0.4, s of difference = 0.2, five paired measurements would give a power of 0.91. The paired t-Test for five paired assays performed on patient specimens with cartridges from a new (U11181A) and an old (U11199) lot, showed mean differences = 0.12 ng/mL (95% CI: -0.28, 0.52) which was not statistically significantly different from zero (P = 0.44). However, the plot of the differences by the value obtained with the old lot showed that while for four specimens the differences were between -0.01 and 0.03 ng/mL for one specimen with value 8.9 ng/mL, as determined with the old lot, the difference was 0.67 ng/mL and it exceeded the upper acceptance limit of 0.4 ng/mL.

Conclusions: The observations for six paired lots of cartridges showed a small and fairly stable variability of the difference between new and old lots. The interpretation for the single difference exceeding the acceptance limit, was limited by the small number of observations. This may have been due to operator error, malfunction of one cartridge of either lot, or calibration of the new lot. Since the value of this observation (8.9 ng/mL) vastly exceeded the threshold of 0.15ng/mL for detection of myocardial infarction and the error caused by the difference would have not altered the interpretation of the result by the ED physician screening for MI , this lot was accepted without any further investigation. However, if these two lots of cartridges were used interchangeably for sequential patient testing, this variability may have affected patient care and further investigation may have been necessary. Statistical software offered computational convenience. However, if it is not available, inspection of the graphic display of the paired differences by the value as obtained with the old lot of cartridges may be sufficient for detecting differences between two lots of cartridges; for power analysis, approximations of the number of observations for the desired power may be obtained with appropiate tables.

D-47

Performance of a New Gluocse Meter Designed for Minimizing Infection Risk

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According to the Centers for Disease Control and Prevention (CDC), finger-stick devices, blood glucose meters, and the hands of health care workers are potential vehicles for the indirect transmission of blood borne pathogens in clinical settings. Advancements in technology for Assisted Monitoring of Blood Glucose (AMBG) might reduce the risk of bloodborne pathogen transmission among persons receiving diabetes care. The OneTouch Verio™Pro+ glucose meter incorporates an onboard test strip ejector that facilitates hands-free test strip disposal and minimizes health care provider (HCP) contact with blood-dosed test strips. This feature, along with the meter's compatibility with common disinfectants, may enhance infection control practices and may help reduce the risk of infection in clinical care settings.

In addition to infection control, glucose meters used for AMBG also require quality control (QC) tests to be performed regularly. The OneTouch Verio™Pro+ meter provides on-screen prompts to remind HCPs to perform QC tests and automatically recognizes when control solution is applied. After reading the Operator's Guide, 100% (38/38) of assessed HCPs reported that they knew how to correctly respond to a control solution prompt and 89.5% (34/38) stated that reminders to conduct a QC test will help them to be compliant with their institution's regulatory requirements.

Table 1 shows the results of a study conducted to ascertain meter performance at 3 clinics and 1 hospital with 189 capillary samples (hematocrit range: 22.9-52.1%), 177 venous samples (hematocrit range: 22.9-54.7%), and 200 arterial samples (hematocrit range: 23.0-59.8%) compared to YSI reference values. The OneTouch Verio™Pro+ glucose meter shows excellent analytical performance across the glucose range and incorporates many important clinical attributes required for AMBG and clinical settings.

Table 1. OneTouch Verio™Pro+ Performance with Arterial, Capillary, and Venous Blood Sample Tests Performed by HCPs at 4 Clinics Compared to YSI reference values.

	Glucose < 100 mg/dL1 Data within ± 12 mg/dL n/N (%)	Glucose ≥ 100 mg/dL1 Data within ± 12.5 % n/N (%)	Data within ± 12 mg/dL or 12.5 %1 n/N (%)
Arterial	27/27 (100%)	164/173 (94.8%)	191/200 (95.5%)
Capillary	21/23 (91.3%)	163/166 (98.2%)	184/189 (97.4%)
Venous	25/26 (96.2%)	149/151 (98.7%)	174/177 (98.3%)
All Blood Sample Types	73/76 (96.1%)	476/490 (97.1%)	549/566 (97.0%)

1 Accuracy Limits (± 12 mg/dL for glucose < 100 mg/dL or 12.5 % for glucose ≥ 100 mg/dL) have been proposed by CLSI POCT12.

D-48

Use of Cytokines as Sytemic Inflammatory Response Markers to Assess the Outcomes of Patients from Mechanical Ventilation

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Background: Mechanical ventilation (MV) has been used in situations where there is a need to fully or partially substitute the ventilatory work accomplished by the patient's muscles. Despite the life saving potential of this assistance, it imposes a considerable amount of mechanical stress on the lung. Studies have shown that inflammatory process triggered by mechanical ventilation resulting in pulmonary and systemic cytokine release may give valuable information on patient outcome. We hypothesized that the systemic cytokine response can predict the outcome of patients from MV.

Methods: Thirty patients (age >18 years) admitted to the intensive care unit (ICU) of a tertiary care hospital and who required volume controlled continuous mechanical ventilation for atleast 24 hours were enrolled for the study. Patients with neuromuscular diseases, left ventricular dysfunction, chest or abdominal trauma and history of exacerbation of chronic respiratory failure during the last 3 months were excluded from the study. Data collected from the patients record included age, sex, underlying disease, Sequential Organ Failure Assessment (SOFA) score at 24 hours after the patient was connected to MV, documentation of the need for MV, and duration of MV. Three ml of the venous blood was collected at 24 hours after the patient was put on MV and used for the estimation of Interleukin (IL)-6, IL-8 and Tumor Necrosis Factor (TNF)-α using a solid-phase enzyme-linked immunosorbent assay method. On ICU admission, patients were continuously sedated, remained supine and were ventilated with volume controlled continuous mechanical ventilation. Respiratory rate, and fraction of inspired oxygen (FIO_2) were adjusted to maintain arterial oxygen saturation >90%, $PaCO_2$ of 35 to 45 mmHg and pH>7.25. Tidal volumes of all the patients were between 10 to 12 ml/kg of the predicted body weight. PEEP was kept at 5 cmH_2O. The inspiratory: expiratory (I: E) ratio was 1:2. Duration of MV was calculated from the time the patient was intubated and connected to ventilator till he was disconnected from the ventilator. Patients were classified as survivors if they were successfully weaned and discharged without any episode of re-intubation. Based on their outcome from MV the patients were divided into 2 groups; Survivors (Group 1) and non-survivors (Group 2).

Results: Out of the 30 patients, 17 patients survived (Group 1) and 13 patients expired (Group 2). Serum levels of IL-6 and IL-8 were significantly increased in group 2 when compared to group 1 (p<0.0001 and p<0.001 respectively). However, TNF-α did not show any significant difference. IL-6 >111.9 pg/ml or IL-8 >88.9 pg/ml at the start of MV increases the probability of mortality by factor 2.40 or 2.62 respectively. An increase of IL-6 by 1 pg/ml significantly increases the relative probability of mortality by a factor of 1.004 (95% CI, 1.0003-1.0078, p=0.0001).

Conclusions: Estimating the levels of IL-6 and IL-8 at 24 hours of connecting the patient to MV will help in predicting the outcome of the patient.

D-49

Evaluation of the Fisher Sure -Vue Signature Mono, Inverness Medical Acceava Mono Cassette, and Beckman Coulter ICON Mono test kits

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Background: This evaluation was conducted to assess the performance characteristics of three FDA-waived, Point-of-Care (POC) devices designed for non-laboratory staff to test capillary whole blood for heterophile antibodies (mononucleosis).

Methods: Waived whole blood methods evaluated included the Fisher Sure-Vue Signature Mono (Fisher HealthCare, Houston, TX), Inverness Medical Acceava Mono Cassette (Inverness Medical, Princeton, NJ), and Beckman Coulter ICON Mono (Beckman Coulter, Fullerton, CA). The reference method was EBV VCA IgM antibody on the Bio-Rad BioPlex 2200 System (Bio-Rad Laboratories, Hercules, CA). To assess the accuracy of the waived tests, 20 normal donors were tested using whole blood and serum on both Acceava and Sure-Vue Signature tests. On the ICON, the same 20 normal donor serum samples and 15 of the normal donor whole blood samples were tested. In addition, 22 serum samples (16 clinical samples, 6 spiked samples produced to challenge the cut-off range of the BioPlex) with measurable EBV VCA IgM were tested by all methods. Each whole blood method was also assessed by a formal survey of laboratory technologists and a POC coordinator who conducted usability testing.

Results: The overall percent concordance was 95% (59/62) for Sure-Vue Signature, 98% (61/62) for Acceava, and 95% (54/57) for ICON. The Sure-Vue Signature had three discrepant samples, which included a single normal donor testing positive using the serum sample but negative using capillary whole blood. The ICON had three discrepant samples, which included a normal donor testing positive by both serum and capillary whole blood. The remaining discrepant samples for the Sure-Vue Signature and the ICON, in addition to the one discrepant Acceava sample, occurred near the assay cut-off for the BioPlex IgM test. An "ease of use" (sample application and test resulting) analysis was also performed for each method. Users concluded that the cartridge-based kits (Acceava and ICON) were easier to read/result compared to the dipstick method (Sure-Vue Signature). The Acceava's disposable, one-piece pipette collection system was determined to be the easiest to use for sample application.

Conclusions: Our data demonstrates that each of the evaluated POC tests shows high concordance (≥95%) with an external reference method (BioPlex IGM) for the detection of heterophile antibodies in cases of suspected mononucleosis. Furthermore, the cartridge-based assays were found to be easier to use in regards to sample application and test interpretation.

D-50

A1c Gear: Laboratory Quality HbA1c Measurement at the Point of Care

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Background: Glycosylated hemoglobin (HbA1c) reflects the average glucose levels over a 3-4 month time frame and is an important part of assessing the diabetic control. Numerous studies have shown that HbA1c levels have a strong predictive value of diabetic complications. Since the use of point-of-care (POC) devices for monitoring HbA1c is increasing, it's important to determine how these devices compare to the central laboratory.

Methods: One hundred twenty patient samples with HbA1c levels between 4.0-12.5% submitted to hematology for analysis on the Bio-Rad Variant™II were run on 2 POC Analyzers (Sakae A1c Gear and Siemens DCA Vantage™). Although all analyzers are NGSP certified methods we obtained an additional 40 samples from a NGSP secondary reference laboratory for comparison on all analyzers. All NGSP samples were run in singlet. All patient samples were run on each POC analyzer (duplicate for A1c Gear and singlet for DCA) over 20 days by two phlebotomists or one MT. Three patient sample pools containing low(~5%), medium(~7%), and high(~10%) HbA1c levels were prepared, frozen, and run along with 2 levels of commercial QC (Quantimetrix Corp.) on each of the 20 days. Three reagent lots and 3 instruments were evaluated for the A1c Gear while 1 reagent lot and 1 instrument was evaluated for the DCA. All statistical calculations were by MedCalc, v12.2.0.0, Mariakerke, Belgium.

Results: All instruments showed excellent correlation with NGSP samples (R^2>0.96) with means=7.62%, 8.01%(p<0.0.001), 7.63%(p=0.88), 7.78%(p=0.002) for NGSP, Variant™II, A1c Gear, and DCA, respectively. The 120 patient samples also showed excellent correlation when compared to the Variant™II for both POC analyzers (R^2>0.95 for both methods). HbA1c levels obtained using the Variant™II (mean=8.06%; 95% CI=7.70-8.42%) and DCA (mean= 8.08% C.I. = 7.72-8.44%) instruments were found to have no statistical mean difference (p=0.56), while the A1c Gear (mean=7.81%; 95% C.I.=7.44-8.18%) was lower (p<0.001). For Variant™II HbA1c results ≤7% the correlation was acceptable for both POC analyzers (R^2>0.89) with means=5.84%, 5.68%(p<0.001), 6.02%(p<0.001) for Variant™II, A1c Gear, and DCA, respectively. Changing reagent lots or instruments did not impact the clinical interpretation for the A1c Gear. The low, medium, and high patient pools intra-day imprecision was between 0.9-1.34% and 2.56-3.3% for A1c Gear and DCA, respectively. Inter-day imprecision for the patient pools was 1.6-2.5% and 2.2-2.4% for A1c Gear and DCA, respectively. Total imprecision for the patient pools was 1.40-2.63% and 3.5-4.1% for A1c Gear and DCA, respectively.

Conclusions: Only the A1c Gear was statistically the same when compared to NGSP values with the average bias for the A1c Gear (0.01% HbA1c) being less than that found for either the DCA (0.16% HbA1c) or Variant™II (0.39% HbA1c). For the patient samples the mean HbA1c levels for the DCA were statistically the same as the Variant™II while the A1c Gear was significantly lower although the average bias was 0.25%HbA1c. The A1c Gear meets the criteria of total CV<3% while the DCA had a total CV<4.5% leading us to the conclusion that the A1c Gear can give results as precise as the laboratory at the POC.

D-51

Ferritin Assay using Novel Dried Instant Plasma Spot Collection Technology

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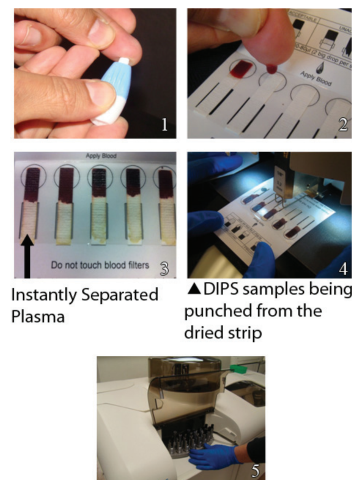
Background: There is currently no accurate, efficient, and convenient blood collection-separation technology available that can be directly substituted for routine, laboratory serum or plasma separation techniques. The new dried instant plasma spot (DIPS) technology developed by our laboratory uses whole blood spotted on one end of a filter strip, so that the plasma and red cells rapidly separate before the blood has time to clot, leaving dried plasma at one end of the card and residual dried red cells at the other.

Methods: Blood samples were collected from 52 volunteers. Venous blood was drawn into serum separator tubes and non-additive tubes. DIPS were prepared immediately by spotting the blood from non-additive tubes on the collection cards and dried. Serum was separated by conventional methods and stored frozen until analyzed. Two 6.0 mm punches from the dried plasma part of each card were rehydrated and the supernatant removed from the filter paper by centrifugation and transferred into tubes for testing. Both serum and extracted plasma were analyzed for ferritin by the Siemens Immulite auto-analyzer. Ferritin results obtained from the DIPS were corrected for total protein to take into account differences in saturation of the filter paper.

Results: Results showed an excellent correlation between DIPS and serum for ferritin (R^2=0.9). Intra-assay and inter-assay precision for three samples spanning the reference range were <7%.The mean recovery of ferritin from DIPS was excellent at 97.5%.

Conclusions: The convenience of sample collection, storage and transport of DIPS will save considerable costs in blood collection, handling and shipping especially in remote areas without phlebotomists or the equipment necessary to prepare blood serum or plasma, This new self-collection method is ideal for large scale clinical/research studies due to convenience of point of care combined with the accuracy of testing by auto-analyzers



D-52

Effects of austere environmental conditions during disasters on quality control for point-of-care testing

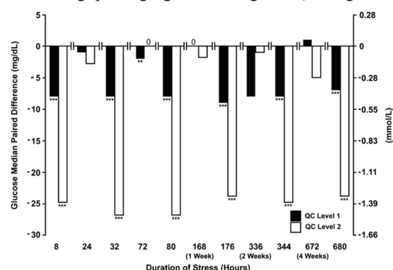
C. S. Tang, W. J. Ferguson, R. F. Louie, J. H. Vy, S. L. Sumner, G. J. Kost. *University of California - Davis, Davis, CA*

Background: Quality control (QC) is essential for validating the performance of devices and the competency of operators. Previous studies demonstrated that test strips for glucose meters were affected adversely by conditions experienced during Hurricane Katrina. Our objective here is to characterize the effects of dynamic temperature and humidity on QC reagents used for point-of-care (POC) glucose testing.

Methods: Glucose meters, test strips, and control (unstressed) QC solutions were stored and operated within manufacturer environmental specifications. We generated a dynamic 24-hour temperature and humidity profile modeling climatic conditions in New Orleans 7 days before and 24 days after Hurricane Katrina. QC solutions were placed in an environmental stress testing chamber that simulated disaster conditions. QC solutions stressed 24, 72, 168 (1 week), 336 (2 weeks), and 672 (4 weeks) hours (designated "cold testing" points) were evaluated at 23°C and 90.6% humidity. "Hot testing" points (8, 32, 80, 172, 344, and 680 hours) of 45°C and 31% also were examined. Paired results for stressed versus unstressed (control) QC solutions were obtained at each time point using two QC levels. We used the Wilcoxon signed-rank test to determine statistically significant median paired differences at each time point.

Results: The Figure shows that median paired differences for stressed QC solutions were depressed significantly at hot testing points (P<0.01) at both QC levels for all durations of stress. Only one cold testing time point paired difference was significant (P<0.01, 72 hours, QC1).

Conclusions: Dynamic temperature and humidity stresses affected the performance of QC results. To ensure the validity and consistency of POC testing in disaster settings, proper monitoring, packaging, and storage of QC reagents must be ensured.



D-53

Hematocrit Effects Lead to Inadequate Glycemic Control and Insulin Dosing in Adult Burn Patients

Z. R. Godwin, J. Bockhold, L. Bomze, N. Tran. *University of California Davis, Davis, CA*

Background: Intensive insulin therapy (IIT) for tight glycemic control (TGC) significantly reduces mortality and morbidity in critically ill patients. Inaccurate glucose measurements during IIT precipitate inadequate TGC and increase the frequency of hypoglycemic events. Glucose monitoring system (GMS) inaccuracies are derived from confounding factors including abnormal hematocrit. Burn patients frequently experience altered metabolism and manifest abnormal hematocrit levels due to pathologic and iatrogenic mechanisms. Hemoconcentration is common during the acute burn shock phase. Alternately, patients with burns >=20% total body surface area (TBSA) lose nearly half of their blood volume during wound excision and grafting. *We hypothesize that automatic hematocrit interference correction in GMS improves TGC the critically ill.*

Table 1. GMS-1 and -2 Results

	GMS-1 Group	GMS-2 Group	P-Value
Mean Age (SD, np)	35.7 (6.2, 6)	40 (15.1, 6)	0.585
Mean %TBSA (SD, np)	44.5 (6.5, 6)	57.8 (12.4, 6)	0.273
Mean MODS (SD, nm)	5.4 (4.3, 413)	5.4 (5.1, 251)	0.985
Mean Hematocrit Percentage (SD, nm)	26.1 (4.9, 263)	25.3 (5.2, 424)	0.777
Mean Bias (SD, nm)	-1.9 (9, 113)	5.48 (11.1, 419)	< 0.001
Number of Hypoglycemic Events	2	14	< 0.001
Percentage of Hyperglycemic Events	11% (119/1,088)	23% (1,846/8,027)	< 0.001
Median Glycemic Variabilitya	22.49	26.19	0.015
Mean Insulin Rate (units/hr) (SD, nm)	2.66 (1.8, 2,312)	4.02 (3.68, 4,641)	< 0.001

Footnote: aGlycemic variability determined by the continuous overall net glycemic action method (standard deviation of differences between hourly glucose measurements)
Abbreviations: MODS, multiple organ dysfunction score; nm, number of measurements; np, number of patients; SD, standard deviation; TBSA, total body surface, area.

Methods: To test our hypothesis, we conducted a pilot randomized controlled trial in burn patients comparing a modified glucose oxidase-based GMS that automatically corrects for hematocrit and other confounding factors (GMS-1) versus a glucose dehydrogenase-based GMS (GMS-2). The hospital laboratory analyzer provided reference glucose measurements. Twelve adult (age >=18 years) burn (>=20% TBSA) patients were randomized (1:1) to receive IIT based on GMS-1 or GMS-2 measurements. The IIT protocol targeted a TGC range of 111-151 mg/dL. Glycemic variability was determined by calculating the standard deviation of differences between hourly glucose measurements during IIT. Insulin rates were recorded. Hypo- and hyperglycemia are defined for our purposes as blood glucose of <70 and >151 mg/dL, respectively.

Results: GMS-1 patients had improved glycemic control versus GMS-2 patients. Table 1 shows between group results.

Conclusions: Critically ill patients exhibit confounding factors that cause GMS inaccuracy. GMS inaccuracy places patients at greater risk for poor TGC, inappropriate insulin administration, and even worse, increased risk for hypoglycemia. GMS-2 reported higher average glucose measurements, higher instances of hypo- and hyperglycemia, and greater glycemic variability than GMS-1. GMS-1 accuracy appears to improve IIT, TGC, and reduce adverse glycemic excursions.

D-54

Hemostatic behavior of the patient in the surgical field

A. I. Alvarez-Rios, J. Romero-Aleta, A. Leon-Justel, J. M. Guerrero. *H.U. Virgen del Rocio, Sevilla, Spain*

Background: Recently a team of thromboelastometry (TEM) has been introduced to the surgical area of our hospital. The TEM is a diagnostic method whose purpose is the detection, few minutes, all the major alterations hemostasis that may impact on coagulation. The TEM is a device that measures the viscoelastic properties of the blood dynamical and globally. Is based on measuring the elasticity of the blood by charting the consistency of a clot during its formation and fibrinolysis later. It is also the GOLD STANDARD for studying the fibrinolysis. Knowledge of the function of coagulation patient will be essential for the proper management of bleeding.Cardiovascular surgery is a good example of the importance of monitoring the patient's bedside, for the need for an effective anticoagulant, early identification and appropriate management of coagulopathies blood products. It is a Point of Care testing (POCT) and the results are obtained more quickly than when samples are sent to the laboratory. The analyzer whole blood clotting ROTEM ® is based in thromboelastometry (TEM) and is based on measurement of the elasticity of the blood by a continuous graphical recording of the consistency of a clot during coagulation (factors and inhibitors clotting, platelets and fibrin) and subsequent fibrinolysis. Objectives: To determine if you could advance the behavior as to the firmness of the clot seeing the value that gives us the A10 (amplitude clot after 10 minutes) because the test most common coagulation (PT and PTTA) need 30 - 50 minutes for its determination and are not usable in the surgical field.

Methods: We analyzed a total of 360 test, the which 175 are Extem ® (Global Test with activation of extrinsic, since the coagulation cascade to consolidation of the clot), and 185 are in-TEM ® (Global Test with activation of the intrinsic system, since the cascade coagulation to the consolidation of the clot). The analyzer whole blood clotting ROTEM ® allows a diagnosis complete hemostasis using citrated whole blood.

Results: In the test Extem if we take as minimum value of A10 = 40.5 mm and we look MCF that the value is above 50 mm, obtain a sensitivity of 97.8%, 95% (92.4% - 99.4%) and a specificity of 89.2%; 95% (80.7% - 94.2%). With a ratio false negative of 2.2% (0.6% - 7.6%). In the test if we take the value INTEM minimum of A10 =

39.5 mm and we set the MCF value is above 50 mm, obtain a sensitivity of 95.3%, 95% (90.1%

- 97.8%) and a specificity of 86.2%; 95% (75.1% - 92.8%). With a ratio false negative of 4.7% (2.2% - 9.9%).

Conclusions: Although the normal range given by ROTEM ® for Extem A10 is 43mm and the INTEM A10 is 44 mm, we found that in our area, Extem A10 values over 40.5 mm and INTEM A10 over 39.5 mm relate to MCF exceeding 50 mm (minimum value of firmness), with all this could advance the behavior patient's hemostatic in the surgical field.

D-55

Comparative Performance of Two Whole Blood Point-of-Care Lipid Analyzers to CDC-Certified Laboratory Methods

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Background: Lipid point-of-care (POC) devices are utilized in clinic, hospital and community settings to screen for cardiovascular risk. We assessed the analytical performance of the Cholestech LDX (Inverness Medical Innovations, Orlando, FL) and the CardioChek PA (Polymer Technology Systems, Indianapolis, IN) systems using capillary whole blood versus CDC-certified laboratory serum methods for total cholesterol (TC), triglycerides, and HDL cholesterol (HDL-C).

Methods: Fasting and post-prandial capillary whole blood samples collected from 26 laboratory donors were analyzed simultaneously on Cholestech Lipid Profile cassettes and CardioChek Lipid Panel strips. Paired serum samples were analyzed with CDC-certified methods for TC (Cobas c501, Roche Diagnostics, Indianapolis, IN), HDL-C ($MgCl_2$/dextran sulfate precipitation) and non-blanked triglycerides (Roche). Non-HDL-C (TC - HDL) and LDL-C (Friedewald equation) were calculated. Percent mean bias (95th percentile confidence intervals) was used to compare differences in whole blood (POC) lipid results to serum reference values. Significant differences in mean percent bias between whole blood devices were defined as $p< 0.05$ using the unpaired t-test.

Results: Six Cholestech cartridge failures occurred; no failures occurred with CardioChek strips. One triglyceride outlier due to hand lotion use was excluded from analysis. NCEP guidelines for bias, as well as the mean percent bias of POC results versus CDC methods for samples analyzed by all three methods are shown in Table 1.

Conclusions: Cholestech was more accurate than CardioChek compared to CDC-reference methods for TC, however CardioChek was more accurate for HDL-C. Both devices exhibited significant variability in triglyceride results, as demonstrated by wide confidence intervals around mean bias. Neither POC device met NCEP bias guidelines for TC or HDL-C. Cholestech-calculated non-HDL-C and LDL-C exhibited less systematic bias and variability compared to directly measured parameters. The optimal approach to whole blood lipid screening may include a combination of total cholesterol with calculated non-HDL-C or LDL-C using the Cholestech device.

Table 1: Lipid Results from Laboratory Methods and POC devices

		Reference Method Results	NCEP Guidelines	CardioChek	Cholestech LDX	Cardiocheck vs. Cholestech bias
	n	Mean (Range)	% Bias	% Bias Mean (95% CI)	% Bias Mean (95% CI)	p
Total Cholesterol	46	190 (126-312) mg/dL	≤ 3%	-13 (-15,-11)	-7 (-9,-5)	<0.0001
Triglycerides	44	120 (54-252) mg/dL	≤ 5%	-3 (-10,3)	2 (-3,7)	0.1651
HDL-C	44	64 (26-113) mg/dL	≤ 5%	-10 (-13,-7)	-15 (-17,-13)	0.0084
Non-HDL-C	44	126 (82-209) mg/dL		14 (10,17)	3 (0,6)	<0.0001
LDL-C	44	101 (52-186) mg/dL	≤ 4%	-16 (-22,-11)	-4 (-7,-1)	<0.0001

D-57

Evaluation of the Enterprise Point-of-Care (EPOC) for Blood Gas, Electrolytes, and Metabolites.

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Background: Point-of-care (POC) devices serve an important role in critical care environments where standard laboratory testing may not be able to meet the required rapid turnaround times. They facilitate close patient monitoring because of their relatively ease-of-use, small sample size requirements, and high degree of portability. Whole blood POC devices are available for analysis of blood gases (pH, pO_2, pCO_2), electrolytes (Na^+, K^+, iCa^{2+}), glucose (Glu), lactate (Lac), hematocrit (Hct), and associated calculated values (cHb, $cHCO_3^-$, cSO_2, $cTCO_2$).

Objective: This study evaluated and compared the analytical performances of two Enterprise Point-of-Care (EPOC) Blood Analysis Systems (Epocal Inc, Ottawa, Ontario, Canada) with an i-STAT System (Abbott Point of Care Inc, Princeton, NJ) and the reference methods from the University of Chicago Medical Center central laboratories.

Methods: POC whole blood devices utilize electrochemical principles (current, voltage, or conductance) from an array of biosensors in disposable test cartridges to determine concentration of critical analytes. Specific measured tests are then used to generate a number of calculated values using well-established equations. Precision studies were conducted using Levels 1 and 3 GAS-ISE Metabolites (Eurotrol Inc, Burlington,MA) and Levels A and B Hct Control (Mission Diagnostics, Holliston,MA) quality control (QC) materials. Analyte measuring range studies were conducted using Calibration Verification Materials (Bionostics Inc, Devens, MA). Interferences studies were conducted using blood samples spiked with increasing amounts of bilirubin. Comparison studies were conducted using 53 de-identified blood samples collected from 28 patients.

Results: Within-run precision for the EPOC devices showed CV ranging from 0.1-6.3 % for all measured analytes. Between-day precision was analyzed two times a day, over 8 days for two QC levels and CV ranged from 0.1-6.7% for all analytes except for pO_2, which ranged from 7.8-16.1%. EPOC measurements for all analytes were linear (r^2 = 0.91-0.99) over the testing ranges of the calibration verifiers with the respective upper measuring limits as shown: pH: 7.9, pCO_2: 84 mmHg, pO_2: 492 mmHg, Na^+: 174 mmol/L, K^+: 12 mmol/L, iCa^{2+}: 3 mmol/L, Glu: 553 mg/dL, and Lac: 15 mmol/L. Bilirubin did not show any significant interference on all analytes tested up to 25 mg/dL bilirubin. Comparisons of most EPOC analyte values were in good overall agreement to i-STAT and to central laboratory analyzer values except Na^+, where constant and proportional biases (Passing-Bablok regression equations of y = 1.12x -13.56 and y = 1.08x - 8.81) were observed when compared to central laboratory analyzer.

Conclusions: The EPOC system showed overall acceptable analytical performance. No significant interference was observed for all analytes for bilirubin up to 25 mg/dL. EPOC analyte measurements correlated well with those from i-STAT and central laboratory analyzer values, with the exception of measurements for Na^+, which displayed an analytically significant overall bias of approximately +4 mmol/L.

D-58

Clinical Performance of the New CLINITEK Novus Urine Chemistry Analyzer

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Background: The CLINITEK Novus™ analyzer* is a new urine chemistry analyzer providing results and performance similar to those of the current Siemens CLINITEK Atlas® automated urine analyzer for the central laboratory. The CLINITEK Novus instrument (CE marked) maintains the level of expected performance offered by the Siemens CLINITEK Atlas and CLINITEK Status® urinalysis analyzers but provides new features.

Methods: An internal evaluation tested 340 specimens on both the CLINITEK Novus and CLINITEK Atlas instruments, and a subpopulation of 260 specimens on both the CLINITEK Novus and CLINITEK Status instruments. These specimens were also tested with reference methods for albumin and creatinine (DCA Vantage® analyzer, Siemens), bilirubin (Ictotest® tablets, Siemens), occult blood and leukocytes (manual cell counts using manual microscopy), glucose (glucose hexokinase assay, Thermo Scientific), and total protein (protein dye-binding assay, Thermo Scientific), and results were compared to the CLINITEK Novus results. Three CLINITEK Novus instruments were implemented in this evaluation of the CLINITEK Novus PRO12 urine cassette.

Results: All urine tests demonstrated an exact clinical block agreement of ≥85% with the Siemens predicate analyzer (CLINITEK Atlas or CLINITEK Status analyzer) except for creatinine at an 80% exact agreement (Table 1). Positive agreement with the predicate was >99% for all applicable reagents, with negative agreement of ≥89%. Overall agreement with the reference methods was acceptable for all relevant CLINITEK Novus reagents, ranging from 58% sensitivity for occult blood vs. manual cell count (reasonable for these different methods) to 97% exact clinical block agreement for glucose vs. the hexokinase assay.

Conclusions: The CLINITEK Novus analyzer is a new urine chemistry analyzer for the central laboratory, offering acceptable clinical performance comparable to that of other Siemens systems and providing measurement of microalbumin.

Table 1. CLINITEK Novus analyzer vs. Siemens predicate system.

Reagent	Predicate	Exact Agreement	Within 1 Block Agreement	Positive Agreement	Negative Agreement
Albumin	Status	85.5%	100%	100%	98.4%
Bilirubin	Atlas	94.3%	99.8%	99.3%	100%
Blood	Atlas	88.9%	100%	100%	100%
Creatinine	Atlas	79.8%	100%	NA	NA
Glucose	Atlas	98.1%	100%	100%	100%
Ketone	Atlas	88.8%	100%	99.7%	89.1%
Leukocytes	Atlas	99.0%	99.9%	100%	99.9%
Nitrite	Atlas	100%	100%	100%	100%
pH	Atlas	85.1%	99.9%	NA	NA
Protein	Status	89.6%	100%	99.4%	100%
Urobilinogen	Atlas	90.7%	100%	100%	88.6%
Color	Atlas	100%	100%	NA	NA

* Not available for sale in the U.S.

D-59

Effect of High Concentrations of Bilirubin and Uric Acid in Commonly Used Glucose Methods

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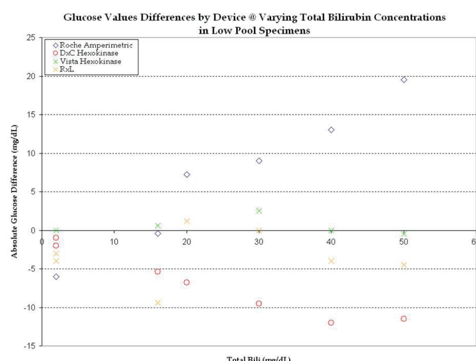
Background: Tight glycemic control protocols have been widely adopted for post-surgical, acutely and critically ill patients. Essential to the success of these programs is availability of rapid and accurate glucose tests. Our institution adopted the AccuCheck® (Roche) point-of-care device. Inaccurate glucose results can result from elevated bilirubin or uric acid (UA) in acutely ill patients. We evaluated the accuracy of the AccuCheck compared to our central laboratory analyzers in specimens with elevated bilirubin and UA.

Methods: To investigate the effects of elevated bilirubin on glucose concentration, 3 pools of patient sera containing in average 87 mg/dL(low), 132 mg/dL(medium), and 293 mg/dL (high) of glucose were created. The pools were spiked with increasing amounts of bilirubin using a high bilirubin specimen (20-50 mg/dL range). The glucose concentrations were measured using the AccuCheck (amperometric) and 3 hexokinase

Methods: the Beckman DxC®, Siemens Dimension Vista®, and Siemens Dimension RxL®. Changes in glucose concentration were plotted against bilirubin concentration. For the UA study, glucose concentration was measured using the Accucheck and the DxC in 18 specimens containing normal or high UA concentration (8-13 mg/dL). The absolute and percent differences in glucose concentration observed in both methods were calculated against the UA concentration in the specimen.

Results: The Accucheck and DxC methods inaccurately measured glucose concentrations in the presence of bilirubin above 20 mg/dL. This effect is more relevant at lower glucose concentrations. The Vista and RxL assays seemed to be unaffected by the bilirubin concentrations measured. High concentrations of UA also seemed to impact the accuracy of the Accucheck when compared to the DxC.

Conclusions: The accuracy of the AccuCheck device will be challenged in critically ill patients, especially in the presence of high bilirubin or UA concentrations. In addition, the DxC assay may also be inaccurate at bilirubin concentrations above 20 mg/dL.



D-61

Comparison of 12 serum biochemical parameters quantified in Reflotron® Plus and clinical chemistry analyzer

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Background: Point of care testing (POCT) is a portable and easy methodology to use and may be an useful tool for evaluating athletes during training sessions. The aim of this study was to compare 12 biochemical parameters applied to monitor athletes using a POCT and a clinical chemistry analyzer.

Methods: Male volunteers (n=103), with an average age of 18 ± 1 years, participated for four months in a regular and controlled exercise program, which consisted predominantly of aerobic activities (three hours daily and five days per week). Blood samples were collected under standardized conditions, after 12 h of fasting, in the morning, in tubes with Vaccuete® (Greiner Bio-One) separator gel. The samples were centrifuged at 1.800x*g* for 15 minutes under refrigeration (4ºC) and separated in two aliquots. The creatine kinase (CK), aspartate amino transferase (AST), alanine amino transferase (ALT), γ-glutamil transpeptidase (GGT), alkaline phosphatase (ALP) amylase activity, and urea, uric acid, glucose, creatinine, total and HDL-cholesterol concentrations were analyzed in Reflotron® Plus (Roche) and RX Daytona™. The Reflotron Precinorm U and Randox internal quality controls were performed and the analytical variation (CV_A) was calculated (Table 1). The paired t test was used to compare the mean values and Spearman's correlation coefficient (r) between Reflotron and RX Daytona data were calculated. Values of $p<0.05$ were considered significant.

Results: All Reflotron parameters were correlated with RX Daytona (Table 1). Urea, creatinine, total and HDL-cholesterol, CK, ALT, ALP presented lower values in Reflotron compared to RX Daytona. Uric acid, glucose, AST, amylase presented higher values in Reflotron compared to RX Daytona. No differences were observed in GGT values.

Table 1. Comparisons between Reflotron Plus and RX Daytona biochemical parameters.

Analyses	RX Daytona (Mean ± SD)	RX Daytona (CVA%)	Reflotron (Mean ± SD)	Reflotron (CVA%)	p (t test)	r
Urea (mmol/L)	5.51± 0.69	2.7	4.59 ± 0.76	4.5	< 0.001	0.725
Uric Acid (mmol/L)	0.37 ± 0.05	3.1	0.38 ± 0.05	3.1	< 0.001	0.977
Glucose (mmol/L)	4.73 ± 0.42	4.3	4.66 ± 0.37	3.7	0.046	0.823
Creatinine (μmol/L)	87.5 ± 8.1	4.8	71.1 ± 8.4	4.5	< 0.001	0.801
Cholesterol (mmo/L)	3.72 ± 0.51	2.1	3.05 ± 0.40	4.2	< 0.001	0.964
HDL (mmol/L)	1.11 ± 0.21	2.4	0.94 ± 0.18	5.6	< 0.001	0.800
CK (U/L)	410 ± 311	3.3	301 ± 190	3.6	< 0.001	0.989
AST (U/L)	19.6 ± 5.4	3.4	26.3 ± 6.1	1.3	< 0.001	0.728
ALT (U/L)	21.4 ± 8.5	1.0	19.1 ± 8.1	4.1	< 0.001	0.939
Amylase (U/L)	59.2 ± 16.4	3.6	66.6 ± 17.6	2.7	< 0.001	0.941
GGT (U/L)	17.7 ± 9.5	1.8	17.1 ± 8.4	5.4	0.431	0.810
ALP (U/L)	219.5 ± 44.5	1.9	72.5 ± 15.2	1.6	< 0.001	0.914

Conclusions: Reflotron can be applied to routine athlete's analysis. However, the results showed that it is crucial to establish specific reference intervals for POCT.

D-62

Evaluation of a method to detect blood in breast discharge

C. B. Williams. *Faulkner Hospital, Boston, MA*

Background: Point of Care Coordinators are occasionally asked to evaluate off label uses for products. FDA defined off label use is subject to the rules of governing laboratory organizations like the College of American Pathologists (CAP). In our facility, radiology oncologists screen breast discharge for blood prior to performing ductograms. This study examines testing for blood in breast discharge using urinalysis reagent strips compared to guaiac reagent cards.

Objective: Medical literature refers to "guaiac" for detecting blood in breast

discharge. However, limitations to the sensitivity of this method occur when testing materials other than stool or gastric fluid. To improve patient care through an improved diagnostic test, urine dipsticks were compared to guaiac cards.

Methods: Human breast milk was dosed with whole blood and titered serially. Samples were tested against two brands of urine dipsticks and three brands of guaiac cards.

Results: This study originated as a 2009 CAP inspection validation. Fresh breast milk was chosen as a matrix similar to breast discharge. 10 ul EDTA whole blood was added to 10ml breast milk, achieving a 2+ visual result for blood using a urinalysis multistick. This equated to approximately 50 erythrocytes per ul of urine and was used as the comparative standard when testing two brands of urine dipsticks with serially diluted breast milk. Validation showed an end point of approximately 3 Ery/ml using Roche Chemstrip 10UA, and an end point of approximately 6 Ery/ml using Siemens Hemastix. Hemastix has a blood detection level of 0.015-0.062 mg/dl of free hemoglobin or 5-20 intact red cells per microliter. Chemstrip detects 5 Ery/ul and hemoglobin content corresponding to 10 Ery/ul. The titration proved that Hemastix detected blood in breast milk as well as it did in urine. This validation was accepted by CAP. Subsequently, literature was reviewed for blood in breast discharge. Several sources cited guaiac as the method. Breast milk from 2009 was thawed and re-titered using 10 ul of packed blood, comparing it to the original urinalysis products and Propper Seracult guaiac cards. The guaiac card was negative at the 1:4 dilution while the urinalysis products reacted positively up to 1:128. Since the breast milk control was negative, the increased titer was believed due to packed cells vs. whole blood. In 2011, the titration was repeated using whole blood in the thawed 2009 breast milk. This study included three brands of guaiac cards; Seracult, Consult Occult Blood Slides- E.S. and HemaPrompt fg (buffered to detect stool/gastric fluid simultaneously). Again, the urine strips titered much further than the guaiac cards (1:4). Chemstrip titered two dilutions higher than in 2009 (1:64). However, Hemastix yielded trace results up to 1:1024 in the presence of a negative breast milk control.

Conclusions: Urine dipsticks are a more sensitive indicator for blood in breast milk than guaiac tests. Hemastix offers a simple, reliable and cost effective method for testing breast discharge. Differences seen in strip endpoints after 2009 may have resulted from breast milk degradation and should be repeated using fresh breast milk.

D-63

How does the Roche Accuchek Inform II perform compared to Reference Methodology in Patients receiving therepies where maltose is present?

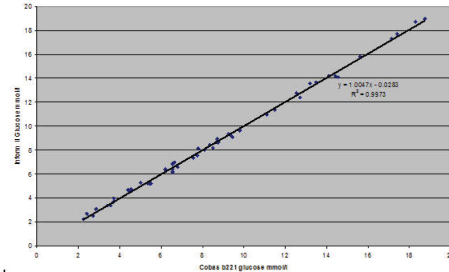
G. M. Creed. *Guy's and St Thomas Foundation Hospital NHS Trust, London, United Kingdom*

Introduction: Maltose and Maltose derivatives have triggered hazard warnings notice with Glucose analysis using POCT meters in both the US and UK. This pilot study sought to investigate whether the Roche Accu-Chek Inform II meter was maltose independent and could be used as a total hospital solution independent of patient treatment regime and care pathway.

Methods: 50 venous whole blood samples from patients receiving maltose/ maltose derivatives as part of their treatment regime were analysed for Glucose concentration via Institutional reference method for POCT testing: Roche Cobas b221 and Roche Cobas Inform II. Maltose concentration was determined for all patients and 3 subsets analysed < 50 mg/dl, >50 - < 100 mg/dl and > 100mg/dl.

Results; Mean maltose concentration= 83.2 ± 39.1 mg/dl. Cobas b221 mean blood glucose = 8.51 ± 4.49 mmol/l, with Roche Inform II 8.52 ± 4.62 mmol/l. Correlation coefficient r^2=0.997 with slope 1.004 and intercept 0.03. Mean Bias for whole patient group 0.01 ± 0.24 mmol/l. Patients with Maltose < 50 mg/dl mean bias 0.03 ± 0.24 mmol/l. Patients with Maltose > 50 < 100 mean bias 0.08 ± 0.22 mmol/l and patients with maltose > 100 mg/dl mean bias -0.07 + 0.24 mmol/l..

Conclusion. Roche Accu-Chek Inform II showed excellent correlation with reference method with no evidence in this patient subset of cross-reactivity with maltose producing erroneous glucose values. Glucose results were independent of patient's maltose concentration as the mean bias between reference and Inform II does not change across the maltose concentration range.



D-64

Performance of CardioChek in lipid testing for health screening purposes

E. Amarose, L. M. Farber, D. F. Soles, H. E. Yu. *Geisinger Health System, Danville, PA*

We evaluated CardioChek for its suitability in measuring lipids for health screening. Using lab method (Roche P-Modular) as the standard, we assessed the ability of CardioChek to properly risk stratified patients base on the Adult Treatment Panel III (ATPIII) of the National Cholesterol Education Program (NCEP).

Number of Miscategorization (Desirable vs Suboptimal Results) when using CardioChek			
	Desirable level (ATPIII)	Falsely Desirable Results	Falsely Suboptimal Results
Total Cholesterol	<200 mg/dL	3/35 patients (8.6%)	4/35 patients (11%)
HDL-Cholesterol	>=40 mg/dL	0/35 patient (0%)	2/35 patients (5.7%)
Triglyceride	<150 mg/dL	1/35 patient (2.9%)	3/35 patients (8.6%)
LDL-Cholesterol (Calculated)	<100 mg/dL	5/32 patients (16%)	3/32 patients (9.4%)

Because patients are advised to follow up with a doctor's visit with suboptimal test results and no medical decision is made base on the CardioChek results in the type of health screen described here, minimal clinical impact is expected for those who got falsely suboptimal results from CardioChek. Falsely desirable results, however, could give the patient a false sense of "wellness".

There was no falsely desirable result when measuring HDL-Cholesterol. The 3 patients who got falsely desirable results of total cholesterol had marginally good levels of 190 - 197 mg/dL(CardioChek); and the 1 patient who got a falsely desirable triglyceride result had a marginally good level of 146 mg/dL (CardioChek). All these results were within the NCEP error limit when compared to those from the lab. Therefore, CardioChek meets our need for the health screen in measuring total cholesterol, HDL-cholesterol and triglyceride.

For 3 of the 5 patients who got falsely desirable calculated LDL cholesterol results, the levels (CardioChek) were marginally good (99 and 98 mg/dL). The other 2 patients had results of 89 and 90 mg/dL (CardioChek), with a 16-20% difference from the lab's; exceeding the NCEP allowable error but considered acceptable using the CAP guideline. Therefore, the calculated LDL-cholesterol by CardioChek is not as accurate, and should be interpreted with care.

D-65

Comparison of whole blood creatinine measurement to serum creatinine measurements in renal dose calculations

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Background: Creatinine concentrations are measured to estimate the glomerular filtration rate (GFR) of patients undergoing pharmacotherapeutic interventions to calculate dosing and to assess their ability to clear the dose of drug administered. Infusion centers where treatment is delivered are increasingly turning to the use of point-of-care (POC) devices to measure creatinine rapidly and for patient convenience. These devices use whole blood (WB) rather than serum and may be based upon a different analytical method than that used in the central laboratory. While method

comparisons indicate the WB methods are suitable in identifying renal dysfunction, there are no studies to support their use in drug dosing calculations derived using serum creatinine concentrations. This study was designed to determine if WB and serum creatinine measurements were interchangeable when calculating dosages for carboplatin and zoledronic acid.

Methods: The patient samples (n=54) used in the study were submitted from the infusion center and consisted of paired whole blood and serum/plasma. Creatinine was measured using both the i-STAT POC device (Abbott Laboratories) and an enzymatic method on the Vitros 5600 (Ortho Clinical Diagnostics). These results were used to estimate GFR using Cockcroft-Gault (CG), the Modification of Diet in Renal Disease (MDRD) and the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equations and subsequently to calculate dosages for carboplatin and zoledronic acid. The patient population included 30 females and 24 males ranging in age from 23-93 years. Patient weight and hematocrit were also documented.

Results: For carboplatin, the use of WB creatinine measurements altered chemotherapy dosing for 65% of the patients tested, with 30% having a dosing change of >10%. For zoledronic acid, WB creatinine measurements altered the calculated dosing for 28% of the patients. The differences could not be attributed to hematocrit, gender, or body mass. Using the CKD-EPI calculation the potential to undertreat was greatest in females (40%) as opposed to males (29%). The opposite was seen using CG and MDRD where the impact was greatest in males (33%, 25%) versus females (23%, 13%). The difference between the CG values calculated using the WB versus the serum sample revealed that the WB sample had an average negative bias (-19.25 mg/dL), while both the MDRD and CKD-EPI had positive biases (+115.2mg/dL and +28.0 mg/dL, respectively).

Conclusions: Our study reveals that an unacceptable percentage of patients would be underdosed if the whole blood creatinine sample were used for dose determination. We conclude that whole blood creatinine measurements should be used with caution in determining doses for carboplatin and zoledronic acid.

D-67

Multi-parameter slide for the point-of-care testing of metabolic profiles in the ICU A new platform for diagnostics

P. Ahuja, B. Hemphill, M. Peshkova, M. Gratzl. *Case Western Reserve University, Cleveland, OH*

Frequent monitoring of metabolites is desirable in many biomedical settings. For example, the metabolic status of patients in the intensive care unit (ICU) is of critical importance and requires frequent monitoring. Current blood test results in the ICU can have time lag due to analysis in a central laboratory. We have developed a reusable sensor array system for *in vitro* point-of-care testing (POCT) of multiple analytes simultaneously from a single drop of blood. The sensor uses an optical sensing scheme based on a polymer multilayer structure and integrated optode membranes that change color in response to analytes' concentration. The slide can be read by the naked eye or digitally with an inexpensive reader. The slide is a self contained unit, thus it does not require any reagents or power supply, when read by the naked eye.

Utilizing a single reusable slide for multiple screenings for the same patient, at the bedside, makes the device more cost-effective and improves turnaround time and compliance for disease management. Opposed to current POC electrochemical technologies that use a single current or voltage value as output, the sensor response provides a spectrum of color, and is therefore more robust. The individual sensors can be tuned to measure pH, K^+, glucose, lactate and other metabolites. A reference system is also included to provide a standard color by which the color changes of the sensing system can be compared. We are currently developing a mass manufacturing procedure for the sensor which allows manufacturing costs of ~$0.01/sensor in series production. For the acquisition of color images of the sensor array, a system of red, green and blue LEDs is used together with an inexpensive monochrome camera. Due to the narrow bandwidths of the LEDs no optical filters are needed. Integrated one-touch software allows for automatic image capture and analysis. The sensor is a tunable platform for other analytes and we are currently developing a test to diagnose cystic fibrosis in newborns based on similar technologies and a home-care sensors for patients with chronic kidney disease.

Sensor response studies in buffer, serum, and whole blood are presented. The sensor array provides a simple, integrated, and reusable system for measuring a number of vital metabolic parameters in a drop of sample in parallel in POCT settings.

D-68

Comparative analysis of lead quantitation via inductively-coupled plasma mass spectrometry and point-of-care LeadCare® II system

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Background: Children with a blood lead level (BLL) greater than 10 mcg/dL are at increased risk for toxicity; additionally, the national health objective for adults with occupational exposure is a BLL less than 25 mcg/dL. Inductively-coupled plasma mass spectrometry (ICP-MS) is a high complexity assay and the gold standard for measuring BLL. A point-of-care whole blood lead assay with ease of use and waived status may be ideal for BLL screening, though potential bias in results may limit its utility. The aim of this study was to compare the analytical performance and clinical concordance of BLL using point-of-care LeadCare® II system (LCS) compared to a reference ICP-MS assay.

Methods: Excess EDTA venous and capillary whole blood samples (n=593) from children (n=531) and adults (n=62) submitted to UMass Memorial Medical Center for routine evaluation of BLL were used. The BLL was measured via ICP-MS (PerkinElmer, Waltham MA) and point-of-care LCS (ESA Biosciences, Chelmsford MA). Overall concordance was calculated as the percent of LCS BLL values falling into the correct BLL category by reference ICP-MS (i.e. below or above 10 mcg/dL or 25 mcg/dL in children and adults, respectively).

Results: Comparative analysis of BLL results above the LCS limit of detection (≥3.3 mcg/dL; n=108) yielded the regression equation: [LCS] = 0.93[ICP-MS] - 0.20, (r^2 =0.95). The mean (± standard deviation) bias between LCS and ICP-MS was -0.84 ± 1.00 mcg/dL. Evaluating BLL from children (n=531), the LCS demonstrated 84.6% sensitivity (11/13) and 100% specificity (518/518) for BLL threshold by ICP-MS of 10 mcg/dL with overall concordance of 99.6% (529/531). In adults (n=62), the LCS displayed 100% sensitivity (6/6) and specificity (56/56) with 100% (62/62) overall concordance for BLL threshold by ICP-MS of 25 mcg/dL.

Conclusions: The point-of-care LCS demonstrated good correlation with blood lead concentrations measured by reference ICP-MS, though systematically underestimates BLL resulting in decreased sensitivity but increased specificity for determination of BLL ≥ 10 mcg/dL in children. The LCS demonstrated excellent overall clinical concordance in children and adults, however, this study was limited by few samples exceeding the BLL thresholds. These data demonstrate that point-of-care LCS is an analytically acceptable alternative method for measuring BLL compared with ICP-MS. This study further supports that lead results measured by LCS near the BLL threshold (10 mcg/mL ± 2 mcg/dL) should be interpreted carefully with confirmation by a different methodology.

D-69

A Method Comparison of Critical Blood Analytes on Siemens Blood Gas Systems

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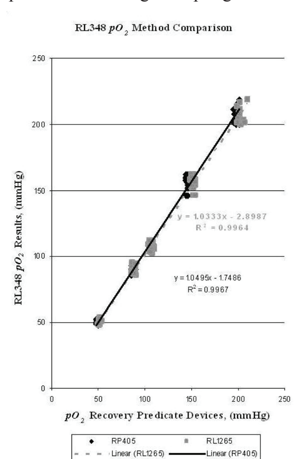
Background: Hospitals may utilize many blood gas analyzers throughout several locations. Each location may have different demands such as throughput, analyte requirements, storage restrictions as well as budget. The Siemens RAPIDLab 348 (RL348) blood gas system is an economical compact analyzer that is designed for critical care sites with low to moderate patient volumes. Shown is an evaluation of analytical equivalence between the RL348 and two cartridge-based Siemens blood gas analyzers.

Methods: A method comparison of whole blood samples was conducted concurrently across two RL348, two RAPIDLab 1265 (RL1265) and two RAPIDPoint 405 (RP405) blood gas systems. For each analyte, heparinized whole blood was prepared to various levels and measured across the six systems. Tested were pH, pCO_2, pO_2, Na^+, K^+, Ca^{++} and Cl^-. A minimum of ten paired specimens were evaluated at each level using syringe devices.

Results: The figure below highlights the pO_2 evaluation at five levels spanning 50-200 mmHg. The simple linear regression for RL348 versus RP405 (solid line) yielded a linear equation of y=1.05x-1.75 (r^2=0.997). For RL348 versus RL1265 (dotted line) the linear equation was y=1.03x-2.90 (r^2=0.996). It was observed that the bias between the RL348 and the other two systems slightly increased at the higher pO_2 concentrations (>150 mmHg). For example, the bias between the RL348 and RL1265 at the 100 mmHg level was 0.4% and 1.9% at the 200 mmHg level. The pO_2 graphical evaluation depicted is one representative of the method comparison data. Comparative data for all other parameters is similar and associated graphical presentation is provided.

Conclusions: The Siemens RL348 blood gas analyzer demonstrates good analytical correlation for all critical care parameters to the Siemens cartridge-based blood gas systems. The RL348 provides an affordable solution for blood gas analysis and may be an alternative backup instrument at high sampling rate sites.



D-70

Expanded Range Point-of-Care Therapeutic Drug Monitoring (TDM assay for FSH

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Introduction: Plasma or serum samples obtained via repetitive venipuncture represent the accepted gold standard for monitoring circulating levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2) and progesterone (P) in published reproductive studies. Unfortunately for research subjects, the burden of frequent venipuncture is high; and, for researchers, venipuncture samples require immediate processing and storage facilities with freezers.

Methods: Rapid and quantitative lateral flow point of care test (POCT) for FSH was developed using proprietary method to achieve increased dynamic range suitable for TDM. Serum samples collected at time of diagnosis of ovarian cancer and from normal individuals were tested for FSH using the POCT assay. Clinical data were analyzed using JMP9 statistical analysis software.

Results: Here, we describe the development of rapid point-of-care tests for detection of FSH that is quantitative and applicable to plasma, serum, as well as urine. The assay was constructed so that it has an expanded dynamic range of 10-10,000 IU/L. The traditional lateral flow method quantitation limit was only 10-200 IU/L. The expanded dynamic range encompasses all possible concentrations of FSH encountered in blood following administration of clinical dose of FSH or during biomarker testing. The cassettes can be read using an optical reader with 2D barcode capability, and the data can be printed out or stored on the reader for uploading onto the hospital database. The reader utilizes confocal optics with a low distance-to-target ratio. The reflectometric measurement is converted to activity units, using an established calibration curve embedded in the 2D barcode. The cassettes are made to be stable for more than 72 hours and can be shipped to a central lab or doctor's office for quantitation if the patient does not have access to the reader. Clinical application of the test against ovarian cancer samples revealed that in the serous adenocarcinoma group, FSH level was higher (median=151.6 mU/ml) vs. normal controls (median of 13.4 mU/ml, p = 0.01, Wilcoxon). FSH progressively increased from normal controls, to normotensive patients, to hypertensive patients with median FSH values of 13.4, 79.3, and 232.2, respectively.

Conclusion: Rapid and quantitative point-of-care testing for FSH has been developed for field deployment directly at home, and the cassettes can be read directly by the patient or can be shipped to the central lab/doctor's office for reading. As TDM, the test should allow for more effective dosing of the patients and thereby improving effectiveness of hormone manipulation therapy. The tests are also patient-centric, inviting better compliance and patient participation in personalizing his/her treatment. The simplicity of the assays would allow for their deployment in underdeveloped regions lacking access to central laboratories with specialized and expensive equipments. The expanded range eliminated the need for dilution of the samples to bring them within working range of the traditional assay.

D-71

Performance Evaluation of the VerifyNow P2Y12 Test in the Physician Office Setting

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Background: The VerifyNow P2Y12 Test (VN P2Y12; Accumetrics, San Diego, CA) is a rapid, point-of-care platelet function test that has been extensively validated as a tool for measuring the antiplatelet effect of P2Y12 receptor inhibitors. The test reports results as P2Y12 Reaction Units (PRU). The PRU result is highly specific for P2Y12 receptor blockade due to the effect of a P2Y12 inhibitor. The test is most commonly performed in the hospital, either in a point of care setting or in the central laboratory. There has been recent interest in expanding platelet function testing into the physician office setting, because this is the most convenient point of contact with patients on long-term antiplatelet therapy. The purpose of this study was to evaluate the ability of untrained physician office laboratory personnel (POL) to obtain VN P2Y12 PRU results that are similar to those obtained by trained laboratory professionals (Expert).

Methods: The study was conducted at three physician office sites, using three POL operators at each site. To be eligible to participate in the study, the POL operators could not have formal laboratory training or prior experience running any VerifyNow tests. Training materials were limited to the standard instructions for use and quick reference guides provided with the VerifyNow P2Y12 Test and VerifyNow Instrument. Three citrated whole blood samples were collected by venipuncture from patients taking Plavix. Plavix was selected as the P2Y12 inhibitor for this evaluation because the high degree of inter-patient variability in response to clopidogrel would yield results across the measurable range of the VN P2Y12 test. One sample was tested by a POL operator and the other two samples were tested by a trained laboratory professional that was stationed in an area separate from the physician office user. The primary methods for assessing comparative performance were Deming regression and error grid analysis. The allowable error limits used for the error grid analysis are based on the VN P2Y12 test 10% CV specification. The limits were set at +/- 1.96xCV, and at least 95% of the results should theoretically fall within the allowable error limits.

Results: A total of 384 blood samples were tested by both the POL operators and trained laboratory professionals. POL operator results were highly correlated with trained laboratory profession results, with Deming regression yielding a slope of 1.00 (95% CI 0.98-1.02) and an intercept of 0.78 (95% CI -2.08-3.64), $r^2 = 0.97$ ($p < 0.0001$). Furthermore, error grid analysis demonstrated that 96.4% of the results were within the allowable error predicted by the CV specification of the test, which is higher than the theoretical goal of 95%.

Conclusions: POL operators were able to obtain results from the VerifyNow P2Y12 Test that were highly correlated to those obtained by trained laboratory professionals. The results from this study suggest that VN P2Y12 results are not significantly influenced by the level of operator training or experience, and the test is suitable for use in a physician office setting.

D-72

Comparative Effectiveness of the VerifyNow P2Y12 Test and Light Transmittance Aggregometry for Assessing the Antiplatelet Effect of Clopidogrel

J. R. Dahlen[1], L. Swaim[1], V. Serebruany[2]. [1]*Accumetrics, Inc., San Diego, CA,* [2]*HeartDrug Research, Johns Hopkins University, Baltimore, MD*

Background: The VerifyNow P2Y12 Test (VN P2Y12; Accumetrics, San Diego, CA) is a rapid, point-of-care platelet function test that has been extensively validated as a tool for measuring the antiplatelet effect of P2Y12 receptor inhibitors. The test reports results as P2Y12 Reaction Units (PRU). The PRU result is highly specific for P2Y12 receptor blockade due to the effect of a P2Y12 inhibitor, and is more specific than light transmittance aggregometry (LTA) due to the presence of PGE1 in the assay, which minimizes the effect of the platelet P2Y1 receptor. The present analysis evaluates the comparative effectiveness of VN P2Y12 and LTA for the detection of a P2Y12 inhibitor effect, measured as reduced platelet reactivity to ADP. The sensitivity of detecting the antiplatelet effect of a P2Y12 inhibitor can be affected by 1) the time since the last dose, 2) the potency of the P2Y12 inhibitor therapy, and 3) inter-individual variability in the response to the drug. Factors such as genetics, concomitant disease, antecedent medication, and compliance can all influence the individual response to antiplatelet therapy. The ability to specifically detect the antiplatelet effect of P2Y12 inhibitors is important whenever the physician wishes to identify an antiplatelet effect in their assessment of the patient.

Methods: Participants eligible for the study had to 1) have a clinical indication to receive a P2Y12 inhibitor (clopidogrel), 2) be taking aspirin at least two days prior to enrollment, and 3) have at least two risk factors for developing vascular disease: family history of vascular disease; sedentary lifestyle; diabetes mellitus; hypertension; morbid obesity; known history of hypercholesterolemia; postmenopausal women; and smoking. VN P2Y12 and LTA measurements were performed from citrated whole blood samples collected prior to clopidogrel ingestion and either 24 hours after ingestion of a minimum 300 mg clopidogrel loading dose or 7 days after starting a 75 mg/day clopidogrel maintenance dose without the use of a loading dose. ROC curve analysis, sensitivity and specificity calculations were based on the ability to correctly identify the presence of a P2Y12 inhibitor.

Results: For the detection of a clopidogrel effect, as evidenced by a decrease in platelet reactivity to ADP, the area under the ROC curve for VN-P2Y12 PRU results was significantly greater than percent aggregation by LTA (0.95 vs. 0.90, p = 0.0067). The optimal decision point from ROC curve analysis for detecting the presence of a P2Y12 inhibitor was VerifyNow P2Y12 Test PRU < 208, which showed 79% sensitivity and 97% specificity for detecting the antiplatelet effect of clopidogrel. The lower limit of the PRU reference range (194) showed 72% sensitivity and 98% specificity for detecting the antiplatelet effect of clopidogrel.

Conclusions: The VerifyNow P2Y12 Test is superior to LTA for detecting the presence of a P2Y12 inhibitor, with significantly greater specificity. VN P2Y12 is suitable for use in clinical settings where it is necessary to identify a measurable effect of a platelet P2Y12 inhibitor.

D-73

Use of a Point of Care PT/INR Assay for Evaluation of Potential Stroke Patients in the Emergency Department

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Background: To meet TAT requirements for stroke certification, PT/INR results must be available within 45 minutes of patient evaluation in the emergency department (ED). A method to reliably provide this service with existing processes was not available in our large medical center, where the core laboratory and emergency department are in different buildings. Point of Care (POC) PT/INR devices are available, but limited in use, for monitoring of coumadin therapy in outpatient settings. An evaluation of a POC platform (Coaguchek XS Pro, Roche) was conducted to determine its suitability in the setting of evaluating stroke patients in the emergency department.

Methods: The validation process included evaluation of POC and central laboratory (Sysmex CA7000) PT/INR results from 39 patients who were being evaluated for stroke or other conditions in the ED. After implementation of the POC test in the ED, the use of the POC assay was monitored for each possible stroke patient and the POC results were compared with the laboratory results in an additional 35 patients.

Results: Initial validation studies in the ED included 15 possible stroke patients, 4 patients on coumadin, and 20 additional patients being evaluated for a variety of conditions in the ED. The correlation of INR in this group was >.99, with biases ranging from -0.4 to 0.2 INR. Among possible stroke patients, the biases ranged from -0.2 to 0.1 INR. After placing the POC assay in service, 35 possible stroke patients have been tested, all with corresponding main laboratory results for followup. The INR correlation is >.975, and using a medical decision cutoff point at an INR of 1.7, two of the 35 patients have had discrepant results (above vs. below the cutoff). In neither of these two cases did the discrepant results lead to a change in care; one patient had a seizure disorder and the other had altered mental status secondary to an infectious process.

Conclusions: We implemented a POC PT/INR device for use in the ED to evaluate stroke patients and meet TAT requirements for stroke certification. The use of the assay has been limited to patients suspected of presenting with a stroke, and our protocol requires another sample to be sent to the main laboratory for eventual confirmation of the POC result. To date, our tracking has shown that the INR correlation is excellent, and patient care has not been affected in two instances where discrepant POC vs. laboratory results have occurred.

D-74

Method Comparisons for Warfarin-Sensitive SNPs Genotyping

P. Jiang[1], S. Lee[1], C. Chen[1], C. Hsu[1], T. Wu[1], C. Chen[1], C. Sung[1], H. Lee[2], J. Tsai[1]. [1]*Industrial Technology Research Institute, Hsinchu, Taiwan, Taiwan,* [2]*National Taiwan University Hospital, Taipei, Taiwan, Taiwan*

Background: Warfarin is the most widely prescribed anticoagulant drug with a narrow therapeutic index. It is now established that genetic differences between individuals play a major role in warfarin metabolism, and single nucleotide polymorphisms (SNPs) are known to impact dose. Common variants in CYP2C9 (*2 and *3) and VKORC1 have been associated with a reduced drug-dosage requirement, and CYP4F2 are associated with higher therapeutic dose requirements in some studies. Turn-around-time for the molecular testing is a critical issue in detecting drug metabolism and infectious diseases. Our objective was to evaluate an assay platform and the sample preparation method, which can potentially be integrated with molecular testing in a point-of-care or resources limited setting.

Methods: In our study, we evaluated the Warfarin-sensitive SNPs genotyping with different assay platforms and genomic DNA extraction protocols. Genomic DNA was extracted from blood cells using QIAamp DNA Mini kit (QIAGEN) and our laboratory developed magnetic beads-based reagent system. Five SNPs of warfarin-sensitive genes including CYP2C9*2 (rs1799853), CYP2C9*3 (rs1057910), CYP4F2 (rs2108622), VKORC1 -1639 (rs9923231), and 1173 (rs9934438) were genotyped using a real-time PCR-based, laboratory-developed assay, Taqman® genotyping assays (Life Technologies) and an isothermal amplification method utilizing loop-mediated isothermal amplification (LAMP) principle. We then evaluated the performance of these methods with sequencing results.

Results: Forty clinical samples were analyzed by the real-time PCR and isothermal-based method, generating 200 SNPs results. The results derived from real-time PCR-based and isothermal amplification were compared, and the unresolved results were repeated and confirmed with the sequencing as the reference. The results in CYP2C9*2 and CYP2C9*3 were consistent but no mutations were detected, which collaborates with low percentage of prevalence in Asian population. More than 90% samples with VKORC1 and CYP4F2 SNPs were consistent using different assay platforms. The results demonstrated the clinical acceptable performance for SNP genotyping of CYP2C9*2, CYP2C9*3, CYP4F2, VKORC1 -1639, and 1173 with the real-time PCR and isothermal-based method. The SNP genotyping was performed in accordance with genomic DNA extracted from commercial kit or our laboratory developed magnetic beads-based reagents. The time from sample-to-result was approximately 4 hours on Taqman, and 2 hours on the isothermal amplification method, respectively.

Conclusions: The short turn-around-time of the isothermal amplification method and comparable performance to the real-time PCR based assays support its potential point-of-care application in genotyping and molecular diagnostics.

D-75

Evaluation of Rapid Antigen Test for the Detection of rotavirus and adenovirus in Fecal Specimens

J. Kim[1], S. Lee[2], K. Park[3], H. Kim[4], J. Kim[4], W. Lee[5], Y. Hong[6], H. Kim[4]. [1]*The Catholic University of Korea, Seoul, Korea, Republic of,* [2]*Neodin Medical Institute, Seoul, Korea, Republic of,* [3]*Department of Laboratory Medicine, Seoul National University, College of Medicine, Seoul, Korea, Republic of,* [4]*Department of Laboratory Medicine, Hallym University College of Medicine, Seoul, Korea, Republic of,* [5]*Department of Laboratory Medicine, Seoul Asan Hospital, Seoul, Korea, Republic of,* [6]*Department of Laboratory Medicine, Korea Cancer Center Hospital, Seoul, Korea, Republic of*

Background: Rapid and accurate detection of rotavirus and adenovirus is essential for the prevention and control of their outbreaks. We compared the performance of a newly introduced rapid antigen test (SD BIOLINE Norovirus; Standard Diagnostics, Korea) for the simultaneous detection of adenovirus and rotavirus with the established ELISA test and multiplex reverse transcription-PCR (mRT-PCR)

.**Methods:** Two hundred and seventy-five stool samples colleted from 6 hospitals and one commertial laboratory were analyzed by rapid antigen test (SD BIOLINE Norovirus; Standard Diagnostics, Korea), ELISA (R-Biopharm, Germany), and mRT-PCR (Seegene, South Korea). Overall percent agreement, percent positive agreement (PPA), and percent negative agreement (NPA) of the rapid antigen test in comparison with ELISA and mRT-PCR were obtained.

Results: For rotavirus, positive rates of rapid antigen test, ELISA, and mRT-PCR were 20.7% (57/275), 14.5% (40/275), and 14.5% (40/275), respectively. Forty samples

(14.5%) showed all positive, and 218 samples (79.3%) showed all negative results by three methods. Overall percent agreement of three methods was 93.8% (258/275). Overall percent agreement, PPA, and NPA of the rapid antigen test in comparison with ELISA and rapid antigen test were 94.0%, 70.2%, and 100%, respectively, and of rapid test with mRT-PCR were 94.0%, 70.2%, and 100%, respectively. For adenovirus, positive rates of rapid antigen test, ELISA, and mRT-PCR were 7.6% (21/275), 5.5% (15/275), and 4.0% (11/275), respectively. Ten samples (3.5%) showed all positive, and 249 samples (90.5%) showed all negative results by three methods. Overall percent agreement of three methods was 94.2% (259/275). Overall percent agreement, PPA, and NPA of the rapid antigen test in comparison with ELISA were 94.9%, 52.4%, and 98.4%, respectively, and of rapid test with mRT-PCR were 95.6%, 47.6%, and 99.6%, respectively.

Conclusions: Rapid antigen test was easier and quicker to perform, and showed high agreement rates with ELISA and mRT-PCR. This test may be useful for rapid screening of rotavirus and adenovirus infection.

Wednesday PM, July 18, 2012

Poster Session: 2:00 PM - 4:30 PM

Proteins/Enzymes

D-76

Comparison of three methods in the estimation of total urinary protein

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Background: The measurement of urine protein excretion is essential for the diagnosis and management of several kidney diseases. The presence of protein in urine by qualitative test is indicative of further investigation by quantifying the levels in order to make a diagnosis of nephritis or nephrosis. The biuret method is the reference method for the determination of total urine protein. This study was set up to assess a microturbidimetric method using benzethronium chloride in alkali medium and a semi-quantitative colorimetric method using pyrogallol red molybdate and using biuret method as the reference method in the estimation of total urinary protein.

Methods: 162 fresh urine samples were obtained after albustix testing (combina 9SG) was done. Samples were assayed for total urine protein using benzethronium chloride, pyrogallol red molybdate and biuret methods. Reproducibility tests were done on freshly pooled urine and assayed for 10 runs in duplicates. Benzethronium chloride/Biuret and Pyrogallol red molybdate/ Biuret assay pairs were used for correlation analysis.

Results: The minimum detection limits for Biuret, Benzethronium chloride and Pyrogallol red molybdate methods using human serum albumin were 50, 25 and 25mg/L and that for human globulin was 150, 50 and 50 mg/L respectively. The reproducibility (CV) for the assays was 4.0, 5.2 and 6.7%. Percentage recovery ranged from 59% for Pyrogallol red molybdate to 83% for Benzethronium chloride. The correlation coefficient for Biuret Vrs Benzethronium chloride and Biuret Vrs Pyrogallol red molybdate were 0.78 and 0.82. The paired CV for Benzethronium chloride and Pyrogallol red molybdate relative to Biuret were 90.0% and 69.1% respectively. The median deviation (SD)for the assays were -0.153 (0.416)g/L for Benzethronium chloride and 0.01 (0.112) g/L for Pyrogallol red molybdate with the limits of agreement as 0.985 g/L below and 0.679g/L above the reference method for the Benzethronium chloride and the same for Pyrogallol red molybdate as 0.217 g/L and 0.231 g/L. The overall median deviations of Benzethronium chloride and Pyrogallol red molybdate from the reference values were -0.0015 (-2.4 -0.56) and 0.0028 (-1.1 -0.67) mg/L respectively. The within-batch coefficient of variation for Benzethronium chloride, Pyrogallol red molybdate and Biuret were 4.0% at 420 mg/L, 6.7% at 340 mg/L and 5.0% at 420 mg/L.

Conclusions: There was consistent over estimation and under estimation by Pyrogallol red molybdate and Benzethronium chloride assays for total urine protein in a similar manner. Benzethronium chloride and Pyrogallol red molybdate gave consistent pattern with Benzethronium chloride giving the highest and the Biuret assay the least concentration value. Overall although the correlation coefficient were high, there was agreement between Benzethronium chloride and Pyrogallol red molybdate with the reference method, however the Benzethronium chloride assay can be useful for estimating total urine protein in albustix negative samples.

D-78

Synthesize the Precursors of Nitro-Containing HNIW Cage Compounds as Inhibitors of AchE and BchE

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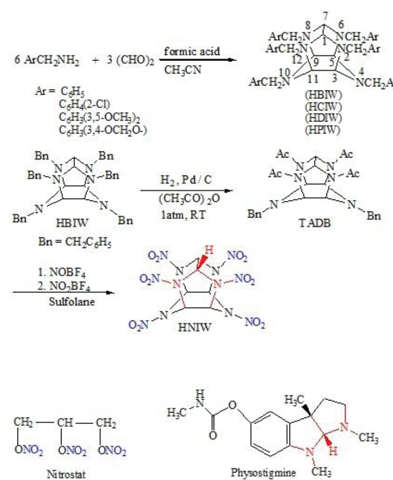
In the presence of a catalytic amount of formic acid, treatment of benzylamine with glyoxal in acetonitrile achieves high yield of HBIW which is a precursor of the cage materials HNIW. Substituted benzylamines such as 2-chlorobenzylamine, 3,5-dimethoxylbenzylamine and (3,4-methylenedioxy)benzylamine also react with glyoxal to afford HCIW, HDIW and HPIW, respectively. These precursors were used as the inhibitors of AchE (Acetylcholinesterase) and BchE (Butyrylcholinesterase). This paper is study on the inhibitory mechanism of AChE and BChE by using cage compounds as reversible inhibitors. From the rate constant (k_2), inhibitory constant (K_I) and biomolecular rate constant (overall inhibitory constant k_i), the results showed that the inhibitory potency for the inhibitor to AChE and BChE was in the order of HNIW > HCIW > HDIW > HPIW > HBIW. This research also illustrated that the inhibitory potency of cage compounds to the BChE was better than that of AChE.



D-80

Evaluation of RBC cholinesterase on Roche Cobas 6000 Chemistry Analyzer

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Background: Acetylcholinesterase is found predominantly in erythrocytes and nervous tissue. Individuals exposed to organophosphorous compounds (eg. in insecticides) exhibit decreased levels of cholinesterase in the red blood cells and serum. Our laboratory has been using the Roche CHE reagent (cat no: 11877763) customized on the Beckman Coulter LX20 chemistry analyzer for reporting patient results. Since the Roche Cobas 6000 became available in our laboratory, we evaluated the same reagent but on the Cobas analyzer and compared patient values with that obtained from the LX20 analyzer

Methods: Cholinesterase reagent, CHE (cat no: 11877763), is used to measure cholinesterase activity by an enzymatic rate method. In the reaction, cholinesterase cleaves acetylthiocholine into acetate and thiocholine. Thiocholine reacts with dithiobisnitrobenzoic acid (Ellman's reagent) to form the yellow-colored 5-mercapto-2-nitrobenzoic acid. The rate of color formation at 480 nm is directly proportional to the cholinesterase activity.

The performance of the CHE reagent was evaluated for imprecision, linearity, analytical sensitivity, carryover and comparison of patient samples (haemolysates) obtained on the Cobas 6000 and LX20 analyzers.

Results: The total within-lab imprecision was found to be less than 5 %. Analytical sensitivity (42 U/L) and linearity (42 - 5000 U/L) were verified to be within the recommended limits. No significant sample carry-over was detected. Using cholinesterase values obtained from haemolysate samples, a Deming regression equation of CHE [Cobas] = 0.94CHE [LX20] - 58.6 was obtained, with n = 98, r^2 = 0.98, range: 97 - 1981 U/L. The Altman Bland plot indicated a negative bias of 98 U/L. To calculate RBC cholinesterase, the cholinesterase from haemolysate requires correction for dilution (11 x) and haematocrit (~50 %). Inclusion of these correction factors will result in a negative bias of about 2156 U/L.

Conclusions: The performance of CHE reagent on Cobas 6000 is within acceptable limits. A new reference interval would need to be established before routinely reporting RBC cholinesterase results for patients.

D-81

Immunoassay standardization for serum cystatin C in Japan by ERM-DA471

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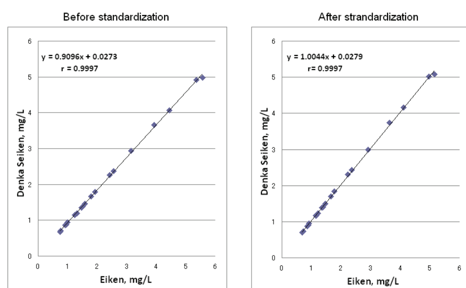
Background: Cystatin C is a key intrinsic serum marker for GFR advantageous over existing markers in its specificity and sensitivity for early detection and prevention of chronic kidney disease. With an introduction of ERM-DA471, international reference material for the protein, JSCC plasma protein committee launched the serum cystatin C immunoassay standardization project and evaluated the effectiveness of the material on international standardization.

Methods: ERM-DA471 was purchased from ReCCS, Japan. Effectiveness of recalibration using ERM-DA471 was evaluated by measuring 20 pooled sera with different concentration.15 immunoassay systems, 14 turbidimetric and one nephelometric methods, commercially available and under development participated in the project.

Results: All the assay systems showed good precisions both for serially-diluted ERM-DA471 and pooled sera with CV less than 2.93%. Proportionality and linearity were overall satisfactory between ERM-DA471 and calibrator of each assay system that made it possible almost all to achieve accurate value transfer. Commutability study was almost satisfactory. Using a given assay as a tentative reference method, the range of the slope with comparative methods was improved from 0.788-1.059 to 0.900-1.033 after standardization. A case is represented in figures shown below. There is one assay system in which antibody relatively weakly reacted with recombinant cystatin C in the material. A few systems showed relatively weak reaction with native cystatin C in serum.

Conclusions: Newly assigned value from ERM-DA471 was thus set in each calibrator. The material is expected to play a central role to international standardization. For further development immunochemical reactions between recombinant or native protein, and assay antibodies should be continuously investigated to minimize the discrepancy. Preparation of prototype reference material (ProBio-S, Sapporo) is under investigation.



D-82

Study of variations in adhesion molecules, cytokines and cytokine related markers in serum of patients presenting metabolic syndrome using simultaneous biochip based immunoassays

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Background. Inflammation is associated to metabolic syndrome. Cytokines and adhesion molecules are involved in the inflammation response. The use of a multi-analytical approach in the determination of these analytes provides more information than single analyte determinations, which is relevant when studying analytes that function as part of complex interacting networks. Evidence biochip array technology enables simultaneous determination of multiple analytes from a single sample, at a single point in time.

Relevance. The aim of this study was to measure adhesion molecules, cytokines and cytokine related markers in human serum using this technology to investigate possible differences between patients presenting metabolic syndrome and controls. This approach leads to a broader overview of biomarker variations from a sample with increased results output and decreased sample/reagent consumption.

Methods. Four biochip arrays were used for the measurement of E-selectin, P-selectin, L-selectin, ICAM-1, VCAM-1, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6,IL-7, IL-8, IL-10, IL-12, IL-13, IL-23, VEGF, IFNγ, EGF, MCP-1, sIL-2Rα, sIL-6R, sTNFRI, sTNFRII, MMP-9. The analytes were quantified by simultaneous chemiluminescent immunoassays on the biochip arrays. The Evidence Investigator analyser was used for all determinations. Serum samples from patients presenting metabolic syndrome as defined by the International Diabetes Foundation (n=90) and controls (n=60) were analysed. The Mann-Whitney U test (significance $p \leq 0.05$) was used to identify analytes that varied in patients when compared with controls.

Results. The five adhesion molecules (E-selectin, P-selectin, L-selectin, ICAM-1, VCAM-1), nine cytokines (IL-1β, IL-6, IL-7, IL-8, IL-10, EGF, IFNγ, MCP-1, VEGF), sIL-6R, sTNFRI and MMP-9 presented significant variations when metabolic syndrome samples were compared with controls.

Conclusion. This investigative biochip array-based study, based on the samples analysed, indicates significant variations of adhesion molecules, cytokines and cytokine related markers in patients presenting metabolic syndrome compared with controls. In the present study, the utilisation of biochip arrays enables a comprehensive investigation of biomarkers. Such an approach can improve the understanding of the divergent signalling pathways that participate in the pathogenesis of metabolic syndrome. This technology represents an analytical tool for the evaluation of multiple biomarkers from limited volumes of clinical samples relevant to a particular disease state.

D-83

Multiplex evaluation of analytes related to metabolic syndrome in serum samples with a biochip array

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Background. Using miniaturized assay procedures, Evidence biochip array technology enables simultaneous determination of multiple analytes from a single sample, at a single point in time. This generates a patient profile for the analytes tested. This study evaluates the variations in serum of male and female patients presenting metabolic syndrome of the analytes C-peptide, ferritin, IL-1α, IL-6, insulin, leptin, PAI-1, resistin, TNFα, by simultaneous determination on a biochip array. These analytes take part in physiological processes such as insulin secretion, glucose regulation, fatty acid metabolism, inflammation, iron homeostasis, energy homeostasis/endocrine regulation and fibrin regulation.

Methods. Simultaneous chemiluminescent biochip based immunoassays were employed and applied to the Evidence Investigator analyser. Serum samples from patients presenting metabolic syndrome as defined by the International Diabetes Foundation (45 male and 45 females) and controls (31 males and 29 females) were analysed. The Mann-Whitney U test (significance $p \leq 0.05$) was used to identify analytes that varied in male and female patients when compared with controls.

Results. IL-6, Ferritin, resistin, insulin, leptin, PAI-1 and TNFα varied significantly both in male and female patients when compared with controls. The variation of C-peptide was only statistically significant when male patients were compared to controls.Conclusion. The data of this evaluation in metabolic patient versus controls indicate the occurrence of significant variations in seven analytes related to metabolic syndrome independent of the genre, whereas the variation of C-peptide was significant for the male samples when compared to controls in this sample collection. The utilisation of a multi-analytical approach can provide new insights into metabolic syndrome research and has the potential to improve the understanding of the pathogenesis of this condition. Moreover, as biochip array technology uses miniaturized assay procedures, the consumption of clinical sample is reduced but the output of test results is increased.

D-84

Development of a rapid kinetic assay for quantifying adenosine deaminase in red blood cells

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Background: Adenosine deaminase (ADA) irreversibly catalyzes the deamination of adenosine and deoxyadenosine nucleosides into inosine and deoxyinosine, respectively, and plays an essential role in the purine salvage pathway. Approximately 20% of severe combined immunodeficiency cases are due to a deficiency of ADA. The immunodeficiency is the consequence of accumulation of adenosine and deoxyadenosine substrates that are indirectly toxic to lymphocytes. Our laboratory uses a calibrated, end-point assay to quantify ADA activity in erythrocytes. The lack of a certified reference material for assay calibration and a 60-minute reaction time prompted us to develop a more accurate and rapid analytical method.

Methods: The conversion of adenosine to inosine catalyzed by ADA was monitored as a continuous decrease in absorbance at 265 nm over 30 minutes at ambient temperatures. Reactions were performed in quartz cuvettes with a 2 mm path length. The molar extinction coefficients of adenosine and inosine as well as the Michaelis constant (Km) and maximum reaction velocity (Vmax) were determined using classic techniques. Accuracy, linearity, imprecision, analytical sensitivity, and enzyme stability were validated using residual samples sent to ARUP Laboratories. Reference intervals were established from 120 self-claimed healthy individuals.

Results: Molar extinction coefficients at 265 nm were 12,680 and 4,917 $M^{-1}cm^{-1}$ for adenosine and inosine, respectively, with their difference (7,763 $M^{-1}cm^{-1}$) used to calculate ADA activity. Km was 0.021 mM and Vmax was 0.002 ΔA/min. A substrate concentration that was 3 times the Km (0.06 mM) was selected for use. Accuracy was evaluated by adding ADA to a hemolysate and calculating recovery. Recoveries were 102.6 and 93.3% at 19.7 and 33.4 U/L ADA activities, respectively. Linearity was determined by adding ADA, performing serial dilutions, and testing each sample in duplicate. The assay was linear to 35.6 U/L. Within-run and total precision were determined by testing two hemolysates in triplicate once each day for 14 days. Within-run imprecision was 5.5 and 8.1% and total imprecision was 12.1 and 16.5% at 1.02 and 0.63 U/g Hb ADA activities, respectively. Analytical sensitivity was 0.5 U/L as calculated from the mean plus 3 standard deviations of 10 replicates of the substrate in the absence of ADA. Enzyme stability was determined in pooled hemolysates at two ADA activity levels. ADA was stable for 15 days at ambient temperature and at 4-8 °C. The reference interval was established as 0.4-0.9 U/g.

Conclusions: The kinetic ADA assay has favorable performance characteristics. Compared to a calibrated, 60-minute end-point assay, the kinetic ADA assay eliminates the calibration requirement and can be performed in 50% less time.

D-85

Development of a highly sensitive enzyme-linked immunosorbent assay for urinary Tamm-Horsfall protein and its usefulness in diagnosing renal dysfunction

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Background: The Tamm-Horsfall protein (THP) is an 80-90 kD glycoprotein synthesized in the thick ascending limbs of Henle's loop of the kidney; it is known to be a predominant component of urinary casts. Although urinary THP level downregulation has been reported in patients with renal calculus or some renal diseases, standard methods for measuring urinary THP levels are not available. The main problem is that these assay procedures require a high dilution of urine samples (>100-fold) to dissociate the polymerized forms of THP molecules but are insensitive to low THP concentrations. Therefore, we aimed to develop a highly sensitive enzyme-linked immunosorbent assay (ELISA) for urinary THP and investigated the usefulness of measuring urinary THP levels for diagnosing renal dysfunction.

Methods: We included 25 untreated patients with different kinds of glomerulonephritis such as IgA nephropathy (male 19, female 6, mean age ± SD = 43.7 ± 15.4 y.o.) and age-matched healthy subjects (male 19, female 6, mean age ± SD = 43.6 ± 18.7 y.o.). THP was obtained from pooled urine samples of healthy subjects (male 3, female 3, mean age ± SD = 30.5 ± 10.2 y.o.) by using diatomaceous earth filtration and was used to raise monoclonal antibodies with classic hybridoma techniques. Polyclonal antibody was also raised in rabbits. This sandwich ELISA measures urinary THP concentrations between 2 layers of these antibodies (the monoclonal antibody (capture antibody, monoclonal antibody; detection antibody, polyclonal antibody). To dissolve the aggregation of urinary THP, the urine samples were diluted to 1:1000~1:10000 with distilled water and were also treated with a diluent to inhibit THP polymerization under acidic urine conditions. The samples were subsequently analyzed by the ELISA.

Results: The range of the ELISA calibration curve was 0-40 μg/L, and the lower detection limit was 0.313 μg/L. The dilution curves of urine samples showed good linearity. The within-run CV was 3.99-6.92% and between-day CV was 5.64-8.00%. The assay recovery was 81-123%. The antibodies reacted strongly with urinary THP, and cross-reaction was not observed. The urinary THP concentrations of the patients with glomerulonephritis were 10.8 ± 2.04 mg/L and were significantly lower than those of the healthy subjects, i.e., 49.2 ± 5.48 mg/L.

Conclusions: The newly developed ELISA assay showed high sensitivity for detecting THP in highly diluted urine samples. The results shoe that urinary THP levels could aid in diagnosing renal dysfunction.

D-86

Determination of amyloid-β1-42 and tau biomarkers in cerebrospinal fluid using the enhanced chemiluminescent technology platform

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Relevance: The use of biomarkers in cerebrospinal fluid (CSF) to identify Alzheimer's disease (AD) is becoming more important and is expected to play a critical role in the diagnosis and treatment of AD. Two biomarkers, amyloid-β1-42 (Aβ42) and tau protein, have been shown to correlate with disease progression, and a number of research use only (RUO) tests are available. The sensitivity requirements of these assays are in the pg/mL range and are challenging for any methodology. These challenges give rise to significant analytical variability, a lack of standardization and differing cutoff levels for decision points from study to study.

Objective: New prototype immunoassays for both Aβ42 and tau proteins are in development on the fully automated VITROS® ECiQ Immunodiagnostic System (VITROS System) with the objectives of ease of use, high precision, and high sensitivity and specificity to enable use in routine clinical laboratories.

Methods: Each of the assays uses an antigen sandwich configuration with two monoclonal antibodies and enhanced chemiluminescence reagents on the VITROS® System. Standard curves of 0 to 2000 pg/mL and of 0 to 4000 pg/mL, were generated for VITROS AB-42 and VITROS Tau assays, respectively. Limit of detection, intra and inter assay precision (n=5 for two days), lot-to-lot variability, and performance with clinical samples (n= 38) were evaluated.

Results: Both tests could distinguish normal control CSF samples (n=20) from patient samples with clinically diagnosed AD (n=18). For Aβ42, tau, and tau/Aβ42 ratio measurements with the VITROS AB-42 and VITROS Tau assays, sensitivity was 83%, 83%, and 89%, respectively and specificity was 85%, 90%, and 95%, respectively.

Preliminary Analytical Data	VITROS AB-42 Assay	VITROS Tau Assay
Time to first result	36 minutes	36 minutes
Limit of Detection (pg/mL)	5 pg/mL	12 pg/mL
Intra-assay precision (%CV)	0.9, 2.9, 1.1	2.9, 0.1, 1.7
Inter-assay precision (%CV)	2.6, 2.8, 1.2	1.1, 0.4, 0.9
Site to site variability with clinical samples (Deming correlation)	R^2 = 0.9557 Slope =1.110 n = 46 samples	R^2 = 0.9982 Slope = 0.989 n = 46 samples

Conclusions: Immunoassays in development for Aβ42 and tau proteins on the VITROS® System exhibit promising analytical and clinical performance toward identifying patients with clinically diagnosed dementia. Automation should make the analysis of these important biomarkers more reliable and routine.

D-87

In vitro reconstitution of human recombinant LD isozymes and the possibility for its application as enzyme reference material

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Background: The human lactate dehydrogenase family contains five isozymes (LD1-5) with tetrameric structures composed of H and/or M subunits. To standardize serum enzyme activity for clinical chemistry, LD1 (H homotetramer) purified from humans, has been distributed as enzyme reference material by the Institute for Reference Materials and Measurements (IRMM). In Japan, however, mixtures of LD1, 2 and 3 purified from human erythrocytes are widely used as the reference material. As reference material, recombinant enzymes constructed using human gene information have advantages over enzymes purified from human tissues, in that they avoid both lot-to-lot variation in the raw material and biohazard contamination. The LD1, 2, 3, 4 and 5 isozymes have been synthesized using dissociation and consecutive reconstitution steps with a mixture of separately purified LD1 and LD5 (M homotetramer) also containing sodium thiocyanate and dithiothreitol (DTT).

Objective: Our aim was to modify the method used for LD reconstitution and apply it to recombinant LD1 and LD5 separately expressed in *E. Coli*. Specifically, we sought to adjust the ratio of the two recombinants so as to enable reconstitution of the isozymes at a ratio similar to that in normal human serum. Then to study the product's fitness to serve as reference material, the reactivity of this artificially reconstituted LD would be compared with that in human serum.

Methods: Recombinant human LD1 and LD5 were separately expressed in *E. Coli* and purified using standard procedures. For in vitro hybridization, a mixture of recombinant LD1 and LD5 was frozen at -25°C for one night in the presence of sodium thiocyanate for dissociation and DTT for reconstitution. The enzymes were then reconstituted by incubation at 37°C for 20 min after thawing. After desalting the product, it was added to a liquid composition containing enzyme stabilizers (e.g. sugar) and lyophilized in vials. Two commercially available reagent kits based on the IFCC and JSCC reference method were used to evaluate the reactivity of the product, as well as that of 50 human serum samples.

Results: All five LD isozymes were successfully constructed using the hybridization technique. Moreover, because the relative amount of each isozyme can be controlled using the mixing ratio of the recombinant LD1 and LD5, we were able to prepare mixtures in which LD1, 2 and 3 dominated, which is common in normal human sera. When the quality of the LD was compared with CRM-001 (certified reference materials containing LD from erythrocytes and used in Japan), the ratios of the activities in JSCC and IFCC were 0.95 for CRM-001 and 0.94 for the product, which was highly consistent with the ratio obtained with serum.

Conclusions: Successful *in vitro* reconstitution of LD was accomplished using a mixture of recombinant LD1 and LD5 via dissociation and consecutive reconstitution steps. The constructed enzymes are thought to be potentially useful as reference material.

D-88

Assessment of the nutritional state using waveform patterning of protein fractionation analysis

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Background: It is well known that activities of Nutrition Support Team can lead to shorten hospital stay by preventing postoperative medical complications. However, it is necessary to perform many expensive diagnostic tests periodically in order to assessment the state of the patient. Accordingly, we examined if the waveform pattern of the protein fractionation analysis can infer quantitative concentration of several nutritional proteins such as Rapid turnover proteins (RTP) and inflammatory proteins by applying waveform analysis technology.

Methods: Protein fractionation tests were performed by "Sebia protein 6" method using inpatient test samples. The relationships between waveform shape of protein fractionation and other measured items were examined.

The waveform was corrected by proportional calculation; fixing albumin and N,N-dimethylformamide (DMF: used as internal standard in pre-examination) positions at 75 and 300, respectively. The size of under curve area of the waveform without internal marker was adjusted to 100,000 when the sample contained 7.0 g/dL of total protein. The correction of the mobility was performed using pattern matching method instead of adding the internal marker, DMF.

Results: Using 3000 healthy test samples, average peak position of nutritional proteins after standardization of the mobility were; (protein fractionation, mobility) ALB at 75, α1 at 144, α2 at 161, β1 at 203, β2 at 222, γ at 249.

The strongest relationship observed between nutritional proteins and peak positions were (mobility, coefficient of relation, number of data): RBP(31, 0.748 ,57), preALB(34, 0.834, 170), HDL(53, 0.325, 886), LDL(86, 0.498, 830), Cu(133, 0.753, 17), SAA(143, 0.612, 148), CRP(144,0.619, 3894), C4(66, 0.576, 151), Tf(205, 0.881, 35) and C3(221, 0.624, 151). Though we also found the possibility of strong relationship about Zn(160, 0.95, 6), number of data remained short to conclude.

Discussion: These data indicated that nutritional related proteins such as RBP, preALB, Cu, CRP, Zn, C3, C4 and Tf could be detected by the protein fractionation analysis, whereas some of the LDL, HDL, SAA and CRP had no significant relationship with peak positions. Thus, several proteins could be detected by analyzing standardized waveform by protein fractionation test and be used as inexpensive objective indicator of nutrition monitoring.

D-89

Automation of total protein measurements for use in a clinical assay of intestinal disaccharidases

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Background: Dietary carbohydrates are the major exogenous source of glucose. Because the small intestine is impermeable to disaccharides, the activity of intestinal disaccharidases is required for their hydrolysis into component monosaccharides. Disaccharidases include lactasephlorizin hydrolase, sucrase-isomaltase, and maltase-glucoamylase. Decreased or absent activities of one or more of these enzymes can result in carbohydrate malabsorption. Measurement of intestinal disaccharidase activities is considered the gold-standard test for the diagnosis of disaccharidase deficiency. Due to high variability in the size of tissue biopsy it is necessary to normalize results to the total protein content of the sample. Our laboratory utilizes a labor-intensive, manual, Lowry-based method for these total protein determinations. The objective of this study was to adapt and validate this method to an automated chemistry analyzer.

Methods: Biopsies of intestinal mucosa were homogenized in saline using a Bullet Blender™ (Next Advance, Inc., Averill Park, NY) and centrifuged to remove cellular debris. Total protein was determined using the Bio-Rad DC Protein assay adapted for use on the Roche cobas c501 chemistry analyzer. The colorimetric assay is based on the Lowry method in which protein reacts with alkaline copper tartrate that subsequently reduces Folin's phenol reagent to produce a final product that absorbs light at 750 nm. Performance characteristics including, analytical sensitivity, linearity, precision, and accuracy were determined. A total allowable error of 25% was determined to be the quality goal.

Results: The limit of blank was determined to be 0.9 mg/dL by testing 10 replicates of the zero calibrator (saline) and the lowest non-zero standard (35.5 mg/dL) and calculated as the concentration that was two standard deviations from the zero response. The limit of detection was determined to be 6.1 mg/dL by testing 10 replicates of a 7.2 mg/dL protein solution and calculated as the mean (4.0 mg/dL) added to three standard deviations (0.7 mg/dL). Linearity was determined by combining low- and high-protein homogenate pools in different ratios to create a set of five samples (range 10-120 mg/dL) that were tested in two replicates. Linear regression analysis produced a slope of 1.0, an intercept of 1.7, standard error of the estimate of 2.5, and a correlation coefficient of 1.0. Precision was evaluated using pooled homogenates tested in two replicates, twice each day, for five days. Within-run and total precision was 5.7 and 6.2% and 2.5 and 3.1% at 40.5 and 73.6 mg/dL, respectively. Accuracy was evaluated by testing 123 patient samples on the c501 and with the manual method. Deming regression produced a slope of 0.93 (95% CI 0.91-0.96), an intercept of 3.3 mg/dL (95% CI 1.8-4.8), standard error of the estimate of 3.2 mg/dL, and a correlation coefficient of 0.99. Total error was defined as systematic error added to three times the highest estimate of random error. At a concentration of 65 mg/dL this was calculated to be 16.6 mg/dL (25%).

Conclusions: The total protein concentration in homogenates of intestinal mucosa can be determined with accuracy and precision using a Lowry-based method adapted for use to a chemistry analyzer.

D-90

Development of highly specific monoclonal antibodies to tropomyosin exons 1a and 9d for the development of a diagnostic blood test for Alzheimer's Disease

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Background. Tropomyosin is a fibrous molecule that consists of two α-helices. It is widely distributed in all cell types, where it regulates the shortening of the muscle filaments actin and myosin. An ongoing clinical proteomics study at the University of Vienna has identified a tropomyosin isoform (S1855) that exhibits a highly significant up-regulation of 35% (P<0.0001) in blood platelets from patients with Alzheimer's disease. Of the eight tropomyosin isoforms expressed in platelets, S1855 and an additional two exhibited a combined upregulation of 20% (P=0.0043) in this neurodegenerative condition. These three isoforms are characterised by the presence of both exons 1a and 9d, the combination of which is specific for Alzheimer's disease. The aim of this project was to generate two highly specific monoclonal antibodies to tropomyosin exons 1a and 9d respectively and to use the resultant antibody pair to develop a diagnostic test for the detection of tropomyosin isoforms related to Alzheimer's disease.

Methods. Sheep were immunised on a monthly basis with peptide sequences corresponding to exons 1a (H-Cys-Leu-Asp-Lys-Glu-Asn-Ala-Leu-Asp-Arg-Ala-Glu-Gln-Ala-Glu-Ala-Asp-Lys-Lys-Ala-Ala-NH_2) and 9d (H-Cys-Glu-Lys-Val-Ala-His-Ala-Lys-Glu-Glu-Asn-Leu-Ser-Met-His-Gln-Met-Leu-Asp-Gln-Thr-Leu-Leu-Glu-Leu-Asn-Asn-Met-OH) conjugated to bovine serum albumin (BSA) via N-terminal cysteine residues. The resulting immunogens were administered to adult sheep on a monthly basis in order to generate a polyclonal response. Lymphocytes were then harvested and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of exon specific antibodies, employing ELISA based assays, in which the microtiter plates were coated with full length protein. Positive hybridomas were cloned to stability. Antibodies generated by the resulting monoclonal hybridomas were purified, characterised by 1 and 2 dimensional western blotting (1D and 2D WB) and employed in the development of a biochip sandwich immunoassay. The assay was applied to the Evidence Investigator analyser, which utilises biochip array technology, based on ELISA immunoassay principles.

Results. 1D and 2D WB confirmed that the antibodies generated to exons 1a and 9d, bound to S1855 and an additional two tropomyosin isoforms carrying those exons. An exon 9d specific capture antibody was selected for immobilisation on the surface of the biochip and an exon 1a specific detector antibody was conjugated to horseradish peroxidase to generate the assay tracer. The biochip immunoassay developed employing these immunoreagents exhibited specificity for the three Alzheimer's Disease related tropomyosin isoforms. Assay sensitivity was <10ng/ml (measuring range 0-700ng/ml) and within-run precision was <10% for standard level concentrations. In isolated blood platelets, levels of up to 12 µg/ml were detected.

Conclusions. The results indicate that the antibodies generated in this study are suitable for detection of individual tropomyosin isoforms, incorporating exon 1a and/or 9d. Combining the antibodies in a highly specific biochip immunoassay enables the detection of Alzheimer's disease related isoforms, which are thought to play a crucial role in the pathophysiology of Alzheimer's disease.

D-92

Vascular cell adhesion molecule-1 (VCAM-1) levels measured with Randox Biochip Array Technology (BAT) correlate with severity and mortality in acute stroke

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Background: Inflammation is implicated in the pathogenesis of acute stroke and the local recruitment of inflammatory cells is likely to exacerbate damage at the site of brain injury. In this inflammatory environment expression of cell surface adhesion molecules such as VCAM-1 are elevated, with resulting recruitment of leukocytes and platelets locally. Consequently, anti-inflammatory therapy constitutes a therapeutic target for the treatment of acute stroke. At present, robust biomarker tests for acute stroke are not available and newly developed tests in this area would complement existing imaging approaches employed by clinicians for stroke diagnosis. In the present study, VCAM-1 levels were measured in acute stroke patients employing BAT. Correlations between VCAM-1 levels and stroke severity and mortality were investigated

Methods: In a prospective study we included 98 patients with acute stroke, 73 ischemic (IS) and 25 intracerebral hemorrhage (ICH). Blood samples were taken at the time of admission and at 24, 48 and 72 hours thereafter. A final measurement was performed on day 7. Sixty healthy subjects served as controls. Stroke severity was measured at the time of admission with the Scandanavian Stroke Scale (SSS). Functional outcome was measured with the modified Rankin scale (mRS) on day 7 and acute stroke patients were categorised into three severity groups (mild, moderate and severe) according to their mRS-score: mild (mRS-score:0-2), moderate (mRS-score:3-4) and severe (mRS-score:5-6). VCAM-1 levels were quantified in EDTA plasma samples employing Randox BAT technology on the Evidence Investigator analyser.

Results: The mean age (SD) of the patients was 75.2 (9.4) years. Forty-two patients (42%) died during a follow-up period of 1 year. The mean time (SD) between the onset of neurological symptoms and hospital admission was 3.22 (1.58) hours. At admission, mean VCAM-1 levels were significantly elevated in both IS (655ng/ml) and ICH (733ng/ml) when compared to healthy controls (452ng/ml) (p<0.00001 anova test). The diagnostic accuracy of a single VCAM-1 measurement upon hospital admission for diagnosis of stroke is high [AUC=0.80 (95%CI 0.72-0.95), P<0.0001]. Plasma levels increased during follow-up peaking at 72hours for both stroke subtypes (903ng/ml for ICH vs 730ng/ml for IS). Mean VCAM-1 levels were significantly increased among non-survivors (785ng/ml) compared to survivors (589ng/ml) (p<0.005). This difference was observed during the whole period of follow-up. Mean VCAM-1 levels increased with severity and this biomarker pattern was apparent upon admission (mild=589ng/ml, moderate=718ng/ml, severe=776ng/ml) (p<0.005 anova-test) and during follow-up with values peaking again at 72 hours (673vs785vs890ng/ml respectively).

Conclusions: The presented data suggest that the determination of VCAM-1 biomarker levels upon admission can facilitate in stroke diagnosis and serve as a predictor of severity and mortality. Our results indicate that there is an association between low levels of VCAM-1 and better outcome among acute stroke patients.

D-93

Physical properties of cuvette material determine serum lipase activity

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Background: Despite striking similarity among colorimetric lipase assay recipes, marked differences are noted in the lipase values obtained with analysers of different brands. In the present study, the impact of physical properties of the cuvette surface on the measured serum lipase activity was investigated.

Methods: Lipase activity of 131 serum samples was measured concomitantly in cuvettes of a Vista (Siemens), a Modular (Roche) and a Synchron (Beckman Coulter) analyzer using the same colorimetric reagents (Roche). The contact angle of the cuvette material was determined using a modified Zeiss type 44024 projection microscope. Also, the effect of various characteristics of serum (biochemical parameters, surface tension) and the surface/volume ratio of the cuvettes was examined.

Results: Analyzing serum lipase activity using the same colorimetric methylresorufin assay showed marked differences, depending on the cuvettes used. On average, lipase activities in Siemens cuvettes were 4.58 times higher than in Roche cuvettes and 4.81 times higher than in Beckman cuvettes. Relative differences appeared to be dependent on the total lipase activity and less important in the higher activity range. Other biochemical parameters (bilirubin, alkaline phosphatase, total cholesterol, HDL, LDL and triglycerides) and serum surface tension dit not affect these results. However, physical properties of the various cuvettes, more specifically the solid/liquid contact angles, showed remarkable differences. We measured contact angles of 5, 67 and 99 ° for the Beckman Coulter (pyrex glass), Siemens (H-12 polymethyl methacrylate) and Roche (cyclo olefin polymer) cuvette respectively.

Conclusions: Serum lipase activity is affected by the physical properties of the cuvette surface. This novel finding is important when comparing lipase data from various platforms and when considering standardisation of lipase assays.

D-94

Evaluation of serum protein immunoturbidimetric assays on the new Beckman Coulter AU5800® Clinical Chemistry System.

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Background: Proteins are useful in the clinical laboratory for the detection and monitoring of inflammation and changes in the normal polyclonal mixture of serum immunoglobulins. The AU5800 is a series of ultra-high throughput clinical chemistry systems with a test menu that includes serum proteins.

Objective: The purpose of this study was to evaluate the performance of serum protein immunoturbidimetric assays on the new Beckman Coulter AU5800 Clinical Chemistry System in comparison to the AU5400 using patient specimens from random blood and urine donors.

Methods: All 21 of the currently available AU serum protein immunoturbidimetric reagents were evaluated as part of this study. Evaluation protocols for precision were based on CLSI EP15A2 methods. Accuracy was evaluated using samples spanning the dynamic range based on CLSI guideline EP15-A2. Linearity protocol was based on CLSI guideline EP6-A.

Results: Total precision targets were met for all serum proteins, as well as within-run targets. Total %CV's ranged from 0.8-4.0%. Linearity met the Beckman Coulter claimed dynamic range in all cases. Representative results from 12 of the 21 comparison studies between the AU5800 and the AU5400 are presented in the table.

Conclusions: With respect to precision, linearity, and accuracy of serum protein assays, the Beckman Coulter AU5800 Clinical Chemistry System produces results that are consistent with the AU5400.

Representative results from comparison studies between the AU5800 and the AU5400							
Analyte	Units	N	Slope	Intercept	R	Bias	Range
β2-microglobulin	mg/dL	113	0.975	0.003	0.9993	-0.002	0.04-1.50
Ceruloplasmin	mg/dL	115	0.965	6.30	0.9995	-4.66	42-1900
C3	mg/dL	71	0.995	-2.06	0.9989	-2.75	39-484
C4	mg/dL	71	0.993	-0.40	0.9988	-0.64	5-146
CRP	mg/L	122	0.967	-1.90	0.9997	-3.75	6-486
Ferritin	mg/mL	62	1.030	-0.87	0.9993	2.67	8-445
IgA	mg/dL	82	0.999	-3.65	0.9994	-3.87	1-664
IgM	mg/dL	82	0.968	2.39	0.9995	-1.24	18-481
Microalbumin (U)	mg/dL	147	1.010	-0.014	0.9997	0.06	0.5-29.5
Prealbumin	mg/dL	72	1.006	-0.42	0.9978	-0.25	7.6-64.4
RF	IU/mL	125	1.008	-1.67	0.9920	-1.44	3-183
Transferrin	mg/dL	101	0.988	-0.93	0.9915	-4.59	109-720

D-95

Performance evaluation of Bioporto Diagnostics' neutrophil gelatinase-associated lipocalin (NGAL) assay on automated clinical chemistry analyzer Hitachi 7600

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Background: The ideal biomarker for diagnosing acute kidney injury (AKI) should provide early and precise information on the acute injury and severity of damage to the kidney. NGAL is normally expressed at very low levels in several human tissues, including lung, stomach, colon, and kidney. Renal expression of NGAL increases dramatically in kidney injury from a variety of causes and NGAL is released into both the urine and plasma. In the present study, the performance of the automated Bioporto Diagnostics' NGAL assay was validated.

Methods: We studied the precision and linearity of the NGAL Test™ (Bioporto Diagnostics', Denmark), compared it with another test, and evaluated its reference interval. Two controls were measured with 4 replicates and 5 runs for the precision test. The linearity range experiment was performed using 5 equally spaced concentrations prepared by Clinical and Laboratory Standards Institute EP-6 dilution methods. Thirty nine samples were tested for comparing the Bioporto Diagnostics' assay with the Triage® NGAL test (Biosite, USA), and 129 samples (male : female = 63 : 66; age range, 21-59 years) were tested for evaluation of the reference interval.

Results: Precision: Means of each low and high level control material were 210.6 and 514.0 ng/mL, respectively. Within-run coefficient of variation (CV) and total CV were 2.8% and 3.3% at low level and 1.4% and 1.8% at high level, respectively.

Linearity: The linearity of the assay was found to be acceptable in the range of 57.0 - 3182.0 ng/mL (r = 0.999).

Comparison: A method comparison between Biosite's assay and Bioporto Diagnostics's assay was made (Passing and Bablok fit; y = 2.33x - 206.96; x, Biosite; y, Bioporto; n = 38; y range; 250-2541 ng/mL). (Passing and Bablok fit; y = 1.94x - 65.29; x, Biosite; y, Bioporto; n = 31; y range; 250-1308 ng/mL). The correlation was linear at less than 1500 ng/mL, but not beyond this limit.

Reference interval: The 2.5 and 97.5 percentile of the reference range for the samples were 42.4 ng/mL and 132.6 ng/mL, respectively.

Conclusions: This Bioporto Diagnostics' NGAL assay represents a rapid, automated, and precise method for the determination of NGAL in EDTA plasma. The reference interval was 42.4-132.6 ng/mL. A good correlation was noted between the Bioporto's and Biosite's systems, but the same cut-off or reference interval of Triage® NGAL might not be acceptable for the Bioporto Diagnostics' assay. The measurement of NGAL by the Bioporto Diagnostics' assay may be useful for the prompt diagnosis of AKI.

D-96

Quantification of Tyro3, Axl ve Mer (TAM) receptors on platelet surfaces in patients with type 2 diabetes

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Background: Several reports revealed that Gas6 has been involved in the regulation of immunity/inflammation, which is related to the pathogenesis of type 2 diabetes. Evidently Gas6/TAM system represents an important mechanism for diabetes mellitus and its complications. The objective of this study is quantification of TAM receptors (Tyro3, Axl, Mer) on platelet surface in patients with type 2 diabetes and compare the results with the control.

Methods: 24 patients with type 2 diabetes and 21 healthy volunteers were enrolled in the study. None of the study participants were on anticoagulation therapy or vitamin K supplementation. Platelets were isolated from citrated blood samples and then the density of TAM receptors on resting and ADP-activated platelet surfaces were analyzed by flow cytometry and quantified by QIFI kit using specific antibodies against each receptor.

Results: The following values were obtained in terms of the density of TAM receptors on resting platelets: Tyro3=9,308±4,261, Axl=8,667±3,333, Mer=8,380±2,980 in healthy controls (n=21); Tyro3=9,442±7,288, Axl=12,517±12,035, Mer=8,937±5,336 in the diabetic group (n=24). In the control group, the density of the receptors Tyro3 (Sky) and Mer increased significantly after activation of platelets with ADP (p=0.004 and p=0.033, respectively) but not Axl (p=0.243). In the diabetic group, activation of platelets with ADP did not cause any significant increase in the density of all three type of receptors (p>0.05). When the diabetic group was compared to the control in terms of Tyro3, Axl and Mer, there were no significant differences for resting platelets and also after activation with ADP (p>0.05). In diabetic patients, an inverse correlation was observed between serum CRP and the density of receptor Mer on activated platelets (r=-0.518), and also Tyro3 (Sky) (r=-0.654) on resting platelets. A correlation was observed between fasting plasma glucose levels and the density of the receptor Axl on activated platelets from diabetic patients (r=0.855).

Conclusions: In conclusion, this preliminary study, for the first time, showed that the changes of the density of the TAM receptors on platelet surfaces may be different in patients with type 2 diabetes than the control group when platelets were activated by ADP. Because of interactions among platelets, endothelial cells, erythrocytes, and leukocytes, any problem with platelets may affect the homeostasis of the vascular system. Gas6/TAM signaling may play a potential role in the pathogenesis of micro- and macro-complications of type 2 diabetes. Further studies are required to elucidate the role of Gas6/TAM signaling in complications of diabetes.

D-97

Reference Interval for Plasma Gas6 (Growth Arrest Specific-6)

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Background: Gas6, which is a member of the vitamin K-dependent protein family, is the ligand for 3 different receptors, Tyro 3, Axl and Mer (TAM receptors. Gas6 is secreted by platelets, contributing to platelet degranulation and aggregation. Gas6-deficient mice seem to be protected from thromboembolism but do not suffer from bleeding. The lack of increased bleeding in the treated mouse model suggests that blockage of the Gas6 system may be both an effective and safe means of anticoagulant therapy. The Gas6 pathway and soluble TAM receptors may be a promising new pharmacological target for the treatment of thrombosis. Gas6 expression is widespread in many tissues, including endothelial cells, vascular smooth muscle cells, and bone marrow cells. Many studies have been published suggesting that Gas6 might be related to various disorders, like rheumatoid arthritis, nephrotoxic nephritis, breast cancer and cardiovascular diseases. The presence of Gas6 in plasma was examined by several research groups and different results have been reported in connection with plasma concentration. We need well determined reference values for interpretation of plasma Gas6 levels in several diseases. The purpose of this study is to determine the reference range [[Unsupported Character -]][[Unsupported Character -]]of plasma Gas6 in healthy adults in Turkey.

Methods: We constructed reference values for Gas6 among healthy adults of ages 18-52 for male and 18-60 for female. 115 healthy, nonsmoking individuals (58 female, 57 male) were included in this study. All participants gave informed consent. Fasting citrated plasma samples were obtained in the morning. Plasma Gas6 levels were assayed by ELISA (R&D). The purity of concentrated calibrator was examined by HPLC. To eliminate the effect of the matrix, Gas6 depleted plasma was prepared. The ELISA method has been optimized. The analytical characteristics were determined.

Results: The mean values for plasma gas6 was 10.79±4.65 ng/ml (min 2.54; max 25.01) and 9.94±4.79 ng/ml (min 1.76; max 34.19) in female and male, respectively. There was no statistically significant difference between male and female (p>0.05).

Conclusions: Therefore, we obtained the first preliminary reference interval values for Turkish population. Further results will be obtained based on an increased sample according to CLSI standards. Similar studies for other age groups will be very helpful in clinical interpretation. Recognizing the importance of new vitamin K dependent proteins will stimulate new areas of research and offer potential therapeutic interventions.

D-98

NGAL vs. other Biomarkers of Kidney Injury during Lithotripsy

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Mechanical / hydraulic influence causing fragmentation of kidney urine concrements in lithotripsy also leads to injury of renal parenchyma. Therefore it is necessary to control the degree of kidney tissue damage at lithotripsy, especially after repeated treatment sessions. NGAL is considered to be a very promising biomarker for the detection of renal tubular damage, along with others like beta-2-microglobulin, IL-18, Kim-1 etc.; unfortunately most of them still rarely use in practice.

The objective of our investigation was to determine the diagnostic value of NGAL and other biomarkers of kidney tissue injury in repeated lithotripsy sessions in patients suffered from urolithiasis.

65 patients with urine stones were examined. Extracorporal shock-wave lithotripsy (ESWL) was performed in 46 patients; 16 of them were treated in one ESWL session, 14 - two sessions, and 16 - three sessions. Contact ureterolithotripsy (CLT) was performed in 19 patients. The control group consisted of 14 healthy donors. All patients were examined of NGAL, microalbumin, and beta-2-microglobulin level in morning urine samples by ELISA (BioPorto, Portugal; Orgentec, Germany). To consider the effect of possible ureteral occlusion on the severity of kidney tissue damage during ESWL all patients were retrospectively divided into the group who had fragments of concrements in urine after the first session (n=28), and the group without any stone pieces in urine (n=37).

The level of microalbuminuria in nephrolithiatic patients was much higher than in controls (192.6±28.1 ug/ml vs. 4.3±0.5 ug/ml), and did not change significantly after ESWL. Urinary level of microalbumin in patients with ureteral stones was 75.3±10.1 ug/ml before CLT, increasing up to 237.6±52.4 ug/ml after surgery. There were no significant changes of beta-2-microglobulin urinary level in nephrolithiatic patients before and after ESWL and CLT.

The urine NGAL level before surgery was 0.68±0.11 pg/ml in patients with kidney stones, and 2.72±0.18 pg/ml at ureteral stones (0.40±0.05 pg/ml in controls). After the first ESWL session, the five times rise of urinary NGAL was found (up to 3.46±0.32 pg/ml), after the second session - extra 1.6 times (up to 5.66±0.72 pg/ml), and after the third session - extra 1.7 times (up to 9.78±0.64 pg/ml). The urine NGAL after CLT increased 2.4 times (up to 6.56±0.89 pg/ml). Urinary NGAL was 6.12±0.71 pg/ml in patients with stone fragments in urine after ESWL (eq. to temporary ureteral occlusion) vs. 3.82±0.45 pg/ml in patients without stone pieces.

Thus, serious nephron tubule damage develops as early as after the first ESWL session, and the level of injury increases after each stones re-crushing procedure (likely due to redoubling of already developed violations). The relatively smaller rise of NGAL excretion after the second and third sessions may be explained by limitation of a shock-wave impact zone size in renal parenchyma by a focal spot, which is usually of similar size and location at subsequent ESWL sessions as at the previous ones. The above data allow suggesting NGAL as informative biomarker of tubular injury at ESWL and of related occlusive complications.

D-99

Inquiry on available biological variation data for AST, ALT and GGT

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Background: the Biological Variation (BV) theory is based first on the notion that the concentration of each biological component fluctuates around a homeostatic point in each individual (within-subject biological variation, CVi), and second on that variation of the homeostatic points of each quantity in individuals of the same population (between-subject biological variation, CVg). BV data are frequently used to set analytical quality specifications. The aim of this work is to verify the validity of the currently available BV data for three liver enzymes: alanine aminotransferase (ALT), aspartate aminotransefrase (AST) and gamma glutamyl transferase (GGT).

Methods: the BV references for ALT, AST and GGT from 1970 to the present day were retrieved according to the following approach. As preliminary data we considered all the references cited in the review of Gambaro et al. (Eur J Clin Chem Clin Biochem 1997;35:845-52) and in the database of Ricos (http://www.westgard.com/biodatabase1.htm) Then, without time limitations, we searched PubMed for papers on the three enzymes with the following keywords: biological variation, biological variability, CVi, CVw, CVb, and CVg. Finally, the validity of the papers retrieved was evaluated according to the following criteria: recruitment of healthy subjects, duration of the study and sampling frequency, sampling storage, method and protocol of samples analysis, statistical analysis. These criteria derived from Fraser's et al protocol (Crit. Rev. Clin. Lab. Sciences 1989;27,409-37).

Results: 21 papers were found, 5 of which (2 in Italian and 3 in Spanish) are not included in Medline. Not all the papers reported results of CVg and the protocols used in these papers were very different. The main differences are summarized hereafter: number of subjects (ranging from 10 to 274), number of blood drawing per subject (2 - 16), frequency of phlebotomy (daily - monthly), number of analytical replicates, statistical methods , type of subjects (healthy or sick), subject's age and gender, sample storage. We found only one paper that performed the measurements using an IFCC optimized method: most of the references were very old and dated from before the 2002 IFCC optimization. The inevitable consequence is the great heterogeneity of the BV data obtained. In fact the ranges of CVi found in our review are very wide: ALT: 11% - 58%; AST: 3% - 32% and GGT 3.9% - 16%. The results present in the Ricos database, currently used to establish analytical quality specifications (ALT: 18%, AST: 11.9% and GGT: 13.8%) are close to the medians of the values found. On comparing these studies with Fraser's criteria almost all studies showed limitations : we found only 3 valid papers for GGT, 2 for AST and none for ALT, and in any case only for the CVi data.

Conclusions: the available BV data for AST, ALT and GGT are not reliable. New studies for the production of reliable BV values, following well detailed protocols, are needed.

D-100

Constructive analysis of urinary proteins using cellulose acetate membrane electrophoresis for predicting renal damages

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Background: Our previous study showed that the urinary protein patterns observed during cellulose acetate membrane (CAM) electrophoresis coupled with the results for highly sensitive silver staining constitute a useful predictor of renal damage (e.g., glomerular, tubular, or a combination of such injuries) [1]. Here, we established a method for extracting proteins from fractions obtained during CAM electrophoresis and compared their levels in patients with typical glomerular injuries and those with tubular injuries.

Methods: Urinary samples were obtained from a patient who had IgA nephropathy with a glomerular pattern and a patient who had tubulointerstitial nephropathy with a tubular pattern. Each urinary sample was added to 10 lanes in a CAM and electrophoresed. Lanes 1 and 10 were cut and were silver-stained. Protein fractions were cut off from the remaining unstained CAM region (lanes 2-9) by using the stained membrane as a guide, and these sections were further fragmented into 5-mm2 sections. The fragments were incubated with 1.5% SDS, which contained 0.5 mol/l Tris-HCl buffer (pH 6.8), for protein extraction. The extracted proteins were separated by non-reducing SDS-PAGE, and the gel proteins were stained with silver. The proteins were identified by MALDI-TOF/MS.

Results: The proteins identified from each fraction differed between the patients (Table 1), including those with similar relative mobility. In the glomerular pattern, the major bands of albumin and hemopexin corresponded to the albumin and α1 fractions, respectively; however, these bands were detected in other fractions also, indicating post-translational modification of these proteins. In the tubular pattern, characteristic fractions, i.e., slow α, slow β, and slow γ, were detected.

Conclusions: Our method helped clarify differences in the protein contents on the CAM according to the renal region affected. These results may increase the clinical value of CAM electrophoresis.

1. Sakatsume M, et al. Nephrology 2007;12:191-6.

List of the identified proteins

Fraction	Relative mobility	Glomerular pattern	Tubular pattern
preAlb	1.13	-	transthyretin
Albumin	1.00	albumin	albumin
α1	0.90	albumin, hemopexin	-
slow α1	0.83	-	α1-microglobulin
α2	0.72	α2-glycoprotein, vitaminD binding protein, albumin, hemopexin	α1-microglobulin
fast β	0.66	-	retinol binding protein
β	0.53	transferrin, albumin, hemopexin	β2-microglobulin
slow β	0.38	-	β2-glycoprotein, apolipoprotein A-1
γ	0.32	immunoglobulin, albumin, hemopexin	protein of 52.7 kDa
slow γ	0.18	-	protein of 51.6 kDa, lysozyme

D-101

Development and evaluation of the performance characteristics of a new immunoturbidimetric assay for HbA1c on Beckman Coulter AU® Clinical Chemistry Systems*

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Objective: The objective performance of this study was to evaluate a next- generation HbA_{1c} assay on AU Clinical Chemistry systems.

Relevance: HbA_{1c} is used for long-term assessment of diabetic glycemic control. Method: Human whole blood samples were pre-treated off-line with hemolyzing reagent prior to analysis. HbA1c concentration, relative to that of total hemoglobin, was determined by immunoturbidimetric inhibition assay of HbA1c and colorimetric measurement of total hemoglobin. The assay was standardized to the IFCC reference method. and results converted into National Glycohemoglobin Standardization Program (NGSP) %HbA1c units.

Results: The HbA1c assay demonstrated acceptable observed within-run and total imprecision of </= 1.4 %CV and </=2.1%CV, respectively, across all analyzers tested (AU480/640/680/2700) in the range 5 -10 %HbA1c. Method comparison versus the current AU HbA1c assay OSR6192 shows substantial correlation with the existing method; r = 0.9966, slope = 1.036 and intercept of -0.4021 %HbA1c, when tested with a panel of patient samples (n=116; Range 4.7 - 12.1% HbA1c).

No significant interference (</=6%) was observed from bilirubin, Intralipid** (Kabivitrum Inc.), and ascorbate up to concentrations of 30 mg/dL, 400 mg/dL, and 50 mg/dL respectively. Rheumatoid Factor interference observed was also </=6% up to 1,000IU/mL. Calibration stability was determined to be14 days, with on-board reagent stability 30 days. NGSP sample testing 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), with CAP samples recovering within ±6% of NGSP target.

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

* Assay currently under development and not for clinical use

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D-102

Evaluation of a New Generation Hemoglobin A1c Assay on Beckman Coulter Unicel® DxC Synchron® Clinical Chemistry Systems*

Z. Zhu, L. Eguchi, L. Wong, M. Necula. *Beckman Coulter, Inc., Brea, CA*

Objective: The objective of this study was to evaluate the performance of a next generation HbA1c assay on UniCel DxC Systems.

Relevance: Hemoglobin A1c (HbA1c) is used to monitor long term glucose control in patients with diabetes mellitus.

Methodology: HbA1c and total hemoglobin (THb) concentrations were measured in human whole blood by immunoturbidimetric inhibition and colorimetric assays, respectively, on Unicel DxC Synchron Clinical Chemistry Systems. HbA1c concentration was reported as a ratio of HbA1c to THb concentration. The assay was standardized to the IFCC (International Federation of Clinical Chemistry) reference method and results were converted in National Glycohemoglobin Standardization Program (NGSP) %HbA1c units.

Validation: In development studies, within-run imprecision was ≤ 1.2% CV and total imprecision was ≤ 1.4% CV, respectively, in the range 5 -10 %HbA1c NGSP, A1c ≥ 0.4 g/dL (NCCLS EP5-A2). Linearity was demonstrated between 0.3-2.7 g/dL HbA1c, 6-24 g/dL THb, and 4 -17 %HbA1c NGSP (NCCLS EP6-A). Methods comparison (Deming regression) against the existing HbA1c assay yielded y = 1.041x - 0.286, r = 0.997, n = 118 (CLSI EP09-A2). HbA1c and THb reagent calibration stability was determined to be 7 days and on-board stability was determined to be 30 days (recovery within 6% from control data point). Sensitivity, defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence, was 0.3 g/dL for HbA1c and 1 g/dL THb (CLSI EP17-A). No significant effect of HbS, HbD, HbE, and HbC was seen with this assay. Accuracy results to NGSP method were within ± 0.53 %HbA1c (development studies using NGSP protocol, criteria ± 0.75 %HbA1c). Accuracy results to CAP (College of American Pathologists) method were within ± 6% (development studies using CAP samples).

Conclusions: In development studies, the next generation HbA1c assay on UniCel DxC systems demonstrated acceptable correlation to existing method, precision, linearity, sensitivity, and reagent stability. Accuracy to NGSP and CAP met current criteria.

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

D-103

Analytical and Clinical Validation of Beta-Trace Protein, a New Marker of Glomerular Filtration Rate.

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Background: Beta-trace protein (BTP) is a low molecular weight protein synthesized in the central nervous system (CNS), generally used as a clinical marker of liquorrhea. As BTP is freely filtrated in the glomerules and cleared by the tubules, its amount in plasma depends mainly from its renal clearance. Thus, different Authors have proposed to use BTP as a marker of renal function. However, little is known regarding the analytical performances of its determination, as well as its stability in serum.

We thus aimed to validate BTP determination, to evaluate its stability at different temperatures and times of storage and to compare its clinical characteristics with different well-established markers of the Glomerular Filtration Rate (GFR).

Methods: First we have assessed the accuracy and precision (according to the CLSI EP 5A-2 guideline) of BTP determination using the Siemens BN2 (Siemens Diagnostics, Tarrytown, NY) nephelometer. We built the accuracy profile on 10 serum pools (ranging from 0,62 to 20,4 mg/L). We calculated the β-expectation limits with β=0.95 and considered the method as valid if they were comprised in the ±15% interval. For stability studies, 10 samples were assayed in duplicate at T0 and after 1, 4, 7 and 14 days at +4°C. Stability after storage at -20°C was studied by assaying in duplicate the 10 same pools at T0 and after 1,3,6 and 12 months. Then we evaluated BTP performance as a marker of renal function compared to cystatine C (CysC; Siemens Diagnostics) in a population of patients for whom the GFR had been previously determined with a reference method (plasma iohexol clearance).

Results: Repeatability did not exceed 3,1% and the intermediate precision 5,4% in the studied concentration range. 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 ±15% in the studied range. BTP was shown to be stable after 14 days of storage at +4°C, and up to 1 year at -20°C. BTP and CysC showed the same classical exponential profile when compared to the GFR determined with the reference method. However, we observed a grey-zone in the range 60-80 mL/min and we could not determine which parameter was the earlier to rise.

Conclusions: BTP determination on BN2 is a reliable method that presents interesting analytical characteristics. However, 15% of total variability should probably be improved, compared to the analytical performance of some other GFR markers. BTP was shown to be a very stable molecule at +4° and -20°C. Finally, studies with a reference method in a greater number of patients presenting a GFR in the grey-zone of 60-80 ml/min are needed in order to see if BTP could be an earlier marker of renal impairment compared to CysC and creatinine.

D-104

Development of an Enzyme-Linked Immunosorbent Assay for the Detection of Platelet Hyaluronidase 2

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Background: Hyaluronan (HA) is a major extracellular matrix glycosaminoglycan that is widely distributed through most tissues. In addition, HA plays a crucial role in inflammation. HA's deposition increases in inflamed tissues; and the degredation of HA results in proinflammatory fragments. HA fragments can induce inflammatory responses in a size dependent manner, and induce macrophage cytokine and chemokine production. The fragments can be generated by specific enzymes called hyaluronidases (HYAL) such as HYAL1 and HYAL2. HYAL2, a GPI- anchored protein, is found on the surfaces of many cell types. Our lab has previously shown that HYAL2 is found on the surface of platelets, and platelet-derived HYAL2 cleaves HA into fragments that stimulate leuckocytes to produce proinflammatory cytokines. Rationale: Little is known about the normal parameters of HYAL2 expression on human platelets. Using immunoblot assays standardized to total protein, we determined that patients with Inflammatory Bowel Disease (IBD) have lower levels of HYAL2 expression than normal controls. Therefore, we wanted to develop a rapid, quantitative screening assay for HYAL2 in platelets, and establish the normal range of HYAL2 expression in a control population.

Methods: To measure the amount of HYAL2 present on platelets, we developed an "indirect" ELISA assay. Platelets from normal donors were isolated, washed and counted. Purified HYAL2 protein standards as well as lysates from 10^6 platelets were added to replicate wells of high-binding 96-well plates and then dried at 37°C. After blocking, mouse anti-human HYAL2 primary antibody was added and detected with alkaline phosphatase rabbit anti mouse IgG secondary antibody.

Results: This ELISA assay has the ability to measure HYAL2 levels reproducibly in as little as 10^6 platelets. In analysis of a limited set of normal donors, a range of 40-90 ng/10^6 platelets was detected.

Conclusions: A sensitive quantitative assay to measure HYAL2 on human platelets has been developed, and determining the normal range of platelet HYAL2 levels is now possible with a larger donor cohorts. Further research will determine the sensitivity of this method and its physiologic usefulness for screening patients with Inflammatory Bowel Disease.

D-105

Traceability of ARCHITECT Enzyme Assays to IFCC Reference Methods - An International Study

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Background. The ARCHITECT clinical chemistry systems enzyme assays determine catalytic concentrations by measuring the rate of substrate conversion using a constant calibration factor (k-factor). This study demonstrated metrological traceability by optimizing the k-factors for agreement of measurement results to the published IFCC primary reference measurement procedure (RM). The assays included in the study were: AST, LDH, AMY and AP (Alkaline Phosphatase).

Method. In study phase 1, optimized k-factors were derived from testing serum pool panels with assigned target values by manual RM on two ARCHITECT *ci*8200 systems. Confirmation of the optimized k-factors was performed in phase 2, in which individual serum samples were tested on two ARCHITECT *ci*8200 systems and by the automated RMs. Phase 3 of the study served to further demonstrate the validity of the optimized k-factors by testing using multiple ARCHITECT instruments in multiple laboratories in Europe, Canada, and the U.S. Aliquots of two serum samples per assay were tested by 25 laboratories using a combination of ARCHITECT *c*8000, ARCHITECT *c*16000 and ARCHITECT *c*4000 instruments. The results were compared with the manual RM target values.

Results. Phase 3 confirmation runs were performed using the optimized k-factors on 36 ARCHITECT instruments. The mean results were in agreement with the respective RMs, as shown below:

Assay	Level 1 %bias	Level 2 %bias
AST	3.4%	2.2%
LDH	-3.9%	1.7%
AMY	7.5%	4.1%
AP	0.7%	1.4%

The %CV values for the ARCHITECT results from all instruments were all <5%. The % difference between mean results of the three ARCHITECT cSystems were all <5%, demonstrating excellent comparability for the ARCHITECT family of analyzers.

Conclusions. An international study demonstrates the alignment of the previously optimized k-factor values to the respective IFCC reference methods for AST, LDH, AMY and AP. Performance data from phase 3 of the study demonstrates the precision and accuracy of the respective assays among reagent lots, among instruments, among ARCHITECT cSystems family members and among laboratories around the world.

D-106

Towards an Assay-Independent Cystatin C-Based GFR-Prediction Equation.

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Background: Several different cystatin C-based GFR-prediction equations have been and are used, which delay the general use of cystatin C as a marker for GFR. The main reason for the variation is the use of different cystatin C calibrators by different diagnostic companies. Recently, an international certified reference material for cystatin C (ERM-DA471/IFCC) was produced and is now used as the "gold" standard for the cystatin C assays offered by many diagnostic companies. Although this has improved the agreement, there still remain significant differences between the results of different commercial assays. This is caused by differences in the parameter setup for several of the reagents, when they are used on the different platforms. In a joint effort between IFCC-WG-SCC and five diagnostic companies (Dako, Gentian, Roche, Sentinel/Abbott, and Siemens) these differences have been minimized creating the foundation for an "Assay-Independent Cystatin C-Based GFR-prediction equation". Such a prediction equation is now being established using these assays and about 4400 plasma samples from patients with measured GFR.

Methods: GFR was determined by plasma clearance of iohexol. A preliminary GFR

prediction equation was established based on the cystatin C levels in the about 1000 initial plasma samples determined by two, or more, of the improved cystatin C assays by Dako, Gentian, Sentinel/Abbott, Roche and Siemens. The accuracy was assessed as the proportion of estimates within 10% and 30% of measured GFR ((P10 and P30) in cross-validations (100 replications, 80:20 split).

Results: The preliminary GFR prediction equation, $eGFR = 94 \times \text{Cystatin C}^{-1.30} \times 0.92$, if female, had high accuracy [median (2.5 - 97.5 percentiles)]: P30 = 82% (78-87%), P10 = 37% (32-42%). The accuracy did not differ among males and females, but was somewhat lower among children below< 14 years of age and at low measured GFR.

Conclusions: The groundwork has been made to an "Assay-Independent Cystatin C-Based GFR-Prediction Equation" with well-characterized diagnostic performance, allowing the efficient use of cystatin C as a marker for GFR in clinical settings. Additional plasma samples will facilitate more detailed validations and make it possible to customize equations for e.g. children.

Wednesday PM, July 18, 2012

Poster Session: 2:00 PM - 4:30 PM

TDM/Toxicology/DAU

D-107

Receiver-operator characteristics of adjusted urine oxycodone measurements to distinguish between three different rates of drug administration

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Background: Use of urine oxycodone measurements to assess their relationship to prescription regimens under controlled conditions has been investigated by Couto et al. ([1]: *J Opiod Management* 2009;5(6):359-64). In that study, subjects in three groups (A-C) were administered oxycodone at fixed rates (A: 80 mg/day; B: 160 mg/day; C: 240 mg/day); after steady-state, urine oxycodone concentrations (ng/mL) were measured by LC-MS/MS and adjusted according to a proprietary algorithm based on urine pH, urine specific gravity, and lean body mass. Analysis of distributions of adjusted concentrations for groups A, B, and C showed that the 95% confidence intervals of the medians of the distributions were non-overlapping between groups. The analysis did not, however, extend to an examination of whether measurements for individual subjects could correctly classify them with respect to inclusion/exclusion within groups A, B or C. To that end, we performed receiver-operator characteristic (ROC) analyses for the data from [1], by calculating the extent to which any assumed reference range for each group was inclusive for members of that group's distribution (sensitivity), vs. the extent to which the same reference range was exclusive for members of each of the other two group's distributions (specificity).

Methods: Distributions of algorithm-adjusted urine concentrations for groups A, B, and C were tabulated from [1] (bin intervals: 2050 ng/mL; range: 450-41450 ng/mL). For each group, variation in assumed reference ranges (which defined sensitivity) were obtained by variation in the percentage of central results (i.e., a percentage of results centered on the distribution median) that were included in the reference range. For example, use of the central 95% of results for A as the reference interval for A defined a 95% sensitivity for members of A, and allowed calculation of the corresponding reference interval-dependent specificities for members of B and C, according to the extents to which their distributions were excluded by the reference interval for A. Each assumed reference interval thus defined one point on the ROC curve for A relative to B, and one point on the ROC curve for A relative to C. Correspondingly, we calculated six complete ROC curves from the three distributions A, B, and C: A vs. non-A (A(B), A(C)), B vs. non-B (B(A), B(C)), and C vs. non-C (C(A), C(B)).

Results: Area-under curve (AUC) for the six ROC curves were as follows: A(B): 0.653; A(C): 0.764; B(A): 0.692; B(C): 0.548; C(A): 0.790; C(B): 0.552. Overlaps of the original distributions A, B, and C were so large that none of the ROC curves exceeded AUC of 0.8. The curve with best AUC (C(A)) had at its most favorable point a sensitivity of 70% for C with specificity of 71% for A.

Conclusions: ROC analyses of data from [1] demonstrate that, even under experimentally controlled conditions, urine oxycodone concentrations could not be used reliably to correctly categorize individual patient results according to known drug administration rates in the range of 80-240 mg/day. In routine practice, urine oxycodone measurements are unlikely to be useful to quantitatively assess oxycodone prescription compliance/diversion in individual patients.

D-108

Application of the Ark-Methotrexate assay to Ortho Clinical Diagnostics® Vitros 5.1 analyzer and comparison with Abbott and LC-MS.

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Introduction: Methotrexate (MTX), widely used for cancer treatment, is known to cause renal toxicity. Therapeutic drug monitoring is important during high dose therapy to determine leucovorin required for rescue, confirm clearance of drug, and discharge the patient. Projected discontinuation of the MTX assay on the Abbot TDx led us to pursue a rapid immunoassay adaptable to another existing autoanalyzer. Currently, there is no application for MTX on Vitros 5.1 analyzer.

Objective: The objective was to develop a user-defined protocol for MTX on the Vitros 5.1, and evaluate performance by comparison with Abbot Immuno Assay and Liquid Chromatography/Mass Spectrometry (LC-MS). We also evaluated the MTX assay using a lower sample volume (4.2 µL) in order to overcome the effects of potential metabolite interference.

Materials and Methods: The Ark MTX assay is a new (November 2011) FDA cleared homogenous enzyme immunoassay for quantifying MTX in serum/plasma. The assay was evaluated on Vitros 5.1 analyzer using an open channel user defined method. It was calibrated using a six point calibration curve (0.05-1.25 µmol/L) and two levels of quality control. Performance of the assay was evaluated for linearity, inter and intra-assay precision, and recovery using two different sample volumes (8.5 µL and 4.2 µL). Ark assay performance was correlated by linear regression with the Abbott TDx and LC-MS using patient specimens (n=65).

Results and Discussion: Linearity of the Ark assay was acceptable between 0.05 and 1.25 µmoles/L with both sample sizes. Within run precision was 8.3% at 0.09 µmoles/L and 4.7% at 0.86 µmoles/L (8.5 µL sample) and 24.7% at 0.06 µmoles/L and 2.34% at 0.77 µmoles/L (4.2 µL sample). Patient correlation showed an approximate 12% positive bias with 8.5 µL sample volume and <1% bias with 4.2 µL sample. At the 4.2 µL sample size the correlation with Abbot and LC-MS was acceptable (R^2 = 0.978, 0.99 respectively). Per manufacturer's procedure, the Ark immuno assay shows cross reactivity of <0.07% with the major metabolite 7-OH-MTX and cross reactivity of 64-100% with the minor metabolite DAMPA (2,4-diamino -methylpteroic acid), which accumulates with renal failure. Abbott's TDX shows 0.6% cross reactivity with 7-OH-MTX and 26% cross reactivity with DAMPA. At the cut-off value of <0.1 µmoles/L for MTX clearance (used at our institute for discharging the patients), 12 patients with low MTX levels would be evaluated as cleared (discharged from hospital) by Abbott TDx vs 10 patients by the Ark 4.2 µL assay. Only 4 of these patients would be identified as cleared by the Ark 8.5 µL assay.

Conclusions: The Ark MTX assay using a 4.2 µL sample volume is comparable with Abbot TDx in terms of measuring clearance of MTX; the Ark assay using an 8.5 µL sample volume is not. The user defined application for Ark MTX assay with sample size reduction was acceptable and might help to overcome the metabolite interferences.

D-109

Khat-induced Mortality Following Khat Use: A Report from the Emergency Department of a Large Teaching Hospital

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Background: Coronary artery disease is the leading cause of death worldwide and most patients die from complications of atherosclerosis. The aim of this study was to determine the association between the levels of biochemical markers and the risk of inducing myocardial infarction as well as severity of MI among khat and khat non-chewers.

Methods: The study included 152 patients with myocardial infarction (MI) admitted to cardiac care unit (CCU) and intensive care unit (ICU) ward of Al-Thawora hospital, the largest cardiac hospital in the Yemen. History of chewing khat, hypertension, diabetes mellitus, hyperlipideamia and cigarette smoking, demographic characteristics were recorded, blood samples were drawn from each patients for cardiac enzymes and blood glucose levels, serum lipid samples were obtained after 12 hours an overnight fast. Statistical Analysis: The data were analyzed by T test, Mann whiteny and chi square test with SPSS version 15.0.

Results: A total of 75% of all admitted patients to the emergency room were khat chewers, 24.3% were no-khat chewers, 80.3% were male, 19.7 were female, 56.0% were smokers and 35.5% were hypertensive, 30.3% were diabetic and 5.3% had hyperlipideamia. S T-elevation (STEMI) with higher troponine, CK, CK.MB than NSTEMI (1.52±1.00 vs. 0.87±0.57, P<0.0001), (1900 (IQR: 1254.24-314.75) vs. 870 (IQR: 539-1901.4), P< 0.0001), (212.8 (IQR: 120.25-338.5) vs. 112.5 (IQR: 73.45-199.75), P<0.0001). Serum TG and HDL-C was higher among STEMI (P<0.003), while TC, LDL-C did not differ between STEMI and NSTEMI. Khat chewers patients were younger than non khat chewers (55.41 ± 12.77 vs. 59.65 ± 13.56 years, P<0.047). Troponin, CK, CK.MB were none significantly higher among khat chewers than non-khat chewers. Serum lipid profile did not differ between khat and non khat chewers group. Serum glucose was significantly higher among khat chewers than non khat chewers (10.83 ± 6.01 vs 8.46 ± 3.24, P<0.02) respectively. Mortality rate

represent 5% and was higher among STEMI than NSTEMI (6% vs. 4%) respectively. Mortality was twice higher among khat chewers as compared to non khat chewers (6.1% vs 2.7%) respectively. Cardiac complication was more frequent among khat chewers (complete heart block (CHB), acute mitral regurgitation (AMR), reinfarction, ventricular fibrillation (VF), heart failure (HF), whereas cardiogenic shock and atrial fibrillation (AF) more frequent among non khat chewers.

Conclusions: In this study khat chewing was more prevalent among patients admitted to the emergency room. The findings of this study show that Khat chewers are more than two folds higher likely to develop cardiac complications than non khat chewers. Therefore, Chewing Khat could be a risk factor for the development of MI with subsequent worsen health outcome and mortality.

D-110

Evaluation of Hexobarbital as an Alternative Internal Standard using an HPLC Method for Quantification of Six Antiepileptic Drugs

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Background: Quantification of antiepileptic drugs (AED) by high performance liquid chromatography (HPLC) often uses 5-ethyl-5-p-tolybarbituric acid as an internal standard (IS) which is no longer commercially available. The factors that we considered when searching for a new IS were chemical structure, pKa, polarity, cost, commercial availability, and not a DEA controlled substance. As a result hexobarbital was selected. The objective of this work was to validate hexobarbital as an IS in an HPLC method for quantification of six AEDs (Table 1). Method: Serum or plasma (100µL) was precipitated with IS solution (250µL; hexobarbital in methanol), vortexed, and centrifuged. Supernatant (15µL) was injected onto a monolithic reverse phase column (3.0X100 mm) maintained at room temperature. Analytical run time was 20.2 min. The analytical measurement range (AMR) was determined by serial dilution of spiked drug free serum extracted and analyzed in triplicate. The acceptance criteria for the AMR was recovery with 100±20% and CV <20% for each level. Precision was determined based on CLSI EP10-A3 guidelines by running the sequence mid-high-low-mid-mid-low-low-high-high-mid twice a day for five days. Patient samples (n=10-40) were analyzed using both IS (5-ethyl-5-p-tolybarbituric acid and hexobarbital) at the same runs. Statistics were performed using EP Evaluator.

Results: AMR ranged from 0.3 to 177.6 µg/mL and analytical recovery ranged from 81.5 to 109.9% for the six AEDs (Table 1). Total CV ranged from 1.5 to 8.6% and intra-assay CV ranged from 1.1 to 3.7% (Table 1). Deming regression of patient results showed R-values (0.9844-0.9998), slopes (0.925-1.013), intercepts (-0.15-0.65), standard error of estimate (0.06-4.35), and mean percent bias (-6.8-1.8). **Conclusions:** Hexobarbital was validated as a suitable replacement of 5-ethyl-5-p-tolybarbituric acid as the IS for quantification of all six AEDs tested.

Table 1. AED AMR, Revcovery, and Precision for 6 AEDs using Hexobarbital as IS

	Felbamate	Lamotrigine	Oxcarbazepine	Zonisamide	Carbepoxide	Pentobarbital
Analytical Measurement Range (µg/mL)	2.5-177.63	0.3-38.1	0.8-49.9	2.6-88.2	0.5-17.6	2.8-109.0
Analytical Recovery (%)	81.5-94.8	85.0-100.3	83.7-92.4	83.7-92.4	85.3-90.7	88.5-109.9
Total CV (%)	1.9-4.1	1.5-4.1	1.8-3.6	2.0-3.4	2.9-8.6	2.1-4.7
Intra-Assay CV (%)	1.2-1.4	1.3-1.7	1.8-3.7	1.1-1.5	1.8-3.2	1.1-1.5

D-112

The Effect of Paraproteins and Rheumatoid Factor on Four Commercial Immunoassays for Vancomycin: Implications for Laboratorians and Other Health Care Professionals

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Objective and Relevance: Paraproteins, immunoglobulins (Ig) which are elevated in various autoimmune disorders, are known to interfere with various laboratory immunoassays, including vancomycin (VANC). Rheumatoid factor (RF), a known immunoassay interferant, may cause falsely elevated results. The aims of this study were to: 1) evaluate the effect of three paraproteins (IgA, IgG, and IgM) on four commercial VANC immunoassays [fluorescence polarization immunoassay (FPIA); enzyme multiplied immunoassay (EMIT); two particle-enhanced turbidimetric inhibition immunoassays (PETINIA)]; 2) determine the concentration at which the effect is obtained, and 3) examine the influence of rheumatoid factor (RF) on the VANC methods.

Methodology: Separate serum and plasma pools from patients prescribed VANC were prepared with levels at approximately 20 mg/L as determined by the Unicel DxC 800 analyzer (Beckman Coulter, Inc., Brea, CA, USA). Drug-free serum was pooled then spiked with a VANC stock solution (50 mg/mL) to obtain a concentration of 20 mg/L. These pools were each mixed 1:1 with individual patient specimens containing IgA (6-63 g/L), IgG (6-54 g/L), IgM (3-30 g/L)(n = 4 for each Ig) and a patient RF pool (196 IU/L). The mixtures (n=39) were split and distributed for VANC analysis at three Alberta Health Services sites. VANC analysis was performed as follows: Calgary Laboratory Services on the Roche cobas c201 (Hitachi High-Technologies Corporation, Tokyo, JP) using an EMIT assay and on the Roche Integra 800 analyzer (Roche Diagnostics, Laval, QC, CA) by FPIA; Red Deer Regional Hospital Centre by the Siemens Vista PETINA assay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA); and University of Alberta Hospital Laboratory using the Beckman Coulter DxC 800 PETINA method.

Results: The presence of IgA and IgG at concentrations of 3.0 to 31.5 g/L and 3.0 to 27.0 g/L respectively, in serum or plasma did not affect any of the VANC immunoassays. RF added to plasma specimens did not interfere, but in serum, elevated vancomycin results were observed. IgM did not affect the FPIA and EMIT methods but did attenuate VANC concentrations by both PETINIA assays (Siemens, Beckman Coulter), with a more pronounced effect on the latter, producing concentrations more than 20% lower than expected in the patient serum and spiked plasma pools. The effect was progressively negative at effective IgM concentrations of 10 and 15 mg/L.

Conclusions: VANC monitoring in patients with elevated RF levels should not be compromised if plasma is used but may be if serum is the specimen of choice. Elevated IgA or IgG levels should not affect VANC analysis by any of the four immunoassays investigated. However in patients with elevated IgM levels, PETINA VANC assays can be affected with the possibility of results below the limit of quantitation but detectable attenuated results as well. This phenomenon is a major analytical and clinical issue which must be communicated to health care professionals caring for patients receiving VANC, so optimal therapy is achieved.

D-113

Comparison of Two Selected Reaction Transitions and Two Internal Standards for the Quantification of Everolimus by a Liquid Chromatography-Tandem Mass Spectrometry Method

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Background: Everolimus is an immunosuppressant drug approved by FDA for kidney transplantation. Liquid chromatography-mass spectrometry (LC-MS) methods offer specific and sensitive results while immunoassays suffer from interference especially from rapamycin. In most LC-tandem mass spectrometry (LC-MS/MS) methods, only one selected reaction transition is monitored with either an analogue or isotope replaced everolimus as internal standard. The objective of this study was to compare two transitions (975.6 → 908.7 and 975.61 → 926.9) and two internal standards (32-desmethoxyrapamycin and everolimus-d4) for the quantification of everolimus in whole blood.

Methods: Whole blood (100 µL) and an internal standard solution (300 µL; desmethoxyrapamycin and everolimus-d4 in acetonitril/0.1 M zinc sulfate solution 70/30) were vortex mixed and centrifuged. 50 µL of the supernatant was purified by online turbulent flow technology and analyzed on a reverse phase column in an LC-MS/MS system. Analytical cycle time was 3.5 minutes per injection.

Results: No ion suppression or interference were observed for both transitions and both internal standards. No carryover was observed with specimens at 104 ng/mL for the primary transition 975.6 → 908.7 while the carryover was observed with the same specimens for the secondary 975.6-926.9 transition. As shown in Table 1 the primary transition showed superior accuracy and precision compared to the secondary transition regardless of the internal standards used as indicated by the linearity (serial dilution of a spiked patient pool) and precision studies (based on CLSI EP10-A3 guidelines). All four combinations compared favorable with a reference laboratory LC-MS/MS method which showed mean difference of -8.2%-2.5% with the primary transition showing the smaller mean differences. Interestingly the two internal standards showed comparable performance across the entire validation process.

Conclusions: The primary transition showed better validation outcome while the two internal standards had comparable performance for the quantification of everolimus in whole blood by the LC-MS/MS method.

Table 1. Everolimus AMR, Revcovery, and Precision for Two Different Transitions and IS

	Everolimus (975.6→908.7) / d4-Everolimus(979.6→912.7)	Everolimus (975.6→908.7) / 32-desmethoxyrapamycin (901.6→834.7)	Everolimus (975.61→926.9) / d4-Everolimus(979.6→912.7)	Everolimus (975.61→926.9) / 32-desmethoxyrapamycin (901.6→834.7)
Analytical Measurable Range (ng/mL)	1.0-54.1	1.0-50.9	1.1-54.3	1.1-51.2
Analytical Recovery (%)	98.3-108.1	100.3-103.6	104.2-119.7	101.3-115.8
Total CV (%)	4.3-7.2	5.1-5.9	7.8-12.8	8.1-12.7
Intra-Assay CV (%)	3.8-5.1	3.5-5.3	7.2-9.0	5.9-9.5

D-115

Development of a monoclonal antibody for the highly specific and sensitive detection of ethyl glucuronide

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Introduction Ethyl glucuronide (EtG) is a direct metabolite formed in the body by glucuronidation of alcohol following alcoholic beverage consumption and is a useful biomarker in forensic toxicology. Even after ingestion of small amounts of alcohol, EtG becomes positive and can be detected through body fluids (serum/blood), tissues and hair up to 80 hours after the completion of alcohol elimination from the body. EtG is used as a biomarker to test alcohol use, particularly within situations where consumption of alcohol is prohibited (e.g. the armed forces, patients recovering from alcohol addictions). Urochloralic acid (metabolite of chloral hydrate) is the "*most probable cause*" of interference with the EtG immunoassay and the production of "false positive" results. Thereby, the availability of rapid and reliable immunoassays specific for the target analyte without interference with urochloralic acid represents a useful analytical approach for application in test settings.

Relevance The aim of this study was to develop a monoclonal antibody specific for ethyl glucuronide and presenting no significant cross-reactivity with urochloralic acid. This antibody can be useful for the development of more specific immunoassays for testing alcohol use.

Methodology Sheep were immunized with ethyl glucuronide hapten conjugated to a carrier protein bovine thyroglobulin (BTG). Lymphocytes were collected and fused with heteromyeloma cells. The resulting hybridomas supernatants were screened for the presence of antibody using competitive ELISA based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by competitive ELISA. Absorbance was read at 450nm.

Results The analytical evaluation of the developed monoclonal antibody showed specificity, expressed as % cross-reactivity, 100% for ethyl glucuronide and 0.7% for urochloralic acid. The half maximal inhibitory concentration (IC50) was 86ng/ml. The intra-assay precision (n=3), expressed as %CV was <10% for different concentration levels.

Conclusion Data indicate very good specificity and sensitivity of the developed monoclonal antibody to ethyl glucuronide with no significant cross-reactivity with urochloralic acid. This antibody can be used in the development of efficient immunoassays for application to tests of alcohol use.

D-116

A simple, no-extraction, liquid chromatography tandem mass spectrometry method for the assay of free phenytoin

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Background: Phenytoin (diphenylhydantoin) is an anticonvulsant drug frequently prescribed for the treatment of generalized tonic-clonic (grand mal) or complex partial seizures, and status epilepticus. Once absorbed, phenytoin readily binds to albumin (90-95%). Concentrations of free phenytoin correlate with pharmacological effects of the drug. Since many conditions such as altered protein concentrations, renal dysfunction and co-administration of drugs that bind to albumin can effect free phenytoin concentration, the measurement of free phenytoin in these conditions is warranted. Abbot Diagnostic's TDx/FLx kit for free-phenytoin quantitation had been widely used to monitor free-phenytoin levels. Since this kit has been discontinued in December 2011, laboratories have been scrambling to develop and validate alternative testing methods. We present a simple liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the assay of free phenytoin.

Materials and Methods: The calibrators were prepared in phosphate buffered saline and the controls were from Biorad. 500 µL of sample (serum or plasma or controls) was filtered through Millipore Protein Filter using centrifugation (1800 g for 10 minutes at room temperature). To 20 µL ultrafiltrate, or calibrators, 100 µL of acetonitrile containing internal standard (phenytoin-D10) was added. The mixtures were vortexed and kept at room temperature for 5 minutes, and centrifuged at 10,000 g for 5 minutes. The supernatants were transferred to autosampler vials. The analysis involved injection of 5 µL supernatants, Restek Ultra Biphenyl 5µm 50x2.1 mm column, Shimadzu UFLC and Applied Biosystems 4000 QTrap MS-MS. Electrospray ionization and multiple reactions monitoring (MRM) mode were used for MS-MS analysis and the following transitions were used for quantification: phenytoin (253.0/182.1 and 253/104.0) and phenytoin-D10 (263.1/192.1).

Results and Conclusions: The method was evaluated for reportable range, accuracy, within and between-run imprecision, limit of quantification and ion suppression. Reportable range (linearity) of the method was from 0.1 to 4.0 µg/mL with recovery of >95%. Within-run and between-run imprecision were <5 and <10% respectively. Lower limit of quantification (inaccuracy of <20% of target value) was 0.1 µg/mL. The method was compared with Abbot Diagnostics TDx/FLx and published liquid-liquid extraction methods. The method compared well with both Methods. the regression equations were y=1.02x-0.05 and y=0.93x+0.17 for for TDx/FLx and the liquid-liquid extraction methods respectively. 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 simple, no-extraction, LC-MS-MS method for the quantification of free phenytoin. The method has clinically acceptable reportable range, accuracy, imprecision and limit of quantification.

D-118

Determination of Nicotine and Related Alkaloids in Urine, Serum, Plasma, and Whole Blood by Supported Liquid Extraction Coupled with LC-MS/MS

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Background: Nicotine is widely recognized as the primary factor in tobacco use dependence. Measurements of nicotine, its metabolites, and the related alkaloid anabasine have been used as biomarkers of tobacco use. However, reports vary on the utility of each of these compounds, suggesting the need for an inclusive method. An LC-MS/MS-based assay could provide simultaneous measurement while ensuring adequate sensitivity and specificity. Extraction of these alkaloid compounds is essential to providing a robust and sensitive method. Supported liquid extraction (SLE) is a unique solid phase extraction (SPE) method combining the automation of SPE with the improved sample cleanup of liquid-liquid extraction. Coupling SLE with LC-MS/MS analysis, we developed a robust method for simultaneous analysis of nicotine, cotinine, nornicotine, norcotinine, 3-hydroxycotinine, and anabasine. This method was validated for use with serum, plasma, whole blood, and urine matrices.

Methodology: Serum, plasma, whole blood, or urine specimens are mixed with isotopic internal standards for all analytes and buffer. Aliquots are then transferred to a Biotage ISOLUTE SLE+ 96 well plate. After elution, the eluate is evaporated and reconstituted for analysis by gradient elution on a HILIC 2.0 mm x 100 mm analytical column. Method validation studies included linearity, limit of quantitation (LOQ), accuracy, and precision. A sample correlation study was completed using patient as well as spiked specimens.

Results: All analytes eluted from the column within 1.2 minutes. No ion suppression/matrix effects were observed. The assay had a lower LOQ of 2.0 ng/mL and an upper limit of linearity of 2,000 ng/mL for all analytes. Total CVs ranged from 2.0% (nicotine) to 6.0% (nornicotine) for serum/plasma/whole blood. Urine total CVs ranged from 1.6% (nicotine) to 5.1% (nornicotine). Accuracy (as % bias) ranged from 0.5% (nornicotine) to 4.8% (3-hydroxycotinine) in serum/plasma/whole blood. Urine accuracy ranged from 1.8% for norcotinine to 4.8% for 3-hydroxycotinine. The correlation study found excellent correlation between the new method and a method used by a reference lab.

Conclusions: This SLE method provided effective cleanup for the analysis of

nicotine, its metabolites, and anabasine in a variety of sample types. The specificity of the LC-MS/MS permitted rapid and sensitive analysis of all analytes simultaneously without compromising precision and accuracy. Overall, this method is a valuable tool for the identification and quantitation of biomarkers of tobacco use.

D-119

Direct Determination of Dabigatran in Human Plasma Using LC-MS/MS

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Background: Dabigatran (Pradaxa®, Pradax™, Prazaxa®) is a promising new oral anticoagulant from the class of univalent direct thrombin inhibitors. It is prescribed as dabigatran etexilate mesylate, which is the prodrug of dabigatran. Once converted into dabigatran and its glucuronides, this drug binds to the active site of both free and clot-bound thrombin. Dabigatran is the first oral anticoagulant since warfarin to be approved by the FDA for prevention of stroke in patients with non-valvular atrial fibrillation. Unlike warfarin, dabigatran does not require frequent blood monitoring as it exhibits predictable pharmacokinetic and pharmacodynamic characteristics. However, monitoring may be useful in certain situations such as during dose titration; during treatment in patients with renal dysfunction, who may need dose adjustment; and when differentiating treatment failure from noncompliance in patients with poor treatment response. Here we report a sensitive and reproducible method to analyze dabigatran in human plasma by LC-MS/MS.

Methodology: Samples were extracted by combining 50 µL of plasma, calibrators, or controls with 200 µL of methanol in a 96-well plate. The resulting precipitate was then agitated for 2 min at 1600 rpm using an orbital shaker, followed by centrifugation at 4000g at 15 degrees C for 30 minutes. For analysis, 10 µL of the supernatant was injected onto a Phenomenex Kinetex C18 column (4.6 mm i.d. x 50 mm length) operated at a flow rate of 1 mL/min, with a mobile phase A of 0.1 % formic acid in water and a mobile phase B of 100% methanol. The HPLC gradient was as follows: 1) 5 min linear ramp from 5 to 25 % mobile phase B, 2) 2 min isocratic elution at 95% mobile phase B, and 3) 2 min isocratic re-equilibration at 5 % mobile phase B. Positive electrospray ionization was employed to measure the ion transition from 471.5 Da to 289.1 Da.

Results: Sample analysis was accomplished in 9 minutes. While the LOQ (0.03 µg/mL with 6% CV) was verified to be comparable to that of an existing assay platform, LC-MS/MS could theoretically achieve a much lower LOQ. The upper limit of linearity was 0.48 µg/mL. A series of patient samples were analyzed with this method and compared to the all-lab mean of results from several testing organizations. The comparison yielded a curve with a slope of 1.008 and a correlation coefficient of 0.99. Evaluation of commercially available calibrators and QC material yielded an average deviation from target of -2.99%.

Conclusions: Dabigatran can be measured by our LC-MS/MS method in 9 minutes using only 50 µL of human plasma. This method offers outstanding precision and accuracy with a linear range of 0.03 µg/mL to 4.8 µg/mL, comparing very well to other established methods. Overall, this technique would be useful for any clinical laboratory performing dabigatran analysis.

D-120

Determination of Zolpidem and Zopiclone in Urine by LC-MS/MS

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Background: Zolpidem (Ambien®) and zopiclone (Lunesta®) are categorized as hypnotic/sedative drugs and are pharmacologically related to benzodiazepines. Zolpidem (ZD) and zolpiclone (ZC) have a rapid onset of action and short half-lifes. Because of their potential for addiction, the DEA has listed both drugs as Schedule IV controlld substances. In addition, ZD and ZC have been used in combination with both prescription and illicit recreational drugs to either enhance the euphoric effects or moderate the disphoric effects of those drugs. Therefore a rapid and sensitive test method is an essential tool for clinical laboratories performing urine drug screening.

Methodology: Urine specimens were mixed with acetonitrile containing ZD-d6 and ZC-d4 (deuterated internal standards). After centrifugation, the supernatants were diluted and injected into a liquid chromatography tandem mass spectrometer (LC-MS/MS). Analyte separation was accomplished by gradient elution on a biphenyl 2.1 mm x 50 mm analytical column. Mobile phases consisted of aqueous 25 mM ammonium formate and 0.1% formic acid with 0.1% formic acid in acetonitrile as the organic phase. Method validation studies included: linearity, limit of quantitation (LOQ), accuracy, and precision. A sample correlation study was completed using patient specimens. Sample stability was evaluated for various storage conditions.

Results: Sample analysis was accomplished within 2 minutes for both analytes. The LOQ was established for each drug at 5.0 ng/mL. The upper limit of linearity was 10,000 ng/mL. Total CVs were 1.4 - 1.8% for ZD at three concentrations (25, 100, and 250 ng/mL). ZC demonstrated CVs of 3.1 - 4.5% at three concentrations (25,100, and 250). The ZD correlation study with a reference lab using LC-MS/MS yielded a curve with a slope of 0.987, an intercept of 59.15 and 0.996 as the correlation coefficient. For ZC, the same correlation study yielded a curve with a slope of 1.260, an intercept of -112.16 and 0.985 as the corelation coefficient. ZD was stable in urine for 7 days at room temperature, 30 days refrigerated, while ZC was only stable 2 days at room temperature, but 30 days refrigerated.

Conclusions: Both ZD and ZC can be measured simultaneously by this method within two minutes. This dilute-and-shoot LC-MS/MS method offers excellent precision and accuracy for both ZD and ZC measurements with a linear range that spans 5.0 - 10,000 ng/mL. Overall this method would be useful for any clinical laboratory performing ZD and ZC analysis.

D-121

Development of the first generic and high sensitivity enzyme-linked immunosorbent assay kit for the determination of synthetic cannabinoids and their metabolites in blood and urine.

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Introduction After the discovery of synthetic cannabinoids substances in “Spice”-like herbal mixtures marketed as ‘incense’ or ‘plant fertilizer’ the active compounds (e.g., JWH-018, JWH-073, JWH-081, JWH-200, JWH-019, AM-2201 and C9-47, 497) have been declared as controlled substances in several countries. The prohibition of these synthetic cannabinoids has let to a rise in new compounds in herbal mixtures that create THC-like psychotropic effects when smoked and have been found to act as the agonists of cannabinoid CB_1 and CB_2 receptors. Several compounds of aminoalkylindole type have been detected so far in herbal mixtures but still their consumption cannot be detected by commonly used drug-screening procedures, encouraging drug users to substitute cannabis with those substances. There is an increasing demand on the part of police authorities, hospitals and toxicology laboratoriess for detection and quantification of synthetic cannabinoids in biological samples. Initial studies of the metabolism of the JWHs have highlighted metabolic processes such us aromatic mono- and dihydroxylation, alkyl residues hydroxylation and carboxylation, N-desalkylation and monohydroxylation.

Relevance This study reports the development of the first generic and sensitive competitive enzyme-linked immunosorbent assay (ELISA) kit for the detection of synthetic cannabinoids “aminoalkylindole type” and their main metabolites in urine and blood with minimal sample preparation, which is of interest for applications in test settings.

Methodology Immunogen comprising JWH-018-hapten conjugated to bovine thyroglobulin (BTG) was administered to adult sheep and target-specific polyclonal antisera for the detection of synthetic cannabinoids and their metabolites were generated. The resulting antibody was used as capture in the development of a competitive ELISA for the determination of synthetic cannabinoids and their metabolites in human urine and blood. After centrifugation for 1 minute, the urine samples are ready for analysis. The blood samples require dilution. For both matrices, 50 µl of sample are required. After incubation of 1 hour at room temperature (+15 to +25^{0}C), to allow the competition reaction between the analytes and the horseradish peroxidase labelled conjugate for the antibody binding sites, the microtitre plate is washed and the enzyme substrate is added. Following incubation of 20 minutes at room temperature (+15 to +25^{0}C), the reaction is stopped and the optical density is measured at 450nm. The signal is inversely proportional to the concentration of the analyte.

Results The analytical evaluation of the developed ELISA showed limit of detection (LOD) values for JWH 018 of 0.7ng/ml in urine and 2.5ng/ml in blood. The specificity, expressed as % cross-reactivity, was as follows: 100% (JWH-018), 215% (JWH-018 6-hydroxyindole), 184% (JWH-018 N-5-hydroxypentyl), 135% (JWH-073), 130% (5-hydroxy JWH-018), 127% (JWH-200), 119% (AM-2201), 106% (JWH-N-(3-hydroxybutane), 96% (JWH-073-N butanol), 85% (JWH-018-N pentanoic acid), 56% (JWH-018 5-hydroxyindole), 37% (JWH-073 N butanoic acid), 35% (JWH-019), 23% (JWH-018 4-hydroxyindole). The intra-assay precison (n=12), expressed as %CV, was ≤ 5 for different concentration levels. Conclusion Data show applicability of this first developed ELISA to the generic and sensitive determination of synthetic cannabinoids “aminoalkylindole type” and their main metabolites in urine and blood with minimal sample preparation. This represents an optimal analytical tool in test

settings for the screening of these compounds.

D-122

Optimization of Enzyme Immunoassays for the Qualitative and Semi-quantitaive Analysis of Methadone and Barbiturates on the Medica EasyRA Chemistry Analyzer

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Background: While medications can be effective when they are used properly some can be addictive and dangerous when abused. Determining what drugs or combination of drugs a person may have taken through medical screening is vital in order for them to receive appropriate treatment. Among the most commonly abused prescription drugs are barbiturates and methadone. Using analytical methods that are suitable and rapid is warranted for those who may be tested for drugs for medical reasons. This study validated the performance of both the Thermo Scientific Methadone assay and the Lin-Zhi International Barbiturate assay on a Medica Corporation EasyRA Chemistry Analyzer in Qualitative and Semi-quantitative modes.

Methods: Methadone and barbiturate assays are homogeneous enzyme immunoassays based on competition between the drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase. Within-run and Total precision were evaluated at the cutoff and ±25%, ±50%, ±75% and ±100% according to CLSI EP5-A2. Analytical recovery (linearity) was determined by spiking the appropriate analyte, methadone or secobarbital into drug-free human urine at levels spanning nearly 50 - 1000ng/ml. Method comparison (accuracy) was determined by analyzing over 125 urine specimens, comprised of samples near and well above and below the cutoff. The results were compared to those acquired by LC/MS. Limits of detection (sensitivity) and comparison to API Survey materials were also evaluated for as well as calibration and on-board stability.

Results:

	Methadone	Barbiturates
Qual. Prec. Range (Within-Run %CV)	0.31-0.54	0.32-0.52
Qual. Prec. Range (Total %CV)	0.47-0.69	0.60-0.85
Semi-Quant. Prec. Range (Within-Run %CV)	0.98-2.75	2.52-5.88
Semi-Quant. Prec. Range (Total %CV)	1.92-5.04	4.95-9.38
Dilution Recovery %	96-107	95-110
Method Comparison % Agreement-Positive % Agreement-Negative	 100 96.3	 95.8 100
Sensitivity	7ng/ml	30ng/ml
Calibration Stability	30 days	7 days
On-board Stability	30 days	30 days

Conclusions: Assays for both Methadone and Barbiturates for the EasyRA Chemistry Analyzer provided excellent precision, accuracy and linearity and are suitable for analyzing urine specimens in both qualitative and semi-quantitative modes.

D-124

Performance Evaluation of Free Phenytoin Assay on Beckman Coulter AU 680 Analyzer.

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Background: Free Phenytoin direct measurement is clinically important in situations when patient's clinical response does not correlate with total drug concentration or toxicity is suspected. This study examines the performance of free phenytoin assay on Beckman Coulter AU 680 Analyzer to replace the current method on Abbott TDx.

Methods: The phenytoin assay evaluated on the Olympus AU 680 is an enzyme-multiplied immunoassay technique (EMIT), which is a homogenous enzyme immunoassay based on glucose-6-phospahte dehydrogenase activity measured spectrophotometrically. We used Roche Free Phenytoin calibrators and controls to achieve the assay sensitivity. Free phenytoin was separated from the bound by preparing ultrafiltrate using a Milipore Centifree ultrafiltration device, centrifuged at 1000g for 20 minutes at 25^{0}C. The performance for EMIT assay was evaluated for precision, linearity, and accuracy against Abbott TDx assay.

Results: Within run precision CVs (n=20), using three levels of Roche controls were 9.9, 6.5 and 4.5% and total precision CVs were 12.4, 11 and 8.4% respectively at 1, 2 and 3 ηg/ml. Assay showed good linearity across the reportable range of 0 to 4ηg/ml with a slope of 1.05 and intercept of 0.086. The limit of detection was verified to be 0.5 ηg/ml. Method comparison results for patient specimens (0.2 to4.0 ηg/ml) to Abbott TDx free phenytoin gave Passing Bablok regression equation: Beckman AU = 1.16[TDx] - 0.16. (Table. 1)

Conclusions: Free phenytoin method on Beckman AU 680 analyzer shows good performance and meets the Clinical need to replace the current Abbot TDX method.

Free Phenytoin Method Comparison		
Passing Bablok Fit		95% CI
Slope	1.16	(1.00 to 1.25)
Intercept	-0.16	(-0.28 to -0.00)
Bias at Decision Levels		
1 ηg/ml	0.00	(-0.15 to 0.22)
2 ηg/ml	0.16	(-0.05 to 0.41)
Difference Plot		
Bias	0.03	(0.06 to 0.13) SE = .047

D-125

Performance Assessment of ARK Diagnostics Immunoassay for Methotrexate on Beckman Coulter DXC Chemistry Analyzer.

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Background: Methotrexate (MTX) is an antineoplastic drug effective against malignancies with rapid cell proliferation such as acute lymphoblastic leukemia and certain carcinomas. It can safely be administered over a wide dose range as chemotherapy and also for the treatment of several nononcologic diseases as rheumatoid arthritis or psoriasis. Moderate to high doses of methotrexate can be favorably administered with leucovorin rescue by closely monitoring MTX serum levels to prevent potentially toxic effects. Accurate measurement of MTX levels at ≤ 0.5 μmol/L, therefore, enables determination of nontoxic status.

Methods: The ARK methotrexate assay is a homogenous enzyme immunoassay based on competition between drug in the specimen and methotrexate labeled with glucose-6-phospahte dehydrogenase activity measured spectrophotometrically. Assay was evaluated on Beckman DXC chemistry analyzer using manufacturer provided 6-point calibrators and three level controls. Performance of assay was determined by assessing precision, linearity, limit of quantitation, accuracy and method comparison to Abbott TDx methotrexate assay.

Results: Within run precision CVs (n=20), for three levels of controls were 6.4, 2.1 and 2.6% and between day precision (20 days) CVs being 10.2, 5.2, 7.5% respectively. Assay showed good linearity across 0.1 to 1.2 μmol/L range with a slope of 0.97 and intercept of 0.009. The limit of blank, limit of detection and limit of quantitation were verified to be 0.00, 0.02 and 0.04 μmol/L. Method comparison results for patient specimens (n=56, 0.02 to 960 μmol/L) to Abbott TDx methotrexate gave Passing Bablok regression equation: Beckman DXC = 1.07[TDx] + 0.04. (Table 1). No clinically significant interference was observed up to 500 mg/dL hemoglobin, 500 mg/dL lipids (20% Intralipid), and 30 mg/dL unconjugated bilirubin.

Conclusions: Overall ARK methotrexate method on Beckman DXC analyzer demonstrates acceptable analytical performance.

Methotrexate Method Comparison		
Passing Bablok Fit (0.00 to 960μmol/L)		95% CI
Slope	1.07	(1.04 to 1.09)
Intercept	-0.16	(0.00 to 0.07)
Bias at Decision Levels		
0.9 μmol/L	0.01	(0.07 to 0.14)
Difference Plot		
Bias	6.43 (9.3%)	(0.60 to 12.25) SE=2.90
Passing Bablok Fit (0.00 to 0.9μmol/L)		
Slope	0.86	(0.72 to 1.05)
Intercept	0.07	(0.03 to 0.12)
Bias at Decision Levels		
0.9 μmol/L	-0.058	(-0.16 to 0.01)
Difference Plot		
Bias	-0.002 (10.6%)	(-0.049 to 0.046) SE = 0.023

D-126

Drugs of Abuse Enzyme Immunoassay Optimization for the Qualitative and Semi-Quantitative Analysis of Cocaine and Amphetamines on the Medica EasyRA Chemistry Analyzer

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Background: Drugs of abuse testing has become increasingly common in many areas including health care, drug rehabilitation, the workplace, and in the criminal justice system. Preliminary screening tests are typically immunological assays such as a homogenous enzyme immunoassay. The gold standard for confirmatory testing is liquid or gas chromatography in combination with mass spectrometry. Both cocaine and amphetamines are commonly abused drugs today. This study describes the optimization and validation of the performance of the Lin-Zhi International Cocaine and Amphetamines assays on a Medica Corporation EasyRA Chemistry Analyzer in Qualitative and Semi-Quantitative modes.

Methods: The Cocaine and Amphetamines assays are homogeneous enzyme immunoassays based on competition between the drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase. Qualitative data was obtained with a 300 ng/ml cutoff for Cocaine and for a 1000 ng/ml cutoff for Amphetamines. Semi Quantitative data was obtained with 5 point calibration curves for both Cocaine and Amphetamines.

Within-run and Total precision were evaluated at the cutoff and ±25%, ±50%, ±75% and ±100% according to NCCLS EP5-A2. Analytical recovery (linearity) was determined by spiking the appropriate analyte, benzoylecgonine (cocaine metabolite) or methamphetamine into drug-free human urine at levels spanning the linear range. Accuracy was determined by analyzing over 120 urine specimens near and well above and below the cutoff. The results were compared to LC/MS data. Limits of detection (sensitivity) and comparison to API Survey materials were also evaluated. Calibration and on-board stability were determined for both reagents.

Results:

	Cocaine	Amphetamines
Qual. Prec. Range (Within-Run %CV)	0.75-1.38 %	0.39-0.57 %
Qual. Prec. Range (Total %CV)	0.93-1.99 %	0.61-0.76 %
Semi-Quant. Prec. Range (Within-Run %CV)	1.4-5.1 %	1.8-2.9 %
Semi-Quant. Prec. Range (Total %CV)	2.1-5.8 %	4.1-6.2 %
Analytical Recovery %	87-104 %	96-109 %
Accuracy (Method Comparison) % Agreement-Positive % Agreement-Negative	96.6 100	94 100
Sensitivity (LOD)	12 ng/ml	18 ng/ml
Calibration Stability	26 days	11 days
On-board Stability	30 days	30 days

Conclusions: Assays for both Cocaine and Amphetamines for the EasyRA Chemistry Analyzer provided excellent precision, accuracy and linearity and are suitable for analyzing urine specimens in both qualitative and semi-quantitative modes.

D-127

An Automated Real-Time Free Phenytoin Assay to Replace the Obsolete Abbott TDx Method.

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Background: Phenytoin has been shown to be useful in control of seizures, however a narrow therapeutic range and potential toxicity make therapeutic drug monitoring of the total and free phenytoin (FP) levels a requirement. Accordingly, when Abbott Laboratories announced they would no longer support FP assays on their TDx fluorescence polarization immunoassay analyzer, we were forced to consider other options including 1) referring the test to a reference laboratory or preferably 2) modifying our total phenytoin assay to measure the free moiety on our Beckman Coulter (BCI) DxC800 chemistry analyzers. Indeed, our chemistry analyzers are interfaced to our Meditech LIS via BCI middleware with specimens delivered on an automated track; results are released by autoverification in real-time on a 24/7 basis thus ensuring rapid and consistent turn around times. The present study describes our in-house developed method and evaluation of FP measurements *via* bidentate turbidimetric inhibition immunoassay on our DxC800s, using in part BCI's total phenytoin reagents.

Methods: Calibration of our FP assay was performed using saline dilutions of BCI total phenytoin calibrators. A linearity study was performed using serial saline dilutions of commercial total phenytoin control material. FP quality control was performed using Liquichek IAC (Bio-Rad) at two levels with peer group data for comparison. All samples were centrifuged using a Centrifree Ultrafiltration Device (Millipore) at 2,250 g and 25°C for fifteen minutes to produce an free drug ultrafiltrate. A real-time correlation study was performed on ninety-seven serum samples comparing results of our in-house method with those obtained on the TDx.

Results: The linearity study was performed at five levels in triplicate ranging from 0.33 to 5.3 µg/mL with a slope of 1.04(1.02 to 1.06; 95% CI) and y-intercept of -0.08 (-0.13 to -0.03), r^2 = 0.99. Analytical sensitivity was determined to be 0.41 µg/mL. Our recovery studies on assayed controls were as follows: Level 1, 92.8%; Level 3, 103%. Our QC levels and precision data obtained with peer comparisons were as follows: Level 1, n= 136, mean = 1.39 µg/mL(peer: n=639, 1.46 µg/mL), SD =+/- 0.083 µg/mL(peer: +/-0.139 µg/mL), CV = 5.9%(peer: 9.5%); Level 3, n=139, mean = 4.66 µg /mL(peer: n=565, 4.61 µg/mL), SD =+/- 0.301 µg/mL(peer: +/-0.401 µg/mL), CV=6.5%(peer: 8.7%). Comparison studies with the TDx yielded a regression equation DxC800 = 0.93*TDx + 0.10; r^2 = 0.97. Regarding interference, we noted a significant positive bias in one patient with acute renal failure with our DxC800 assay and the TDx.

Conclusions: There is excellent correlation between our DxC800 and TDx methods. Other studies including recovery, precision, interference, and analytical sensitivity suggest that our FP method is an acceptable surrogate for the obsolete TDx method.

D-128

Free Phenytoin: To Measure or To Calculate?

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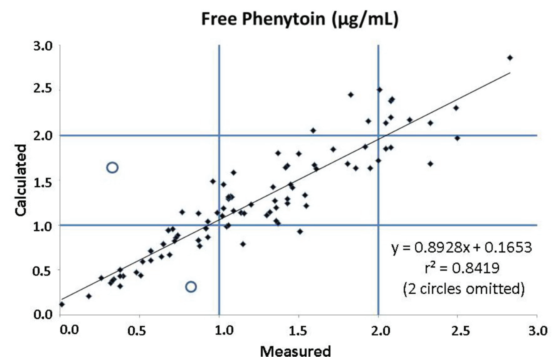
Our objectives were to determine whether free phenytoin levels provide clinically useful information (different from total phenytoin levels) and whether calculated free phenytoin values can reliably replace measured values. On 100 consecutive inpatient samples with total phenytoin levels ordered, we measured free phenytoin, creatinine and albumin. We also determined additional drugs prescribed to each patient. Total phenytoin, creatinine, and albumin were measured using Roche Hitachi MODULAR; free phenytoin, using Roche Integra 800. All assays were performed using the manufacturer's reagents, according to the manufacturer's guidelines. Careful review indicated 2 measured values (circles in graph below) were erroneous and therefore excluded from further analysis. Of the 50 different patients represented in this study, free phenytoin was ordered on 7 (14%). With therapeutic ranges of 10-20 ug/mL (total phenytoin) and 1.0-2.0 ug/mL (free phenytoin), there were 3 samples with high totals (>20), all of which had high free levels (>2.0); 39 samples with therapeutic totals, of which 9 had high and 2 had subtherapeutic free levels; 56 samples with subtherapeutic totals, of which 1 had high and 20 had therapeutic free levels. Overall, 32 of 98 samples (33%) had total phenytoin levels that were potentially misleading. Using an equation developed in a separate study, we compared, on the current samples, free phenytoin calculated from total phenytoin and albumin with measured free phenytoin:



Adding a correction factor for creatinine (19 samples had values >1.9 mg/dL, range of 2.0 to 6.1) led to minimal improvement in concordance. Results using the Sheiner-Tozer equation for calculating free phenytoin gave similar results to those from our own equation. Given that it may not be practical to measure free phenytoin on all samples, we conclude that laboratories should carefully consider offering albumin measurements and calculated free phenytoin levels whenever total phenytoin levels are ordered.

D-129

Screening of drugs of abuse in urine with enzyme immunoassays requiring low reagent and sample volumes on the RX daytona plus analyser

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Background The abuse of legal and illicit substances is escalating worldwide, affecting all layers of society. Drug abuse leads to higher absenteeism, lower productivity and increased accidents in the workplace. In order to control this problem, high quality, accurate drug screening methods are required. This study reports the performance evaluation of nine homogeneous Enzyme Immunoassay (EIA) kits for both qualitative and semi-quantitative analysis of barbiturates benzodiazepines, canabinoids, cocaine metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), methadone, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA) and opiates, applied to the fully automated RX daytona plus analyser. This is of value as a convenient screening tool in test settings.

Methods The principle of the assays is based on competition between drug-labelled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody on the reagent. When free drug is present in the sample, the antibody would bind to free drug; the unbound drug-labelled G6PDH then exhibits its maximal enzyme activity. Conversion of NAD to NADH is measured at 340nm, the absorbance of which is directly proportional to the amount of drug in the sample.

The RX daytona plus uses a maximum sample volume of 7µl (neat urine) for each assay and generates the first result after 14 minutes. Reagent volumes of 73µl and 27µl for the R1 and R2 respectively are required per test. Total precision was assessed by testing 8 samples at concentrations based around the appropriate drug cut-off. These samples were tested for precision in qualitative and semi-quantitative modes. Each sample was assayed two times per run, 2 runs per day, for 20 days. Agreement with GC/MS was assessed by testing a minimum of 80 urine patient samples on the RX daytona plus and by GC/MS. Overall % agreement was calculated. Accuracy by recovery was determined by assessment of replicates of serial dilutions of a drug spiked urine sample with an upper limit that was comparable to the appropriate semi-quantitative top calibrator concentration.

Results Evaluation of the performance parameters, qualitatively and semi-quantitatively, showed that throughout the total precision studies for all 9 assays, all negative and positive samples tested across the 20 days were classified correctly. The method comparison versus GC/MS showed ≥80% agreement for the nine assays. After linear regression analysis, the accuracy by recovery studies for all nine assays gave slopes within 0.90 - 1.10 and r values of ≥0.95.

Conclusion Data shows optimal performance of the EIAs for both qualitative and semi-quantitative determination of drugs of abuse in urine samples on the RX daytona plus analyser. A maximum of 7ul of neat sample per test is required for each assay with a throughput of 270 tests per hour. The low minimum reagent volume needed makes the screening of drugs of abuse on this system more cost effective for the laboratories.

D-130

Validation of a Quantitation Method for Nicotine and Cotinine in Serum by Liquid Chromatography and Tandem Mass Spectrometry

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Background: Tobacco use has long been recognized as one of the most significant public health issues in the US. Increasing numbers of health insurance companies are requiring screening for tobacco use as one of their enrollment requirements. In addition, tobacco use has been shown to delay healing time and increase infection and thrombosis rates after surgical procedures, especially plastic surgery, resulting in unsatisfactory results. This study aimed to validate an accurate and robust liquid chromatography and tandem mass spectrometry (LCMS/MS) method to quantitate nicotine and its major metabolite, cotinine, in serum.

Methods: Deuterated stable isotopes, nicotine-d_4 and cotinine-d_3 were added to all samples, calibrators, and quality control materials as internal standards. All samples underwent solid phase extraction (SPE). The resulting eluents were dried under nitrogen, reconstituted in a 75% acetonitrile solution, and injected onto an Agilent 1100 high-performance liquid chromatography (HPLC) connected to an AB Sciex API 3000 tandem mass spectrometer (MS/MS) for analysis.

Results: The intra-assay and inter-assay precisions were 6.77% and 7.02% for nicotine and 4.76% and 8.37% for cotinine. The assay was linear from 1 ng/mL to 600 ng/mL for nicotine and 1.0 ng/mL to 800 ng/mL for cotinine. The limit of detection (LOD) was 1.0 ng/mL for both analytes. The limits of quantitation (LOQ) were 2.0 ng/mL and 1.0 ng/mL for nicotine and cotinine, respectively. The average recoveries of 98.5% for nicotine and 103.1% for cotinine were observed from three different concentration levels. Method comparison to a similar method from reference laboratory showed regression equations: y=1.0061x-0.3255 (R^2 = 0.9244, n=20) for nicotine and y=1.0524x-4.5045 (R^2 = 0.9379, n =18) for cotinine. No carryover was observed after injecting a sample containing 1000 ng/mL of nicotine and cotinine each. No significant signal suppression effects were seen in the areas of nicotine and cotinine during a flow infusion experiment. The validation data confirmed that the linearity ranges of this method were suited for nicotine and cotinine levels reported in the serum of tobacco users.

Conclusions: The method evaluated is accurate and robust, and correlates well with the results from the reference laboratory. Thus, this method meets the needs of providers in patient care and can be performed as a routine laboratory assay.

D-131

Evaluation of the new Beckman Coulter AU5800® Clinical Chemistry System with Emit*, Emit 2000, and Emit tox therapeutic drug monitoring (TDM) assays.

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Background: The AU5800 is a series of ultra-high throughput clinical chemistry systems with up to four connected units completing up to 8000 photometric tests/hour plus electrolytes and an on-board capacity of 432 reagent positions plus a dual ISE module for sodium, potassium, and chloride.

Objective: The purpose of this study was to evaluate the performance of therapeutic drug assays on the new Beckman Coulter AU5800 Clinical Chemistry System using spiked patient samples from random blood donors in Texas.

Methods: Methods evaluated were 18 Siemens Emit, Emit 2000, and Emit tox therapeutic drug monitoring assays. The AU5800 was compared to the AU5400 according to CLSI guideline EP15-A2 for comparison of patient sample results.

Results: Representative results from 12 of the 18 comparison studies between the AU5800 and the AU5400 are presented in the table.

Conclusions: Based on these studies, we conclude that the AU5800 can effectively perform a broad menu of drug tests, including TDMs. The AU5800 will offer laboratories the opportunity to consolidate tests as a primary chemistry analyzer or as a dedicated specialty analyzer for TDM assays.

* All trademarks are property of their respective owners.

Representative results from comparison studies between the AU5800 and the AU5400

Analyte	Units	N	Slope	Intercept	R	Bias	Range
Acetaminophen	µg/mL	111	1.031	2.51	0.9935	4.84	8-203
Caffeine	µg/mL	140	1.000	-0.77	0.9924	-0.77	1.2-30.5
Carbamazepine	µg/mL	190	1.072	-0.104	0.9787	0.80	2.0-19.9
Digoxin	ng/mL	94	0.978	0.03	0.9922	-0.05	0.28-5.04
Disopyramide	µg/mL	190	1.002	-0.02	0.9973	-0.02	0.45-8.30
Gentamicin	µg/mL	110	1.068	-0.09	0.9942	0.15	0.55-8.81
Lidocaine	µg/mL	191	1.003	0.01	0.9938	0.03	1.0-12.1
Methotrexate	µmol/L	130	0.954	0.05	0.9891	-0.01	0.17-2.42
Procainamide	µg/mL	151	1.021	0.13	0.9908	0.25	0.9-11.8
Salicylic Acid	mg/dL	96	1.082	-1.41	0.9948	1.83	5.3-76.3
Tobramycin	µg/mL	105	1.049	-0.03	0.9960	0.21	0.6-10.1
Vancomycin	µg/mL	183	1.005	-1.60	0.9917	1.38	2.0-49.7

D-132

5-FU Assay Applications for the Thermo Fisher CDx 90® and Roche cobas® c6000

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Background: The pharmacokinetic variability of 5-FU and the relationship between plasma concentration and pharmacodynamic effect have been reported in a number of studies. For many patients dosing based on Body Surface area (BSA) fails to achieve the target therapeutic range for steady state plasma concentrations. Dose management

of 5-FU based on 5-FU blood concentrations could benefit patients when blood levels are sub-therapeutic or toxic. Recently, an immunoassay has been made available to measure plasma 5-FU (The Saladax My5-FU™ Assay). As testing on readily available, routine clinical chemistry analyzers is fully automated and less expensive than physical methods, the immunoassay may provide a more convenient and cost-effective approach to 5-FU dose management.

Objectives: 1. Development of parameters for two clinical chemistry analyzers to measure 5-FU with the My5-FU assay. 2. Validation of the analytical performance of the assay on these instruments and comparison to product specifications.

Methods: CLSI protocols were used to evaluate linearity and precision. Analyzers used were the Thermo Fisher CDx 90 and the Roche c6000 with c501 clinical chemistry module. Accuracy was evaluated with a method comparison of each instrument compared to the Beckman AU400®.

Results: Within run (N=40) coefficients of variation (CV) with low and medium controls and low and high patient pools on the CDx 90 were 2.5%, 1.5%, 2.2%, and 1.1%, and on the c6000 2.7%, 1.8%, 3.5%, and 1.4% respectively. Recovery of 5-FU was within 5% of the theoretical for all samples across platforms. Within-laboratory (intra-assay, total) precision coefficients of variation for low, medium, and high controls on the CDx 90 were 3.5%, 1.8%, and 1.2%; on the c6000 4.4%, 2.9%, and 1.5%. Recovery of 5-FU was within 5% of the expected value on both analyzers. The assay was linear over the reportable range of the assay as evaluated with 11 concentrations from 100 - 1750 ng/mL. Recovery deviated <5% from the theoretical at all concentrations. With patient samples (N=50) there was good agreement between the CDx 90 or the c6000 and the AU400. For the CDx vs. AU400, the slope was 0.982, coefficient of concordance (R) was 0.9985. The c6000 results were also comparable to the AU400: slope = 0.998, R=0.9971.

Conclusions: Based on the performance demonstrated on the CDx90 and c6000, these analyzers could be used for clinical testing of 5-FU plasma levels in routine clinical laboratories on existing instrument platforms.

D-133

Drug Monitoring and Toxicology: A Simple Procedure for the Monitoring of Lacosamide by HPLC-UV Detection

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Background: In a large double-blind, randomized clinical trial of people with poorly controlled partial-onset seizures, lacosamide was found to significantly reduce seizure frequency when given in addition to other antiepileptics. In another trial of people with diabetic neuropathy, lacosamide also provided significantly better pain relief when compared to placebo. Lacosamide was approved in the US on October 29, 2008.

Therapeutic drug monitoring of 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 lacosamide 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 lacosamide 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.

The need for a quick measurement of lacosamide in plasma samples in a simplified manner and the need for a cost-effective procedure prompted the development of a simple HPLC method. Here, a simple and reliable HPLC method is described for determination of lacosamide concentrations in a small volume (100μL) of plasma that is suitable in pediatric practice.

Methods: Sample (100μL) was vortex-mixed with methanol and the internal standard trimethadione for 1 minute and centrifuged at 10,350 g for 10 minutes at room temperature. The supernatant was transferred to an autosampler vial, 10μL of supernatant was injected directly onto the HPLC system. Separations of lacosamide and trimethadione were achieved by using a 5-μm Microsorb-MV reversed-phase C18 column (250 x 4.6 mm) and a mobile phase consisting of methanol (14%) and acetonitrile (17%). The flow rate of HPLC run was at 1.0 mL/min and column temperature at 50°C. Peaks of lacosamide and trimethadione were monitored at 199 nm.

Results: The method achieved a linear concentration range of 1-40 mg/L, which covered the proposed range of 5-10 mg/L for reference. The limit of detection was 0.2 mg/L. Both within-run and between-run precision were lower than 6%. Recoveries ranged between 95% and 103% for spiked and pooled samples. No interferences with other common antiepileptic drugs were observed. The method was compared to a reference laboratory HPLC assay using 30 samples ranging from 1 to 31 mg/L. The correlation showed a slope of 1.05, an intercept of 0.28 mg/L and an r of 0.98.

Conclusions: This method is simple and easy to perform with excellent reproducibility, requires no solid-phase extraction and one step deproteinization prior to chromatography. No interferences with other common antiepileptic drugs were observed. It is suitable for routine analysis of lacosamide in plasma samples.

D-134

Batched extraction for Tacrolimus on the ARCHITECH improves precision and turn-around-times

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Background: The analytical performance of the Abbott ARCHITECH Tacrolimus assay using manual extraction of individual samples was evaluated and compared to the Abbott ARCHITECH Tacrolimus assay using batched off-line extraction. The performance of on-line extraction methods used to measure immunosuppressants compromise precision and sensitivity. However, manual extraction of individual samples is a challenge for achieving turn-around-times when measuring >400 samples daily and often results in hand-fatigue.

Methods: The ARCHITECT assay requires individual whole blood specimens to be pretreated 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. We modified the pretreatment and extraction step with a centrifuge and rack system that could accommodate batched extraction of 20 or 40 samples. Total imprecision was determined by analyzing whole blood (EDTA-anticoagulated) patient pools in duplicate for a total of 5 days. Timing of sample extraction was determined by analyzing whole blood patient samples ranging from 0.0 to 22.9 ng/mL. Functional sensitivity was determined by analyzing seven whole blood patient pools (duplicate analysis, 5 days) ranging in tacrolimus concentrations from 0.2 to 4.0 ng/mL Over one hundred whole blood samples from patients on tacrolimus therapy that had received a kidney allograph transplant 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 ARCHITECH-single extraction was 3.4% (mean = 15.0 ng/mL) and 5.3% (mean = 4.2 ng/mL) for patient pools. Total imprecision for the ARCHITECH-batched extraction was 3.3% and 4.2% for patient pools, respectively. The functional sensitivity (20% CV) as measured at the upper 95% confidence interval was the same for single and batched extraction at 0.5 ng/mL. Patient comparison studies (n = 117) revealed the following relationship: ARCHITECH-batched extraction = 0.992 (single extraction) + 0.13 (r = 0.99, average bias = 0.06). Timed analysis of single compared to batched extraction at 15.0 ng/mL was 888 sec (single extraction) versus 765 sec (20-batched extraction) and 1600 sec (single extraction) versus 1400 (40-batch extraction). This resulted in a 1 min 55 sec (batch of 20) and 3 min and 20 sec (batch of 40) time savings of approximately 10% overall.

Conclusions: The 20 or 40 batched extraction of samples on the ARCHITECT has similar functional sensitivity, correlation and bias but reduced imprecision at low concentrations for Tacrolimus measurements compared to the current single extraction of samples. In addition, batched analysis can resulted in a small time savings and less hand fatigue. The improved precision at low tacrolimus concentrations may improve patient care.

D-135

The evaluation of an application protocol using atomic absorption spectrophotometry for determination of zinc concentration in plasma.

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Background: Zinc (Zn) is an essential trace element for human beings and it participates in several enzymatic processes as cofactor. It is important for DNA synthesis, immune and neurosensory functions and antioxidant activities. The quantification of trace elements in biological samples is important to evaluate the nutritional standpoint, but also for diagnosing and monitoring the environmental or occupational exposure. The aim of this study was to evaluate an application protocol using a flame atomic absorption spectrophotometer for determination of zinc

concentration.

Methods: The analysis was performed on PerkinElmer AAnalyst 100 flame atomic absorption spectrophotometer (PerkinElmer Inc.,USA). The sample is subjected to a high-energy thermal environment in order to produce excited-state atoms. This environment can be provide by a flame or, more recently, a plasma. However, since the excited state is unstable, the atoms spontaneously return to the ground state and emit light. The emission spectrum of an element consists of a collection of emission wavelengths called emission lines because of the discrete nature of the emitted wavelengths. The intensity at an emission line will increase as the number of excited atoms of the element increases.

The assay has been optimized to measure the Zn concentration from 8.0 to 300.0 µg/dL. Two control samples (low and high levels) were evaluated. The detection limit was defined running 20 times for each diluted sample from an original standard sample with Zn concentration of 1.00 µg/dL. The recovery test was assessed adding control sample containing Zn in plasma samples resulting two different final concentrations of 20.0 µg/dL and 97.0 µg/dL. The carryover analysis was evaluated with samples of 9.0 and 320.0 µg/dL, intercalated and assaying into a total of 21 determinations.

Results: The assay was found to be linear up to 435.0 µg/dL. The detection limit was 0.01 µg/dL. The functional sensitivity was 7.5 ± 0.28 µg/dL. The coefficient of variation (CV) of within-run precision ranged from 2.6 % (SD=1.28 µg/dL) to control sample with a concentration of 48.5 µg/dL of Zn and 0.8% (SD=2.71 µg/dL) for concentration of 320.0 µg/dL. The CV of between-day precision ranged from 3.6±1.3% to control sample with 48.2 µg/dL and 4.7% for concentration of 320.0 µg/dL. The recovery test ranged from 99.3% to concentration of 20.0 µg/dL and 97.0% to 97.0 µg/dL. The limit carryover was less than acceptable level of 1.6%.

Conclusions: The flame atomic absorption spectrometry assay is a simple fast, and convenient alternative to evaluate Zn in blood sample, to be useful in the diagnosis of Zn deficiency , especially for patients with concurrent chronic disease, where routine tests of Zn status are compromised including growth retardation and depression of the immune system.

D-136

Application of ultra high-performance liquid chromatography tandem mass spectrometry for the analysis of felbamate, lacosamide and metabolite des-lacosamide and rufinamide in serum and plasma.

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Background: Anticonvulsant drugs are commonly prescribed in the United States and are monitored, to assess patient compliance and toxicity, by liquid and gas chromatographic methods. Runtimes, interferences, and sample preparation for liquid chromatographic methods have been condensed using ultra high-pressure liquid chromatography-MS/MS. A rapid and simple procedure for monitoring felbamate, lacosamide, des-lacosamide and rufinamide was developed using a Waters Acquity system.

Methodology: Concentrations in serum and plasma were determined by ultra high performance liquid chromatography with tandem mass spectrometry detection (Waters Acquity UPLC TQD, [UPLC-MS/MS]). The instrument was operated with an ESI interface, in multiple reaction monitoring (MRM), and positive ion mode. The resolution of both quadrupoles were maintained at unit mass resolution with a peak width of 0.7 amu at half height. Data analysis was performed using the Water Quanlynx software.

Results: Serum samples were thawed at room temperature and a 10-µL aliquot was placed in a tube. Seven hundred microliters of precipitating reagent (acetonitrile-methanol [50:50, volume:volume]) containing the internal standard (0.1 mg/L MDMA_D5) was added to each tube. The samples were then vortexed and centrifuged. The supernatant was transferred to an autosampler vial and 2 µL was injected into the UPLC-MS/MS. Analytes were separated on a Waters Acquity UPLC HSS T3 1.8 um, 2.1 x 50mm column at 25ºC, using a timed and linear gradient of acetonitrile and water, each having 0.1% formic acid. The column was eluted onto a Waters Acquity TQD, operating in a positive mode to detect MDMA_D5 at transition 199.20>165.14, felbamate at 239.10>117.05, lacosamide at 251.20>91.11, des-lacosamide at 237.17>108.08, and rufinamide at 239.10>127.0. Secondary transitions are also monitored for MDMA_D5 at transition 199.20>139.28, felbamate at 239.10>178.18, lacosamide at 251.20>108.08, and des-lacosamide at 237.17>91.11. The run-time was 1.8 minutes per injection with baseline resolved chromatographic separation. The analytical measurement range was 0.5 to 40.0 µg/L for lacosamide and metabolite des-lacosamide and rufinamide. The analytical measurement range for felbamate was 5.0 to 400.0 µg/L. Intra-assay imprecision (%CV) was less than 10% and inter-assay %CV was less than 14% at three different concentrations.

Conclusions: The Waters Acquity UPLC TQD assay demonstrated acceptable assay performance for monitoring the antiepileptic drugs felbamate, lacosamide, and rufinamide in serum and plasma, with a runtime of 2 minutes.

D-137

A Sensitive Method to Screen for Lorazepam in Urine Samples by Utilizing an Online Enzymatic Hydrolysis ELISA Assay

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Objective: To determine if the addition of an on line, automated deconjugation step can reduce the number of false negative results for lorazepam in a screening enzyme linked immunosorbant assay (ELISA) (Neogen Corporation, KY) on the DS2 system (Dynex Corporation, Chantilly, VA) for benzodiazepines.

Introduction/Clinical Relevance: Lorazepam is a high-potency, short-to-intermediate-acting benzodiazepine. Due to the nature of benzodiazepines, many people have become addicted to this class of drug, a major health issue in the USA. Furthermore, the reports of lorazepam use and abuse may be greatly underestimated due to lorezepam's lack of cross reactivity in conventional screening immunoassays for benzodiazepines. The sensitivity of immunoassays to lorazepam is frequently 10-50 times less than oxazepam and other benzodiazepines. In urine, lorazepam is excreted primarily as a glucoronide (85%). This further impairs lorezepam's ability to interact with the anti-benzodiazepine antibody and leads to a greater false negative response. Some laboratories have now adopted a separate screening method for lorazepam.

Methods: Certified negative urine was obtained. Half was fortified with lorazepam glucoronide at 100, 200, 400, 500, 800 and 1000 ng/mL and the other half likewise with lorazepam. Samples were screened for benzodiazepines by the Neogen ELISA with a cutoff of 100ng/mL (oxazepam) using the conventional screening method (method 1) and then analyzed with the additional online step of an incubation with 2000 Fishman units/mL e-coli beta glucoronidase (Sigma-Aldrich, St. Louis, MO) for 2 hours at 37ºC (method 2). Ten forensic samples that had previously screened negative by the Neogen ELISA or EMIT® but confirmed positive for lorazepam by GC-MS were analyzed by using both Neogen ELISA methods.

Results: The results show that by the addition of beta glucoronidase, an increase in the assay sensitivity for lorazepam (Method 2) was gained. When using method 1 (cutoff oxazepam 100ng/mL), all lorazepam glucoronide samples were negative and lorazepam itself showed positive at 200ng/mL. When analyzing both sets of samples with method 2, all samples at 200ng/mL and above were reported as presumptive positive.

The ten forensic samples that had previously screened negative by EMIT® or Neogen ELISA for benzodiazepines were tested using method 2. With the additional step of the beta-glucoronidase nine out of the ten samples screened positive. This is a 90% increase in positivity rate for lorazepam by adding this automated step.

Conclusions: By the simple addition of the β-glucoronidase (method 2), the sensitivity to detect lorazepam increases as it liberates the lorazepam and allows it to cross-reacts with the antibody. All reagents are added by the instrumentation; hence no extra steps by the analyst are needed, making it a more acceptable practice in routine toxicology testing. Even with the supplementary step, the sensitivity for this drug is still less than other bendoiazepines and care should be taken when interpreting benzodiazepine-screening results.

D-138

High-Throughput Analysis of Immunosuppressant Drugs in Whole Blood Using Ultra-fast SPE/MS/MS

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Background: In many clinical research laboratories, liquid chromatography-mass spectrometry (LC/MS) methods of analysis of immunosuppressant drugs have proven superior because of their increased sensitivity and selectivity. We evaluated the ability of an ultra-fast SPE/MS/MS system to analyze tacrolimus, everolimus, sirolimus, and cyclosporin A in whole blood with much faster sample cycle times and similar analytical results compared to LC/MS/MS assays.

Methods: MS methods for tacrolimus, everolimus, sirolimus, and cyclosporin A and their corresponding internal standards were optimized for analysis by QQQ MS. Calibration standards for each analyte were prepared in bovine whole blood. The whole blood samples were mixed with water and precipitated using a zinc sulfate and methanol solution containing the internal standards. Precipitated samples were gently mixed and then centrifuged. Following centrifugation, supernatants were transferred

to a 96-well plate for analysis. Samples were analyzed using a high-throughput mass spectrometry system coupled to a QQQ mass spectrometer. A phenyl column was used for online SPE. Data analysis was performed using RapidFire Integrator software.

Results: Prepared calibration standards were run in triplicate over a series of days to establish both intra- and inter-day precision and accuracy. Tacrolimus, for example, had both intra- and inter-day accuracies within 15% and coefficient of variation values less than 10% for all concentrations within the linear range (2-50 ng/ml). The method for all four analytes had excellent linearity within their respective measured ranges with an R^2 value greater than 0.995. Blank whole blood was treated and analyzed in the absence of internal standard in the same manner as the other samples to measure signal to noise which was found to be greater than 20 to 1. These analytical results are comparable to those using LC/MS/MS, however the analysis time for SPE/MS/MS was approximately 10 times faster. Blinded human samples will be analyzed to further evaluate this method.

Conclusions: Based on these results tacrolimus, everolimus, sirolimus, and cyclosporin A can be accurately and precisely measured in whole blood using ultra-fast SPE/MS/MS at rates of less than 15 seconds per sample. While the analytical results were comparable to LC/MS/MS, the analysis time was approximately 10 times faster. This methodology is capable of throughputs >240 samples per hour.

D-139

A High Sensitivity Benzodiazepine Screening Assay is Inadequately Sensitive for Compliance Monitoring in Patients Treated for Chronic Pain

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Background: 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. Benzodiazepines are commonly prescribed as well as abused; in our pain management population approximately 27% of patients screen positive for benzodiazepines by immunoassay. However, immunoassay-based testing lacks the requisite sensitivity for detecting benzodiazepine use in this population primarily due to their poor cross-reactivity with conjugated metabolites and newer benzodiazepine therapeutics. In our laboratory we observe high rates of false negative benzodiazepine screening results with both the Kinetic Interaction of Microparticles in Solution (KIMS) and Cloned Enzyme Donor Immunoassay (CEDIA) methods. A High Sensitivity CEDIA (HS-CEDIA) assay, which employs a beta-glucuronidase sample pretreatment to covert conjugated benzodiazepines metabolites to free drug prior to analysis, has been shown to perform better than traditional assays, but its performance in patients treated for chronic pain is not well characterized.

Objectives: To determine the sensitivity and specificity of the HS-CEDIA assay, relative to liquid chromatography-tandem mass spectrometry (LC-MS/MS) and traditional screening assays, for detecting benzodiazepine use in patients treated for chronic pain.

Methods: A total of 299 urine specimens from patients treated for chronic pain were screened for the presence of benzodiazepines using the KIMS assay on a Cobas e601 analyzer (Roche Diagnostics, Indianapolis, IN) (positive/negative cutoff = 100 ng/mL) and the CEDIA and HS-CEDIA assays (Thermo Fischer Scientific, Inc., Fremont CA) on the Olympus AU480 analyzer (Beckman Coulter, Inc., Brea, CA) (positive/negative cutoff = 200 ng/mL). All samples were also tested by LC-MS/MS for the presence of free and conjugated forms of 7-aminoclonazepam, alpha-hydroxyalprazolam, lorazepam, nordiazepam, oxazepam and temazepam, using beta-glucuronidase pretreatment, with a reporting concentration limit of 50 ng/mL for all compounds. The sensitivity and specificity of the screening assays for these individual benzodiazepines were determined using the LC-MS/MS derived results as the reference positive/negative determinant.

Results: Of the 299 urine specimens tested, 137 (46%) confirmed positive for one or more of the benzodiazepines/benzodiazepine metabolites quantified by LC-MS/MS. The CEDIA and KIMS sensitivities were 56% (77/137) and 47% (64/137), respectively. The HS-CEDIA assay demonstrated higher sensitivity (79%; 108/137) due to the enzymatic sample pretreatment, which increased detection of lorazepam and clonazepam use. Despite this relatively higher sensitivity, the HS-CEDIA screening assay still missed 29/137 true benzodiazepine-positive urine specimens. The KIMS, CEDIA and HS-CEDIA assays were all >98% specific with 2, 1 and 2 false positive screening results identified, respectively.

Conclusions: While the HS-CEDIA assay has better sensitivity than the KIMS and CEDIA assays, it still missed 21% of positive benzodiazepine samples and therefore is unsuitable for urine benzodiazepine screening of pain management patients. LC-MS/MS quantification with enzymatic sample pretreatment offers superior sensitivity and specificity for monitoring benzodiazepines in patients treated for chronic pain. As a result of these findings, our laboratory now bypasses immunoassay screens and uses LC-MS/MS as the front-line test for detecting benzodiazepine use in patients treated for chronic pain.

D-141

Ultra-fast Analysis of Drugs of Abuse in Urine Using SPE/MS/MS

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Background: Forensic drug testing has traditionally utilized GC/MS and more recently LC/MS as the analytical method of detection. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional technologies. In the present study, we evaluated the ability of an ultra-fast SPE/MS/MS system to screen for some of the drugs of abuse including: opiates, benzodiazepines, benzoylecgonine (the major metabolite of cocaine), and 11-nor-9-delta-9-tetrahydrocannabinol (THCCOOH, the major metabolite of marijuana), in urine with much faster sample cycle times (under 15 seconds per sample) and similar analytical results compared to GC/MS or LC/MS assays.

Methods: Mass spectrometry and SPE methods were optimized separately for panels of opiates and benzodiazepines as well as for benzoylecgonine, THCCOOH, and their respective isotopically labelled internal standards on a high-throughput mass spectrometry system. Drug-free urine was spiked with each drug analyte, hydrolysed in the case of THCCOOH, diluted or prepped via SPE, and injected for analysis. Sample analysis cycle times for all SPE/MS/MS methods were under 15 seconds per sample. Data analysis was performed using RapidFire Integrator software.

Results: Feasibility was assessed using prepared calibration standards that were run in triplicate over a series of days to establish both intra- and inter-day precision and accuracy. For example: benzoylecgonine had excellent linearity within its measured range of 31-4000 ng/ml with an R^2 value greater than 0.995. Intraday accuracies were within 15% and intraday coefficient of variation values were all less than 5% for concentrations within the measured range. THCCOOH also had excellent linearity within its measured range of 10-5000 ng/ml with an R^2 value greater than 0.995. Intra- and interday accuracies were within 10% and intra- and interday coefficients of variation within 5%. Carryover was assessed and found to be <1% of the lowest point of the standard curve. Drug-free urine was treated and analyzed in the absence of internal standard in the same manner as the other samples to measure the signal to noise ratio which was greater than 15 to 1. While this non-separative technology was unable to distinguish isobaric compounds like those in the opiate panel, accurate and efficient screening results could be obtained using SPE/MS/MS to triage samples prior to LC/MS/MS confirmation analysis for this panel of analytes.

Blinded human samples were evaluated to further verify the SPE/MS/MS methods and the results were compared to LC/MS/MS data. Strong correlations were seen between the two analytical technologies. For example, the correlation for blinded human samples containing a variety of THCCOOH concentrations showed an R^2 value of 0.966 with the following equation: $y = 1.0755x + 0.6618$.

Conclusions: Based on these results, many drugs of abuse including opiates, benzodiazepines, benzoylecgonine and THCCOOH can be accurately and precisely screened in urine using ultra-fast SPE/MS/MS at rates of <15 seconds per sample. This methodology is capable of throughputs >240 samples per hour.

D-142

Case of a False Positive MDMA and Methamphetamine

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Objectives: We describe a case where a 34 year old female was brought to the emergency department in a combative and altered state after being ill for the evening. An NCS POCT urine drug screen was falsely positive for MDMA and LC-MS/MS identified methamphetamine, but the amphetamine metabolite was absent. GC-MS analysis identified the interfering compound as phenpromethamine (N-methyl-2-phenylpropan-1-amine), a positional isomer of methamphetamine and a stimulant that can be found in various diet aid supplements. We show how phenpromethamine can produce a false MDMA and methamphetamine result.

Methods: Pure standards of amphetamine, methamphetamine and phenpromethamine were obtained from TRC chemicals (Toronto). The fragmentation patters and retention

times for each chemical was determined and compared using both HPLC-MS/MS (ABSciex) as well as GC-MS (Thermo).

Results: GC-MS analysis demonstrated that methamphetamine and phenpromethamine share the same TMS derivatized ions (m/z 221) as well as some fragment ions (m/z 206, 91). However, there are differences in retention times (3.73 and 3.85 min, methamphetamine and phenpromethamine, respectively) as well as fragment ions (130 for methamphetamine and 116 for phenpromethamine) so that these chemicals can be differentiated. Differences in fragmentation patterns and retention times were also observed with LC-MS/MS analysis.

Conclusions: As demonstrated, phenpromethamine can be misidentified as MDMA when using POCT or immunoassay based screening methods, and misidentified as methamphetamine by LC-MS systems, based on the molecular ion. By targeting other fragment ions, phenpromethamine can be differentiated from methamphetamines. This example highlights the importance of identifying drug metabolites when utilizing broad drug screening.

D-143

Development of QMS Tacrolimus Assay for the Automated Clinical Chemistry Analyzer

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Background: The objective of this study is to develop a sensitive immunoassay intended for the quantitative determination of tacrolimus in human whole blood using automated clinical chemistry analyzers to aid in the management of kidney, heart, and liver allograft patients receiving tacrolimus therapy. Monitoring for tacrolimus is important for effective use to prevent allograft rejection following organ transplantation. The measurement of tacrolimus concentrations in whole blood in conjunction with other laboratory data and clinical evaluation can optimize immunosuppressive effect and minimize adverse side effects for patients.

Methods: The QMS Tacrolimus assay is a liquid stable particle-enhanced turbidimetric inhibition immunoassay. The assay is based on competition between free tacrolimus in the sample and tacrolimus derivative coated onto a micro-particle for anti-tacrolimus antibody binding sites. The tacrolimus-coated micro-particle reagent is rapidly agglutinated in the presence of anti-tacrolimus antibody reagent and the rate of agglutination is inversely proportional to the tacrolimus concentration in the sample. The rate of absorbance change is measured photometrically and is directly proportional to the rate of agglutination of the particles. A concentration-dependent classic agglutination inhibition curve can be obtained to determine the tacrolimus concentration in the sample. The reagents consist of two reagents and an extraction solution for sample pretreatment. The calibrators contain tacrolimus in the human whole blood matrix at concentrations of 0, 2, 5, 10, 20 and 30 ng/mL.

Results: The performance of the QMS Tacrolimus Assay was evaluated on the Beckman Olympus AU680 analyzer. The assay detected tacrolimus concentrations as low as 0.4 ng/mL and was linear up to 30 ng/mL. The functional sensitivity, where the assay could produce inter-assay precision less than 20% and acceptable recovery, was observed at 0.9 ng/mL. Assay precision was evaluated using CLSI guideline EP5-A. Three levels of tacrolimus spikes and patient pools with lowest concentration at 2.9 ng/mL and highest at 25.0 ng/mL were tested twice per run, two runs per day for 20 days. The precision ranged from 1.8 %CV to 4.9 %CV for within-run and 3.9 %CV to 7.5 %CV for total run. The assay accurately recovered spiked tacrolimus samples throughout the assay range. No significant interference was observed with various endogenous substances and other immunosuppressive drugs such as cyclosporine, sirolimus, everolimus, and mycophenolic acid. The assay did not exhibit obvious cross reactivity with tacrolimus metabolites with the exception of M4, 12-hydroxyl tacrolimus.

Patient correlation studies: QMS Tacrolimus vs. Abbott ARCHITECT Tacrolimus yielded a regression equation of $y=1.01x + 0.41$ and a correlation coefficient of 0.96. QMS Tacrolimus vs. LC/MS/MS yielded $y=1.10x + 0.56$ and a correlation coefficient of 0.97. The reagent placed onboard and calibration curve was stable for a minimum of 30 days.

Conclusions: The QMS Tacrolimus Assay enables measurement of tacrolimus in human whole blood with high precision at very low concentrations which is essential for long-term monitoring of transplant patients. Ability to measure trough levels of tacrolimus with high accuracy makes this method a desirable alternative for monitoring tacrolimus in whole blood samples. The QMS Tacrolimus Assay can be applied to various types of automated clinical chemistry analyzers.

D-144

Conversion of Phenobarbital to its Prodrug Primidone: a Novel Finding from a Case Report

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Background: Phenobarbital is one of the oldest antiepileptic drugs still in clinical use. Primidone, a pro-drug of Phenobarbital, has also been widely used as an anticonvulsant. Although reverse conversion of phenobarbital to primidone has not been described, we describe a suspected case of Munchhausen by proxy where this phenomenon was observed; this finding was then recapitulated using an animal model.

Methods: We conducted a retrospective review of several in-hospital patients, including the index case, with high levels of phenobarbital. Also, eight Sprague Dawley rats were injected intraperitoneally with a single dose of phenobarbital using four different doses: 10, 20, 40, and 80 mg/kg. Blood levels were taken at 8 and 24 hrs post-dose and analyzed by HPLC for both phenobarbital and primidone.

Results: All patients' records were reviewed and none had a history of taking primidone. Primidone was detected only in patients whose phenobarbital concentrations were > 150 mcmol/L (therapeutic range 50-180 mcmol/L). The concentrations of primidone ranged from 8.1 to 81.5 mcmol/L and were proportional to the concentration of phenobarbital. Primidone was not detected in rats receiving a loading dose of 10, 20, or 40 mg/kg. However, in rats receiving a high dose of 80 mg/kg, a phenobarbital level of 407 mcmol/L and 224 mcmol/L, and primidone levels of 6.6 mcmol/L and 4.4 mcmol/L were measured at 8 and 24 hrs, respectively.

Conclusions: Our data indicate that high levels of phenobarbital reversely convert to primidone. These findings may have an impact on clinical and forensic toxicological interpretation of results.

D-145

Presence of a Volatile Substance Mistaken for Diethylene Glycol in Plasma in a case of Valproic Acid Overdose

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Objectives: Diethylene glycol (DEG) is an organic compound widely used in various consumer products and is toxic. A 38 year old male with a history of seizures and on valproic acid was rushed to the hospital after being found unconscious. Valproic acid was measured and found to be >5000 µmol/L. Volatile alcohols were negative but DEG was detected. As this finding was inconsistent with expected biochemical abnormalities, we postulated that a component of valproic acid interfered with volatile analysis.

Method: A standard of DEG along with patient samples with high valproic acid levels were analyzed by GC-FID. We also analyzed various components of valproic acid capsules. The GC chromatographs and retention times for DEG standard, patient samples and the components of valproic acid capsules were compared.

Results: DEG has a retention time of approximately 5.92 min; A mixture of pure DEG with the patient-in-question sample showed two closely resolved peaks at the retention time noted above. GC analysis of the gel like inner content of the valproic acid capsule showed no peak between 5.9 and 6.1 min. However, GC analysis of the outer capsule shell showed a large peak at 6.07 min, which was identified by the software as DEG.

Conclusions: Elevated valproic acid levels can lead to a false positive DEG by GC-FID due to a component in the outer capsule shell that has a retention time close to that of DEG. This case highlights the importance of continuously monitoring results for potential interferences in routine toxicology analysis.

D-146

Resolution of Hook-Effect in Roche Partner DRI Oxycodone Immunoassay

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Background: Oxycodone is a semi-synthetic opioid widely used for pain management. We routinely screen urine for Oxycodone using a semi-quantitative application of the Microgenics DRI Oxycodone immunoassay (OXY3S, Roche Cobas Integra; cut-off 300 ng/mL; range 0 - 1,000, extended to 10,000 ng/mL by auto-dilution) and confirm by gas chromatography/mass spectrometry (GC/MS). Oxycodone and its metabolite Oxymorphone, also a prescription medication, are equally detected by OXY3S. We identified a sample with a false negative OXY3S result, with Oxycodone+Oxymorphone >75,000 ng/mL by GC/MS. We hypothesized that we were observing a hook-effect, which is considered unusual with EMIT (Enzyme Multiplied Immunoassay), and so devised experiments to investigate and eliminate the phenomenon.

Objective: This study investigates the hook-effect observed in the DRI Oxycodone EMIT assay.

Methods: Drug-free urine supplemented with Oxycodone, Oxymorphone (500,000 - 122 ng/mL), or Oxymorphone-3β-D-glucuronide (50,000 - 98 ng/mL) (Cerilliant) was serially diluted and measured by OXY3S. Patient samples with OXY3S <1,000 ng/mL and Oxycodone+Oymorphone >10,000 ng/mL by GC/MS were frequently observed (23% of positive tests). Fourteen samples were selected for study, reflecting those containing a majority of either Oxycodone or Oxymorphone, or a mixture of both. Each sample was serially two-fold diluted until a maximal OXY3S result was obtained when factored for the dilution. Full scan GC/MS was used for a comprehensive drug screen, and to determine the Oxycodone and Oxymorphone glucuronide-conjugate concentration in each sample. The OXY3S test parameters were replicated in a development channel, and subsequently modified for the order and volume of reagent and sample addition, and for measurement times. The resulted Oxycodone value in each patient sample was determined for each set of parameters.

Results: OXY3S accurately determined high concentrations of Oxycodone, Oxymorphone and Oxymorphone-3β-D-glucuronide supplemented in drug-free urine up to 500,000 ng/mL, with recoveries near 100%. No drug(s) could be identified in common amongst the samples that might interfere with the immunoassay. The development channel OXY3S performed identically to the manufacturer's application in all 14 patient samples. This assay, when modified to add sample after both assay reagents had been incubated, and using a later kinetic measurement time (OXYDEV), yielded maximal values (>10,000 ng/mL) in 12 (86%) samples. For the two unresolved samples, including the index case, OXYDEV read maximal concentration after a 1:2 dilution. In contrast, OXY3S only achieved maximal results for these two samples at dilutions of 1:8 and 1:16. However, addition of twice the antibody/substrate reagent in either OXY3S or OXYDEV, accurately determined Oxycodone in every sample.

Conclusions: The precise cause of the hook-effect with the DRI Oxycodone EMIT remains unclear, as the phenomenon could not be replicated simply by supplementing urine with Oxycodone, Oxymorphone or Oxymorphone-3β-D-glucuronide concentrations similar to or higher than those in the patient samples. Importantly, addition of more antibody/substrate into the reaction resolved the effect in all 14 samples. The utility of the DRI Oxycodone EMIT as a semi-quantitative assay as defined by Roche on the Integra is questionable, but the modified application presented in this study eliminates the hook-effect, and allows reliable determination of high Oxycodone concentrations.

D-147

Lack of Adequate Specificity in an Approved Urine Oxycodone Immunoassay Results in a Nearly 50% False Positive Rate

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Background. Urine drug testing is a critical tool for clinicians to monitor proper use of prescription pain medications and to detect abuse, substitution, or diversion of opioids. Clinical laboratories must validate reliable, analytically sound tests in order to provide clinicians with correct information to determine each patient's level of compliance. During validation of an oxycodone immunoassay for use in the University of Louisville outpatient laboratory, we found that half of the patient urines that were screen-negative by our validated in-house oxycodone immunoassay (Thermo DRI Microgenics) tested positive using the reagents under evaluation from a second manufacturer (Lin Zhi International, Inc.) *The objective of this study was to determine the reason for the high false-positive rate in the Lin Zhi oxycodone immunoassay.*

Methods. Two sets of de-identified urine samples were provided by AIT Laboratories, accompanied by LC-MS/MS confirmation data for drugs present in each sample. Reagents from both oxycodone immunoassay manufacturers were loaded into open channels on the Siemens Viva E ® Drug Testing System. Testing was performed according to the manufacturers' instructions. Both assays were calibrated in qualitative mode for the 100 ng/mL oxycodone cutoff and passed quality control.

Results. The first set of samples from AIT included 20 oxycodone-positive and 10 oxycodone-negative urines. Both assays detected the positive samples without discrepancies; however, as described above, 5 of the 10 negative urines tested positive by the Lin Zhi oxycodone assay. Differences in calibration material were considered, but we found that calibrators from the two manufacturers were indistinguishable by the DRI method. To test the hypothesis that the Lin Zhi reagents were detecting other drugs in these patient samples, we ran another set of 50 urine samples from AIT that were confirmed negative for oxycodone and oxymorphone, but positive for other drugs (buprenorphine, fentanyl, methadone, tapentadol, tramadol, and/or other opioids). Of these 50 samples, one tested positive by the DRI oxycodone method close to the calibration cutoff. However, 24 of the fifty tested positive by the Lin Zhi oxycodone method; these positive samples had varying, clinically relevant concentrations of morphine, codeine, hydrocodone, and/or hydromorphone. One had a high concentration of buprenorphine. The Lin Zhi reagents did not cross-react with samples containing fentanyl, methadone, tapentadol, or tramadol, unless the opioids listed above were also present.

Conclusions. The oxycodone immunoassay marketed by Lin Zhi International, Inc. was cleared through the FDA 501(k) pathway in 2005 as substantially equivalent to the DRI Microgenics assay. However, we have found that the antibodies in this reagent set will also recognize other opioids if present in patient samples. Low specificity in a screening method leads to increased volume of confirmation testing and subverts clinicians' confidence in drug screening intended for assessment of patients' compliance with pain management regimens. Clinical laboratory professionals should be reminded of the critical nature of in-house validation testing required before releasing patient results using any given test system.

D-149

UGT1A1*28 and UGT2B7*2 Polymorphisms do not Significantly Affect the Formation of Ethyl Glucuronide in vivo.

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Ethyl glucuronide is a minor metabolite of ethanol that can be detected for up to five days after ethanol abuse. Despite the advantage of a markedly increased window of detection, there is substantial controversy over testing for ethyl glucuronide. Numerous studies have demonstrated that incidental exposure to products containing small amounts of ethanol can led to false positives, depending on the cutoff utilized. In addition, several studies have shown enormous between-subject variability in the production of ethyl glucuronide, leading to widespread speculation that the observed variation has a genetic origin. Specifically, the principal enzymes that catalyze the formation of ethyl glucuronide from ethanol, UGT1A1 and UGT2B7, are highly polymorphic and mutations in these genes such as the UGT1A1*28 and UGT2B7*2 have been demonstrated to affect enzyme activity *in vitro*. Although these polymorphisms are common in humans, it is unknown whether their presence significantly alters ethanol metabolism *in vivo*. Objectives: 1) Develop a high resolution mass spectrometry method to quantitate ethyl glucuronide in human urine. 2) Determine the between-subject and within-subject variability associated with ethyl glucuronide production. 3) Determine the effects of the UGT1A1*28 and UGT2B7*2 polymorphisms on the formation of ethyl glucuronide *in vivo*.

Methods: A method for determining ethyl glucuronide concentrations in urine was developed using a Thermo Exactive Orbitrap mass spectrometer operating in negative mode. Genotyping was performed on a BD Max thermocycler for UGT1A1 and an ABI 7500 thermocycler for UGT2B7. For ethanol consumption studies, subjects ingested 0.5g/kg ethanol over a period of twenty minutes. Ethyl glucuronide levels at seven time points over twenty-four hours were measured and normalized to creatinine. The area under the resulting curve was subsequently utilized as a surrogate for total ethyl glucuronide production.

Results: We developed a high resolution mass spectrometry method to measure ethyl glucuronide in human urine. The assay was linear (R^2=0.9994) from 0.4 ug/mL to 150 ug/mL with a LLOQ (S/N of 20:1) of 0.15 ug/mL and a LLOD (S/N 5:1) of 0.075 ug/mL. Within-run and between-run imprecision were 2% and 5%, respectively,

and between-run inaccuracy at 5 ug/ml was 5%. Consistent with previous reports, variability between ten wild-type participants was substantial with a CV of 60%. In contrast, two subjects participated three times in the study and displayed significantly lower within-subject variability (CV of 18%), indicating a low index of individuality. We conducted controlled ethanol consumption studies on 26 genotyped individuals that fell into one of four categories: wild-type, UGT1A1*28 homozygous, UGT2B7*2 homozygous, UGT1A1*28/UGT2B7*2 double-homozygous. The average amount of ethyl glucuronide produced from individuals harboring UGT1A1*28 or UGT2B7*2 alleles was not significantly different from wild-type individuals.

Conclusions: From these data, we conclude that ethyl glucuronide production exhibits a low index of variability and the common polymorphisms found in UGT1A1 and UGT2B7 do not significantly affect the production of ethyl glucuronide *in vivo*. Consequently, testing for ethyl glucuronide as a marker of ethanol ingestion remains a robust test and genotyping for UGT1A1 and 2B7 does not need to be considered.

D-150

Validation of the ARK methotrexate assay on the COBAS 6000 analyzer

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Background: Methotrexate (MTX), is a folic acid antagonist used in the therapeutic regimens for Acute Lymphoblastic Leukemia and osteosarcoma. MTX, as a folic acid antagonist inhibits dihydrofolate reductase, preventing the regeneration of the cellular tetrahydrofolate pool. Tetrahydrofolate in turn is a cofactor for a number of enzymatic reactions including the thymidylate synthase catalyzed conversion of thymidylate (dTMP) from deoxyuridylate. Depletion of the tetrahydrofolate pool prevents the de novo synthesis of dTMP, and inhibits DNA synthesis leading to cellular apoptosis. Because of the potential toxicities associated with this treatment regimen, monitoring the plasma concentration is crucial for patient management. Currently most clinical chemistry laboratories monitor MTX concentration with the commercially available TDx Methotrexate II assay (Abbott Laboratories, Abbott Park, IL). Unfortunately the TDx analyzer, a stalwart analyzer for many clinical laboratories, is slated to be phased out in the near future. In an effort to maintain our current level of service for this assay without the need for additional equipment, we have evaluated a third party reagent, the ARK Methotrexate assay (ARK Diagnostics, Inc, Sunnyvale, CA) on our COBAS 6000 analyzer. The objective of our study was to perform an in-house validation of the ARK Methotrexate assay on our COBAS 6000 analyzer with a split sample comparison to our TDx Methotrexate assay

Methods: Precision studies as well as linear range and carryover studies of the ARK assay on the COBAS 6000 analyzer were performed using available templates in the EP Evaluator software package. Forty patient samples spanning the analytic measurement range of the FDA-approved ARK Methotrexate assay were compared with our current TDx assay.

Results: The ARK assay on the COBAS 6000 analyzer demonstrated acceptable within-laboratory imprecision of 12.9% at a MTX concentration of 0.057 mcmol/L and 4.4% at 0.844 mcmol/L. The day-to-day precision over 20 days was 13.4% and 7.7% at MTX concentrations of 0.075 mcmol/L and 0.844 mcmol/L, respectively. The ARK assay did not demonstrate any significant carryover and patient sample comparisons between the ARK assay and the TDx analyzer yielded excellent concordance with a Deming linear regression equation of ARK= 1.019(TDx) +0.012, R= 0.9954.

Conclusions: Our analysis of the ARK methotrexate assay on the COBAS 6000 analyzer demonstrated excellent inter-assay concordance and precision over a twenty day validation period. Validation of this assay on an open channel on the COBAS analyzer will permit many clinical laboratories an additional option to consider in replacing their TDx assay in a financially challenging laboratory climate.

D-151

Labetalol Interference in a New Fentanyl Immunoassay

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Introduction: Fentanyl is a potent synthetic opioid agonist prescribed for management of chronic pain. Because of the high potential for fentanyl diversion and abuse, pain management practice guidelines recommend the use of urine drug testing to monitor patient compliance. Our laboratory recently implemented an automated fentanyl homogenous enzyme immunoassay (HEIA) (Immunalysis Corporation, Pomona, CA) for compliance monitoring. The HEIA assay is rapid and more cost-effective than non-automated alternatives. However, we have observed a relatively high rate of HEIA false positives, which is suspected to be due to interference from other structurally-related medications.

Objective: To determine the causes of false positive urine fentanyl HEIA screening results.

Methods: A total of 3079 urine samples from patients treated for chronic pain were analyzed by HEIA on the Olympus AU480 analyzer (Beckman Coulter, Inc., Brea, CA) (cutoff = 2 ng/mL). All positive HEIA results were confirmed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (cutoff = 1 ng/mL for fentanyl and its metabolite, norfentanyl). Patient medications prescribed at the time of testing were documented for all HEIA false positives (FP = HEIA positive with undetectable fentanyl and norfentanyl by LC-MS/MS) and 50 HEIA negatives. We focused on therapeutics with similar chemical structure to fentanyl, and trazodone, a previously reported interferent with another fentanyl immunoassay. Cross-reactivity studies were performed by spiking separate aliquots of drug-free urine with labetalol (0.3 - 1250 μg/mL), trazodone (0.06 - 500 μg/mL) and the trazodone metabolite, meta-chlorophenylpiperazine (mCPP) (1 - 1000 μg/mL) and assaying by HEIA in duplicate. Log regression analysis was performed to determine the concentration required to trigger a positive result. Urine labetalol concentrations in four false positive samples were also quantified by liquid chromatography with UV detection (LC-UV).

Results: Twenty-four HEIA FP were identified (FP rate = 12%; 24/203). Eighteen (75%) of the FP specimens originated from patients prescribed labetalol and another 3 (12.5%) originated from patients prescribed trazodone. None of the patients associated with the 50 HEIA randomly selected negative specimens had prescriptions for labetalol; 2 were prescribed trazodone. Labetalol, trazodone and mCPP demonstrated cross-reactivity with the fentanyl HEIA assay (labetalol (0.002%) > trazodone (0.001%) > mCPP (0.0004%)). However, the LC-UV urine labetalol concentrations (0.5 - 1 μg/mL) in the four measured samples were below the estimated urine labetalol concentrations (≥80 μg/mL) needed to generate a positive HEIA result. Trazodone and mCPP spiked at concentrations as high as 500 and 1000 μg/mL, respectively, did not generate positive HEIA results.

Conclusions: Labetalol, trazodone and mCPP were identified as potential HEIA interferents; however, predicted urine concentrations of these drugs/metabolites are insufficient to generate positive HEIA results. Given the substantial labetalol cross-reactivity and the large percentage of FPs originating from patients prescribed labetalol, we suspect that labetalol metabolites such as, 3-amino-1-phenylbutane (APB), may be the major cause of FP HEIA results in our laboratory. The cross-reactivity of APB was not tested due to its caustic nature. Clinical laboratories should be aware of the high rate of the FP results with the HEIA assay and the potential causes, labetalol and its metabolites.

D-152

Development of a dried urine spot screening assay for drugs of abuse and pain management.

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Objective: The purpose of this study is to develop a dried urine spot screening assay for drugs of abuse and pain management that provides superior sensitivity and specificity compared to traditional drugs of abuse immunoassays, while offering advantages in specimen storage and transport.

Background: Dried blood spot (DBS) has been used in clinical laboratories for many years, especially to detect inborn errors of metabolism. DBS offers a very convenient way of collecting specimen on filter paper. DBS eliminates the need for phlebotomists and allows sufficiently for one or two drops of blood to be used for analysis. Most importantly, DBS can be easily mailed to clinical laboratories for analysis, as specimens on DBS are less prone to degradation and hydrolysis, thus offering an advantage during specimen storage and transport. Although DBS has been used for many years, dried urine spot (DUS) has not been introduced into clinical laboratories for routine use. Here we report the preliminary results of the development of a DUS screening assay for a panel of nine natural and semi-synthetic opiates, three synthetic opiates, two sedative-hypnotics, five stimulants, and phencyclidine.

Method: At least six calibration standards for each analyte were made by spiking blank urine with different quantities of certified reference materials (Cerilliant, Round Rock, TX). The 903 Protein Saver Snap-apart Cards (GE Healthcare Life Sciences, Piscataway, NJ) were spotted with samples for each circle, and allowed to air-dry for two hours. DUS circles were cut with a puncher, and analytes were recovered by extracting each punched-out circle with 300 uL of acetonitrile-methanol mixture (1:1) fortified with corresponding deuterated internal standards, followed by brief

centrifugation. Extractants were analyzed by an integrated Shimadzu UFLCXR-Sciex 3200 QTRAP system. A gradient program starting at 5% and ending at 95% of mobile phase B in 20 minutes was developed for this analysis where mobile phase A was water with 1% formic acid and 1mM ammonium formate and mobile phase B was acetonitrile with 1% formic acid. Linearity was tested by establishing calibration curves with at least six calibration standards in duplicate.

Results: Our preliminary results show that all drugs could be detected by this DUS method. Linearity was established for hydromorphone from 50 to 1,000 ng/mL, for hydrocodone from 25 to 1,000 ng/mL, and for benzoylecgonine from 25 to 1,000 ng/mL, with within-day imprecision of 5.7%, 0.3%, and 2.2% at their respective LLOQ. A long-term stability study with the DUS cards, as well as a comparison of our LC-MS/MS method to the traditional GC-MS method, is underway.

Conclusions: We have developed an easy, sensitive, and specific DUS assay with LC-MS/MS quantification as its analytical component. The LLOQ for hydromorphone, hydrocodone, and benzoylecgonine is one order of magnitude lower than those by Syva EMIT II PLUS Drugs of Abuse assays (Siemens Healthcare Diagnostics, Tarrytown, NY). Refinement of this method may eliminate the need for the reflex GC-MS or LC-MS/MS confirmatory tests that are required to confirm positive results from current drugs of abuse immunoassays.

D-153

Development and Validation of a Liquid Chromatography Tandem Mass Spectrometry Method for Quantitation of the Amminoglycoside Arbekacin in Serum

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Background: Arbekacin (MW = 552.62 Da) is a semisynthetic aminoglycoside antibiotic widely used in Japan for treatment of multi-resistant bacterial infections such as methicillin-resistant *Staphylococcus aureus* (MRSA), with specific activity demonstrated against both gram-positive and gram-negative bacteria. Arbekacin is typically administered by intravenous or intramuscular injection over a period of 7 to 10 days. Ototoxicity and nephrotoxicity are the most serious potential adverse effects of this drug, and thus therapeutic drug monitoring (TDM) is needed to maintain drug concentrations within the therapeutic range for an individual patient. Arbekacin is licensed in Japan for the treatment of septicemia and pneumonia caused by MRSA but is not approved for use in the United States. Given the increasing prevalence of multi-drug resistant organisms in the US and the successful use of this aminoglycoside in Japan, arbekacin will be tested in an upcoming clinical trial. This project entails the development and validation of a method for quantitation of serum arbekacin concentrations using liquid chromatography tandem mass spectrometry for TDM support of the clinical trial.

Methods: Following a protein precipitation with 0.3 M perchloric acid containing internal standard dibekacin (Discovery Fine Chemicals) at a concentration of 0.6 µg/ml, human serum samples containing arbekacin (Meiji Seika Kaisha, Ltd.) were analyzed using a Hypersil Gold PFP column and a Transcend UPLC liquid chromatography system (Thermo Fisher Scientific). Elution occurred with a gradient of water and acetonitrile, each containing 0.05% (v/v) TFA and 0.1% (v/v) formic acid. Analytes were detected over a 3.25 minute run time using a TSQ Quantum Vantage tandem mass spectrometer (Thermo Fisher Scientific) with a heated electrospray-ionization source in positive ionization mode with selected reaction monitoring. A limited validation of this method was performed, evaluating simple precision, linearity and recovery, and limit of quantitation. All statistical parameters were evaluated using EP Evaluator 8 software.

Results: The limit of quantitation (the lowest concentration with a CV <20%) for arbekacin was determined to be 0.1 µg/ml for this method. Simple precision was evaluated at three concentrations of arbekacin (low - 0.25 µg/ml, med - 5µg/ml, and high - 20 µg/ml); giving a % CV of 3.7%, 1.5% and 2.2% for the low, medium, and high levels, respectively. The described method was linear from 0.1 µg/ml to 47.3 µg/ml (slope of 0.982). The mean recovery ranged from 94.7 - 103.8%.

Conclusions: This developed and validated LC-MS/MS method allows for the quantitation of arbekacin in serum following a brief protein extraction procedure. Next steps for this project include a full validation of the assay and application of this method for TDM in the clinical trial.

D-154

Clinical Toxicology Results after Supplementation of Commercial GC/MS Libraries

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Background: Mass spectral libraries have been employed in analyses involving gas chromatography/mass spectrometry (GC/MS) for a number of years. However, few industry standards exist in the construction of commercial libraries apart from the application of 70 electron volts in electron impact ionization. As a result, spectral libraries may be composed of low or high analyte concentrations acquired from one or several types of instruments. This may lead to the acquisition of spectra that may or may not correlate with those on other instruments. In this study, we compared the number of additional spectral matches obtained using a commercially-available drug library with and without spectral supplementation.

Objective: The objective of this study was to determine if spectral supplementation significantly affected the number of spectral matches in clinical toxicology analyses. The spectral library of each match with highest purity and fit was also noted.

Methods: One hundred pharmaceuticals listed in the top 200 prescription drugs of 2010 (Thomson Reuters Red Book®) were each diluted into 100, 50, and 10 ug/mL methanolic solutions, and injected onto an ion trap GC/MS (Thermo ITQ, Thermo Fisher Scientific Inc.). All relevant spectra were stored electronically in a spectral library specifically created for this project. Subsequently, 125 urine specimens were extracted via solid phase extraction (Bond Elute Certify, Agilent Technologies Inc.) and injected onto the same instrument used to acquire supplemental spectra. Each specimen was de-identified and stored at -80°C until analysis as approved by the Institutional Review Board at the University of Mississippi Medical Center, Jackson, MS. All spectral analyses were performed by a single medical technologist with >5 years GC/MS experience. Spectral matches were determined by comparing spectra to the Pfleger/Maurer/Weber library and to the experimental library. Cutoff criteria for positive matches were: >600/1000 SI (fit), and >750/1000 RSI (purity). Caffeine was excluded from the results because it was detected in nearly all samples.

Results: A total of 310 drugs were detected using the commercially-available library. Of the 310 drugs detected, 48 exhibited higher purity and fit with the supplemented spectra than with those in the commercial library. An additional 27 drugs were detected with the supplemented spectra. These represented an increase in 8.7% over the total, and included analeptics, antidepressants, antihypertensives, antihistamines, and opiod analgesics.

Conclusions: Direct supplementation of spectral libraries can significantly increase the number of drugs detected in clinical toxicology analyses. The supplementation of spectral libraries should also include spectra of recently approved pharmaceuticals in order to ensure comprehensive analyses.

D-155

Evaluation of the ARK(TM) Levetiracetam Assay on the Beckman Coulter AU680 Chemistry Analyzer

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Background: Levetiracetam [(-)-(S)-α-ethyl-2-oxo-1-pyrrolidine acetamide] is a second generation anticonvulsant medication indicated as adjunctive therapy in the treatment of certain types of seizures in people with epilepsy. It is marketed under the trade name Keppra®. Levetiracetam is a single enantiomer and the precise mechanism(s) by which levetiracetam exerts its antiepileptic effect is unknown. However, high doses of levetiracetam can induce adverse effects, including dizziness, somnolence, asthenia, headache, behavioral problems, depression, and psychosis (Kanner et al., 2004). The therapeutic drug monitoring of levetiracetam concentrations plays an important role as an aid in management of patients treated with levetiracetamfor toxicity issues.

Objective: To evaluate the performance characteristics of the new ARK Diagnostics Levetiracetam Assay on the Beckman Coulter AU680 chemistry analyzer system for routine clinical laboratory use.

Methods: The ARK Levetiracetam Assay is a homogeneous immunoassay used in the quantitative determination of levetiracetamin human serum or plasma. When sample and reagents are mixed, drug in the sample competes with drug labeled by the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody so that the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance

change that is measured spectrophotometrically. The NADH absorbance is directly proportional to drug concentration in the sample. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the bacterial enzyme (from Leuconostocmesenteroides) used in the assay. The ARK Levetiracetam Assay was evaluated using the Beckman Coulter AU680 chemistry analyzer. The assay was calibrated using a six point calibration curve(0 to 100 µg/mL). Performance of the assay was determined by assessing precision, limit of quantitation, spike recovery, endogenous interferences, and correlation studies using Hitachi 917.

Results: Precision on tri-level controls was 7.3% CV (8.11 µg/mL), 3.3 % CV (25.56 µg/mL) and 4.4 % CV (65.83 µg/mL). Limit of Quantitation (LOQ) was 2.0 µg/mL.Linearity was demonstrated from 2.0 to 100.0 µg/mL. No common endogenous substances (Hb, bilurubin, gamma globulin, uric acid, albumin, cholesterol and triglyceride) interference was observed with the measurement of levetiracetam at the levels tested. Recovery experiment using spiked samples showed acceptable recovery. Correlation studies were done using 48 patient samples with levels over the linearity range, from 1.8 to 75µg/mL were analyzed using reference the Hitachi 917 method showing acceptable statistical results (Passing Bablokregression analysis: y (Beckman AU680) = 1.06 (Hitachi 919) + 0.17, $r^2 = 0.99$).

Conclusions: The ARK Levetiracetam Assay is suitable for the quantitative measurement of Levetiracetam in serum and plasma on the Beckman Coulter AU680 chemistry analyzer. This assay correlated with the Hitachi 917and is well-suited for routine TDM use on the Beckman Coulter AU680 chemistry analyzer.

Acknowledgments : We acknowledge the outstanding contribution of Diana Hernandez, M.T. for updating and validating the reaction parameters and precision studies on the AU680 and the excellent correlation studies by Mila Huerta, M.T.

Thursday AM, July 19, 2012

Poster Session: 9:30 AM - 12:00 PM

Hematology/Coagulation

E-02

Evaluation of Cellavision DM96 Analyser in Screening and Quantitating Abnormal Cells (Reactive Lymphocytes, Blast Cells, Immature Granulocytes and Nucleated RBCs)

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Background: Approximately 28% of Full Blood Count requests received by the Department of Laboratory Medicine at Khoo Teck Puat Hospital will require a blood smear for microscopic review, to verify flags indicated by the Hematology Analyzer. In this study, we evaluated the accuracy and reproducibility of the Cellavision DM96 (Cellavision AB, Sweden), an automated digital cell morphology system, in identifying and quantifying abnormal and immature cells. While microscopy remains the gold standard in correctly identifying and quantifying White Blood Cell sub-classes, the methodology is labour-intensive, time-consuming and fraught with variability in technique due to operator differences. By automating this process, we aim to increase analytical productivity and ensure consistency in our reporting.

Methodology: A total of 573 slides of various white blood cell populations from anonymized sources were collected. These were then assessed microscopically for 4 groups of cells, namely Reactive Lymphocytes, Blast Cells, Immature Granulocytes and Nucleated Red Blood Cells. The manual assessment was performed by six medical technologists of varied microscopy skills, representing the range of expertise in the performance of manual differentials. A 200-cell differential crenellation battlement pattern was used. This data served as the reference count. The DM96 automated result was documented and reclassified. Analysis for specificity and sensitivity was performed and linear regression calculation done on quantitative findings.

Results: Of the 4 groups of cells, the DM96 scored best in analysing Blasts while the poorest was Reactive Lymphocytes. For pre-classification, the sensitivity and specificity was 98% and 93% respectively for Blasts, and 75% and 69% respectively for Reactive Lymphocytes. The DM96 pre-classification sensitivity scored between 75% and 98% for all the indices and showed minimal improvement upon post-classification. Marked improvement in specificity was noted where the pre-classification average increased from 79% to 88%. Linear regression analysis in the quantification of these parameters indicated that re-classification was not necessary for immature granulocytes and nucleated red blood cells. However, reclassification is necessary for reactive lymphocytes, where pre-classification findings are $y = 0.1291x + 4.7791$ versus post classification of $y = 0.9116x + 1.0322$. A reclassification for blasts results in an improvement by 15% in slope value. When imprecision was assessed, the DM96 returned a coefficient of variation as low as 4.34% for Blasts with an average of 36%. Manual microscopy at best performed at 64.8% with an average coefficient of variation of 89%.

Conclusions: While microscopic examination of peripheral blood films remains the gold standard analysis method, the Cellavision DM96 system performs well in moderate complexity cases. This automated method excels in its consistency in quantifying standard morphologic sub-classes. However, operator-assisted post-classification is still required and the ability to recognize highly complex cases is presently ambiguous. Nonetheless, the DM96 is very effective in ruling out abnormal films which are flagged during the automated cell count.

E-03

A Comparative Study of Sebia's Capillarys2 and Capillarys2 FlexPiercing for Hemoglobin Electrophoresis

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Background and Objectives: Hemoglobin electrophoresis has evolved from labor intensive agarose methods using cell chambers which were technical dependent, geared toward low volume testing and which had inherent technical and safety issues. Newer, semi-automated agarose systems provided some relief and solutions but still contain similar issues for the laboratory. Current methods have progressed to using fully automated systems such as Sebia's Capillarys2™ (C2). Capillary electrophoresis technology (CE) utilizes a liquid buffer and Electric Osmotic Flow (EOF) as the medium rather than agarose along with temperature controlled fused silica capillaries. The hemolysate is prepared on line from packed red blood cells, plasma previously removed. A second generation CE instrument, Capillarys2 Flex Piercing™ (C2FP), utilizes whole blood and has cap piercing capabilities. The purpose of this study was to compare these two fully automated systems for time efficiencies, precision, as well as method and operational differences.

Methods: A side by side comparison of the Capillarys2 and Capillarys2 Flex Piercing was performed at an Ohio laboratory currently using the C2. Samples were selected from the routine specimens received for hemoglobin variant testing. The time study began at the receipt of the specimens through sample loading to instrument processing. Data was then collected for alternate method and precision studies and includes normal samples as well as those with common hemoglobin variants. Observations were noted for operational differences between the C2 and C2FP. Routine operation and SOPs were followed for both instruments.

Results: Total preparation time for the C2 which included such steps as organizing the specimens from the pending list, aliquotting samples, centrifugation, and plasma removal to the final hands on step of decapping and loading the racks was 29:17 minutes. The C2FP total preparation time was shortened to 5:32 minutes. The front end preparation as found with the C2FP eliminated six steps from this process. The savings reduced preparation steps by 75%, time required for those steps was reduced by 81.2%, and a run time reduction of 24%. Four samples were then selected from the day's run of 15 samples to be included with the data for the method comparison and precision study. The precision study performed on all eight capillaries of the C2FP (using 4 samples) gave an overall CV of 0.36 for HgbA and 1.64 for HgbA2. The method comparison (using 4 samples with normal and variant hemoglobins) showed an average SD of 0.28 for all samples.

Conclusions: The data shows that the C2FP is able to accomplish and attain the same proven quality as confirmed with the C2 while improving workflow. Although the instrument time for sample processing was equal, C2FP improved efficiency by reducing pre-analytical steps and processing time. The testing menu includes all the current available techniques on the C2 which are Hemoglobins, Proteins, Immunotyping and CDT. The data from this study supports that the C2FP is a logical and rather seamless improvement for hemoglobin electrophoresis on the C2.

E-04

Association of Inflammation, Endothelial Dysfunction and Angiogenesis in Patients with Thalassemia Intermedia

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Background: Angiogenic growth factors, such as the vascular endothelial growth factor (VEGF) family of proteins, govern numerous aspects of vessel homeostasis. Placental growth factor (PlGF) is a member of the VEGF family of angiogenic proteins and is expressed in placental, cardiac, and lung tissue. Placental growth factor (PlGF) and its receptor the fms-like tyrosine kinase receptor 1 (Flt-1 or VEGF-R1) are novel therapeutic targets for angiogenic disorders. These growth factors exert pleiotropic effects, potentially beneficial, such as the promotion of angiogenesis, and/or potentially harmful pro-inflammatory effects, such as the promotion of endothelial dysfunction and pulmonary hypertension. von Willebrand factor (vWF) has been proposed as a biomarker of endothelial damage/dysfunction because increased plasma levels have been found in inflammatory and atherosclerotic vascular diseases and is defined as a novel link between hemostasis and angiogenesis.

Patients and Methods: We investigated if alterations in angiogenic growth factors may contribute to endothelial dysfunction in patients with thalassemia intermedia (TI) using peripheral biomarkers. Thirty-four adult patients with TI were included in the study, while 20 healthy individuals served as controls. Markers of inflammation such as high-sensitivity C-reactive protein (hs-CRP), serum Amyloid A protein (SAA), von Willebrand factor, nitric oxide (NO) along with PlGF and soluble Flt-1 were measured in patients and controls by means of immunoenzymatic techniques, while tissue hypoxia was evaluated in terms of hemoglobin oxygen affinity (P_{50}).

Results: The main results of the study showed that: a) plasma levels of vWF, NO, PlGF and sFlt-1 were significantly higher in patients with TI compared to controls (88.0±21.8 vs 71.1±21.5 IU/dL, 101.5±34.7 vs 52.1±8.2 mmol/L, 52.2±20.0 vs 17.2±4.0 pg/mL and 96.5±25.2 vs 76.8±11.5 pg/mL, respectively ($p<0.01$), while angiogenic balance expressed as sFlt-1/PlGF was significantly lower in patients with

TI compared to controls ($p<0.0001$), b) in patients with TI the plasma levels of vWF correlated significantly with: NO (r=0.535, p<0.001), PlGF (r=0.478, p=0.004) and sFlt-1 (r=0.609, p<0.0001), while no associations were found between vWF with Hb and Hb F levels and c) both PlGF and sFlt-1 levels correlated significantly with NO levels (r=0.571, p<0.001 and r=0.482, p=0.004, respectively) and d) sFlt-1/PlGF correlated significantly with Hb F levels (r=0.385, p=0.02) and with P_{50} values (r=0.365, p<0.05).

Conclusions: These results demonstrate for first time the important link between endothelial dysfunction and angiogenesis in patients with TI. The decreased sFlt-1/PlGF ratio in almost all patients with TI suggests that the pro- and anti-angiogenic system is shifted towards the pro-angiogenic state, providing evidence that the factors contributing in this dysregulation are low-grade inflammation and tissue hypoxia.

E-05

Total cell count in body fluids - Comparison of an automated with the manual counting method

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Introduction: The determination of total cell count in body fluids like pleural effusion or ascites with automated blood cell counters is not possible under standard conditions. The hematology autoanalyzers are only configurated for the measurement of human blood. Therefore, for routine cell counting in body fluids the manual method in a counting chamber is the gold standard. On the other hand, automated measurement has some advantages, as no manual dilution is necessary and more cells are counted, thus improving the significance of the achieved results.

In this study we compared the manual cell counting with an automated procedure in different body fluids using a Pentra DX120 analyzer.

Materials and Methods: A total of 85 body fluids were analyzed, 35 pleural effusion samples from 31 patients, 43 ascites samples from 29 patients, and 7 other materials from 6 patients, respectivly. The samples were collected in standard EDTA sampling tubes to avoid coagulation and preserve cells. All samples were analyzed with a Pentra DX120 hematology autoanalyzer. The cell count was calculated using the raw data of the LMNE channel and the following equation:

"Cell count (G/L) = LMNE-count x calibration factor x dilution factor"

Manual counting was performed with a NEUBAUER cell counting chamber. The statistical analysis was performed using Microsoft Excel.

Results: The body fluid samples showed cell counts between 0.008 and 8.676 G/L with the Pentra DX 120 analyzer and 0 and 8.8 G/L with the counting chamber, respectively. Comparing all samples there was a good concordance between the manual and automated cell counting with y = 0.9101x - 0.0791, with "y" is the manual and "x" the Pentra DX120 cell count. Correlation coefficient was calculated with r = 0.9552. In the lower measuring range up to 2.0 G/L concordance was y = 0.8222x - 0.0203 with r = 0.8834

Conclusions: Measuring of total cell count in body fluids with the Pentra DX120 analyzer is a promising tool to improve the accuracy of the results. The results show a good concordance of the automated with the manual method. In the lower range the concordance is only slightly lower but also acceptable. The automatic measurement with the autoanalyzer is easy to perform, overcomes the error-prone manual cell counting, and is therefore suitable as a routine cell counting procedure for body fluids.

E-07

Predictive value of routine hematological parameters for diagnosing renal colics.

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Background: The rate of emergency department (ED) overcrowding is dramatically increasing, so that the use of simple, economical, rapid and efficient laboratory tests might help accelerate the triage of patients. Since renal colic, also known as nephrolitiasis or kidney stone disease, is a widespread disorder with a lifetime prevalence up to 12% in western countries, we assessed whether some hematological parameters provided by routine hematological testing might help the rule in or rule out of this prevalent disorder.

Methods: We retrospectively reviewed results of hematological testing from the database of our laboratory information system from January 1 to December 31, 2010 for 262 patients urgently admitted to the ED with final diagnosis of nephrolitiasis, and 429 blood donors matched for age and sex, referred to our hospital for regular blood donation. The haematological testing was carried out on the same laboratory instrumentation (i.e., Sysmex XE 2100, Sysmex Inc, Mundelein IL, USA). Among the numerous haematological parameters, we selected those which are more useful in urgent testing, i.e., hemoglobin (Hb), white blood cell count (WBC), platelet count (PLT), alon with emerging parameters such as mean platelet volume (MPV) and red blood cell distribution width (RDW). The quality of laboratory results was validated throughout the study period by regular internal quality control and participation in an External Quality Assessment Scheme. Results are shown as mean ± standard error of the mean (SEM) and differences assessed by Wilcoxon-Mann-Whitney test.

Results: As compared with healthy controls, renal colic patients exhibited significantly lower values of Hb (140±10 versus 148±10 g/L; p<0.001), MPV (10.0±0.1 versus 11.1±0.1 fL; p<0.01) and RDW (13.1±0.1 versus 13.5±0.1 %; p=0.01), as well as higher values of WBC (9.2±0.2 versus 6.2±0.1 x 10^9/L; p<0.001) and PLT (242±4 versus 232±2 x 10^9/L; p=0.01). The analysis of each parameter by receiver operating characteristic (ROC) curve analysis yielded the following Results MPV: Area Under the Curve (AUC) = 0.78 (95% CI, 0.74-0.82; p<0.01), best sensitivity (57%) and specificity (86%) at 10.3 fL; RDW: AUC = 0.59 (95% CI, 0.54-0.63; p<0.01), best sensitivity (37%) and specificity (83%) at 13.0%, Hb: AUC = 0.67 (95%CI, 0.62-0.71; p<0.01), best sensitivity (71%) and specificity (57%) at 149 g/L; WBC: AUC = 0.83 (95%CI, 0.79-0.86; p<0.01), best sensitivity (75%) and specificity (78%) at 7.2 x 10^9/L; PLT: AUC = 0.54 (95% CI, 0.49-0.58; p=0.06). As such, after assigning one point to any of the four significant parameters (i.e., one point each if MPV<10.3 fL; RDW <13.0%; Hb <149 g/L and WBC >7.2 x 10^9/L), the resulting score (from 0 to 4) yielded an overall AUC of 0.86 (95%CI, 0.84-0.89; p<0.01) and, even more interestingly, a score ≥2 was associated with a remarkably high negative predictive value (i.e., 89%), coupled with an excellent positive predictive value (i.e., 70%).

Conclusions: The results of this study attest that the combined use of some hematological parameters, which are routinely provided by most hematological analyzers, might be helpful for the fast triage (i.e., especially for the safe rule out) of patients urgently admitted at the ED with renal colics.

E-08

Analytical performance evaluation of the Scanning Capillary Tube Viscometer for measurement of whole blood viscosity

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Background: Whole blood viscosity (WBV) is the inherent resistance of blood to flow and represents the thickness and stickiness of blood. The dynamic range of WBV is relatively large, which highlights the potential utility of this parameter as a biomarker - to the degree that viscosity provides additional incremental prediction of clinical outcomes and is modifiable by therapeutic modalities. We investigated the performance of the new scanning capillary tube viscometer (SCTV) (Bio-Visco Inc., South Korea) for the measurement of WBV.

Methods: We evaluated total imprecision for twenty days using three control viscosity materials of non-Newtonian shear-thinning characteristics like whole blood. We estimated within-day imprecision with the whole blood samples of three different individuals. For linearity evaluation, serial dilutions of a high concentration standard material were used. We compared the results on 227 patient whole blood samples from the new method with those of the Brookfield rotating viscometer (Middleboro, MA) using the Passing-Bablok linear regression for the method comparison.

Results: The viscosity results of the SCTV had a within-run CV of 3.8%, 5.4% and 3.9%, and total-run CV of 4.3%, 5.4% and 4.0% at a low-, mid-, and high-concentration samples, respectively. The within-day CVs were 6.3%, 3.7% and 3.8% at a low-, mid-, and high-concentration samples, respectively. The method showed a good linearity for the range of 84.9 to 558.2 miliPoise. For the comparison study, the data between the new method and the Brookfield rotating viscometer showed interchangeable results in the Passing-Bablok regression analysis (slope 1.08; 95% CI, 0.98 to 1.18 and intercept -3.14; 95% CI, -7.09 to 0.60).

Conclusions: The new scanning capillary tube viscometer showed a stable analytical performance required for measuring whole blood viscosity and had good comparability with the rotational viscometer. This new method could be used in the clinical laboratory for various needs.

Within-run and total-run precision of viscosity by SCTV							
	Level	Within-run			Total-run		
		Mean	SD	CV, %	Mean	SD	CV, %
Viscosity	Low	84.3	3.2	3.8	84.3	3.6	4.3
	Medium	177.5	9.7	5.4	177.5	9.7	5.4
	High	560.5	21.9	3.9	560.5	22.5	4.0

E-10

Development of an assay kit for the determination of 11-dehydro thromboxane ß2 in urine requiring low reagent and sample volumes on the RX daytona plus analyser

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Introduction Activated and aggregated platelets play a key role in the pathogenesis of cardiovascular disease. An important part of antiplatelet therapy in cardiovascular disease is aspirin (acetylsalicylic acid, ASA), which has been known for many years to have antiplatelet activity. Aspirin's effectiveness varies among individuals. It has been known for many years to have antiplatelet activity, however, its effectiveness varies among individuals. It has been estimated that 10-20% of aspirin users have recurrent thrombotic events a phenomenon commonly referred to as aspirin resistance.

Activated platelets produce Thromboxane A_2 (TxA_2), a potent vasoconstrictor and inducer of platelet aggregation. Therefore, measurement of stable metabolites of TxA_2, such as urinary 11-Dehydro Thromboxane B_2 ($11dhTxB_2$), is a means of quantitating TxA_2 production by platelets and thus a direct way to analyze the effectiveness of aspirin therapy.

This study presents the performance evaluation of an ultra-sensitive latex-enhanced immunoturbidimetric assay (ITA) to determine levels of $11dhTxB_2$ in human urine, applied to the fully automated RX daytona plus analyser. This is of value for the qualitative detection of aspirin (ASA) effect in apparently healthy individuals post ingestion.

Methods The assay is a latex-enhanced immunoturbidimetric assay based on the principle of measuring changes in scattered light. The latex particles are coated with $11dhTxB_2$, which in the presence of $11dhTxB_2$ antibody solution, rapidly agglutinate. When a sample containing $11dhTxB_2$ is introduced, the agglutination reaction is partially inhibited, slowing down the agglutination process. The rate of agglutination is inversely dependent upon the concentration of $11dhTxB_2$ in the sample. The change in scattered light is measured as a change in absorbance at 700nm. The actual change in absorbance is inversely proportional to the concentration of $11dhTxB_2$ in the sample. The $11dhTxB_2$ urine values are then required to be normalised using urine creatinine values.

The RX daytona plus uses a maximum sample volume of 11.2µl and reagent volumes of 50µl for both reagent 1 and 2. The first result is generated after 14 minutes.

Results The analytical range of the assay was 0 to 5,000pg/mL with a Limit of Blank (L.o.B.) <250pg/mL and an intra assay precision<5%. Correlation with AspirinWorks Test kit ($11dhTxB_2$ ELISA kit) demonstrated a correlation co-efficient (r) >0.9 (n=69). The AspirinWorks test kit determines the clinical outcome of samples based on normalised values (pg $11dhTxB_2$ per mg of creatinine) and interprets an individual as showing aspirin effect if the normalised value is ≤1500pg $11dhTxB_2$/mg creatinine and a lack of aspirin effectiveness if >1500pg/mg. The clinical outcome obtained on the ITA was compared with that of the AspirinWorks Test kit. The overall percent agreement was ≥90%.

Conclusion Data shows optimal performance of the reported assay kit for the determination of $11dhTxB_2$ in urine samples on the RX daytona plus analyser. A maximum sample volume of 11.2µl of neat sample per test is required per test with a throughput of 270 tests per hour. The low minimum reagent volume needed makes predicting aspirin effectiveness in individuals suffering from myocardial infarction, stroke and thrombosis more cost effective for the laboratories.

E-11

A Comparative Evaluation of PATHFAST D-Dimer Assay and VIDAS D-Dimer Exclusion in Emergency Patients with Post-Hoc Confirmed Pulmonary Embolism

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Background: The early exclusion of deep vein thrombosis (DVT) or pulmonary embolism (PE) is a major indication for D-dimer assays. Aim of the study was to evaluate the diagnostic accuracy of the new point of care assay PATHFAST D-Dimer in comparison to the VIDAS D-Dimer Exclusion assay.

Methods: In patients who presented early to the medical emergency room (ER) with symptoms of PE the diagnosis was established by echocardiography, spiral-CT, and pulmonary angiography. 60 patients were included in the study. After admission to the ER, blood was drawn and processed to obtain Li-heparin plasma samples which were stored at -70°C until D-dimer measurement. We also determined PATHFAST D-Dimer in plasma samples obtained from 102 healthy individuals in whom DVT or PE was excluded and who served as control group Basic clinical data (gender: 23 men and 24 women; age: 19 to 85 years, mean 55 years; BMI: 26.1 +/- 7.8 kg/m) as well as information on the severity of PE according to the Goldhaber classification score were available for 40 patients. Bilateral PE was the final diagnosis in 32 of 48 patients and the severity of PE was classified as massive, moderate, and small.

Results: Mean D-dimer concentration of the control group and of the patient group was 0.28 (95% CI: 0.25-0.31) µg/ml FEU and 1.45 (95% CI: 1.23-1.72) µg/ml FEU, respectively. Receiver operator characteristics analysis revealed an optimized cut-off value of 0.466 µg/ml FEU for the PATHFAST D-dimer assay (AUC = 0.975 (95% CI: 0.938-0.993); Sensitivity: 95% (95% CI: 86-99%); Specificity: 89 % (95% CI: 82-95%)). Therefore we used a rounded up cut-off value of 0.5 µg/ml FEU to examine the diagnostic accuracy of PATHFAST D-dimer to exclude PE. The correlation between PATHFAST and VIDAS results was particularly close for concentrations at or around the critical cut-off value of 0.5 µg/ml FEU, which is the recommended cut-off value to rule out DVT for users of the VIDAS assay. Somewhat higher results were generally obtained with the PATHFAST assay compared to the VIDAS assay especially for higher concentrations above 1µg/ml. PATHFAST and VIDAS assays gave both results <0.5 µg/ml FEU in 4 patients with small PE. The VIDAS assay remained below this cut-off value in another three cases.

Conclusions: Considering that all patients in this study suffered from PE, ultimately confirmed by independent imaging techniques, by use of the PATHFAST D-Dimer assay only 6 of these diagnoses were missed at the time of first presentation compared to 10 diagnoses missed by the VIDAS D-Dimer Exclusion assay. Therefore the PATHFAST D-Dimer assay was more sensitive than the VIDAS D-Dimer Exclusion assay (90% vs. 83%). Moreover, the PATHFAST analyzer allows simultaneous determination of D-dimer and cardiac troponin I within 17 min from whole blood samples. Therefore, this method might be useful at the point-of-care for early diagnostic assessment of patients with symptoms of PE or chest pain admitted to the ER or to the chest pain unit.

E-12

ADVIA Centaur D-Dimer: Multicenter Prospective Clinical Trial To Demonstrate Exclusion Of Pulmonary Embolism

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Background: D-dimer is a fibrin degradation product of clot formation. It is a combination of two "D" fragment moieties that are covalently linked through γ-glutamyl-ε-lysyl cross-linked by factor XIIIa. Increased D-dimer levels correlate with clinical conditions that relate to the formation of fibrin, mirroring an in vivo lysis of formed cross-linked fibrin. Such conditions include deep venous thrombosis (DVT), disseminated intravascular coagulation (DIC), pulmonary embolism (PE), postoperative states, malignancy, trauma, and preeclampsia. This study was conducted to demonstrate in an outpatient setting the performance of the ADVIA Centaur® D-Dimer sandwich immunoassay* versus imaging to achieve an exclusionary claim

to rule out pulmonary embolism.

Methods: For this multicenter study conducted at eight sites, samples (n = 697) were prospectively collected from nonpregnant outpatients able to provide informed consent, with no known history of venous thromboembolism. Subjects were stratified as having low, moderate or high pretest probability using the Wells scoring system. Standard of care (imaging) was performed, along with the Siemens ADVIA Centaur D-Dimer Assay on Na citrated plasma samples. Subjects were followed up 3 months after the initial presentation to determine if PE occurred.

Results: Sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) with 95% confidence intervals, stratified by pretest probability for PE, are presented for the prediction of PE.

Pretest Probability	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	Prevalence of PE
Low	100.0%; 14/14 76.8%-100.0%	33.6%; 98/292 28.2%-39.3%	100.0%; 98/98 96.3%-100.0%	6.7%; 14/208 3.7%-11.0%	4.6%; 14/306
Moderate	100.0%; 36/36 90.3%-100.0%	26.3%; 88/334 21.7%-31.4%	100.0%; 88/88 95.9%-100.0%	12.8%; 36/282 9.1%-17.2%	9.7%; 36/370
High	100.0%; 6/6 54.1%-100.0%	20.0%; 3/15 4.3%-48.1%	100.0%; 3/3 29.2%-100.0%	33.3%; 6/18 13.3%-59.0%	28.6%; 6/21
All	100.0%; 56/56 93.6%-100.0%	29.5%; 189/641 26.0%-33.2%	100.0%; 189/189 98.1%-100.0%	11.0%; 56/508 8.4%-14.1%	8.0%; 56/697

Conclusions: The ADVIA Centaur D-Dimer Assay demonstrated a 100% negative predictive value in ruling out pulmonary embolism in patients presenting with a low to moderate pretest probability. This test can be used as an exclusionary tool for pulmonary embolism when a low- to moderate-risk patient presents with symptoms consistent with pulmonary embolism.

* The ADVIA Centaur D-Dimer Assay is not available for sale.

E-13

Total Protein vs. Serum Viscosity in Evaluating Hyperviscoisty Syndrome in Patients with Paraproteinemia.

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Background: Hyperviscosity syndrome typically results from Waldenstrom's macroglobulinemia or multiple myeloma (conditions with paraproteinemia). This syndrome can also result from elevated WBC/RBC in hyperproliferative disorders such as leukemia or polycythemia. Hyperviscosity syndrome can lead to mucosal membrane bleeding, neurologic, and pulmonary complications. Traditionally, serum viscosity (SV) is manually measured in suspect patients and requires a large volume of serum (3 mL) and time (~15 min/sample); unlike total protein (TP), a cost-efficient, fast test that can be run on automated chemistry platforms. The objective of this study was to determine the correlation between TP and elevated SV in patients with paraproteinemia.

Methods: An Ostwald viscometer (37°C water bath) was used to manually measure SV. A Roche (Hitatchi) method was used for TP (P module). TP and SV results from 2010-11 were retrospectively analyzed for inclusion of 50 patients, half of which had normal (<2.0) SV, and the other half with elevated SV. Medical records of patients with elevated SV were checked for paraproteinemia, and non-elevated WBC/RBCs. An SV cut-off of >=2.0 was used for positivity (positive=viscous serum). ROC /Dot-plot analysis was used to select a TP cut-off that best correlated to the elevated SV results.

Results: Analysis showed that at a cut-off of >=9.1 g/dL TP, 100% specificity and 92.6% sensitivity (Fig.1) was obtained against SV (taken as gold standard). From 50 patients analyzed there was only one without an elevated TP (>=8.6 g/dL) when SV was >=2.0.

Conclusions: For patients with paraproteinemia this study shows that at a cut-off of >=9.1 g/dL, TP can be used in place of more laborious SV to determine if serum hyperviscosity is present. However, SV is often stratified by severity, so this should be established for TP. In a prospective analysis a larger cohort of patients will be examined to stratify TP by severity when paraproteinemia is present.

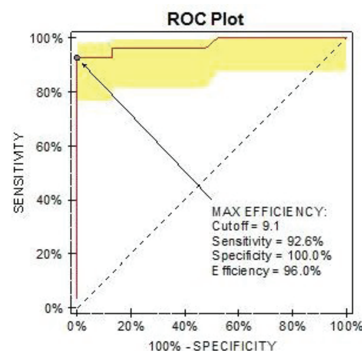

Fig 1 ROC curve for Total Protein response when Serum viscosity ≥ 2.0 is used as the diagnostic criterion for hyperviscous serum. 50 patients included, half with SV < 2.0, and half with SV ≥ 2.0 (all patients with elevated SV were checked for paraproteinemia).

E-14

Evaluation of Sysmex new automated hematology analyzer XN-9000 in routine clinical laboratory

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Background: In induction of automated hematology analyzer, improvement of the work efficiency is important in routine clinical laboratory. We evaluated a new automated hematology analyzer XN-9000 (Sysmex, Kobe, Japan) with basic analytical performance and effects of induction in routine clinical laboratory.

Methods: Blood samples collected in EDTA anti-coagulant from patients were used after receiving informed consent for this study at Toyama University Hospital (Toyama, Japan). We used automated hematology analyzer XE-2100, Cytomics FC 500 (Beckman-coulter, USA) and microscope(Olympus, Tokyo, Japan) for evaluation of a new automated hematology analyzer XN-9000.

Results: Basic analytical performance for XN-9000 with complete blood count (CBC) as follows: within run and day to day precision (CVs) showed 0.2 ~ 1.4 % for normal samples(N=10), 0.8~3.5 % for abnormal samples(N=10), respectively. Especially,since the within run precision for the low concentration of platelet samples(9 x10^9/L) were less than 3.5 %,low concentration platelet samples has been measured with sufficient accuracy. Correlation between XN-9000(Y) and XE-2100 (X) were well (N=189, slope of regression curves 0.98 ~ 1.07, r=0.94~0.99).Furthermore,correlation between XN-9000 and reference method of platelet (CD41+CD61) using Cytomics FC 500 with less than 80 x10^9/L of platelet samples were good (N=30,r=0.98). Concordance rate of WBC fractions between microscopy and XE-2100 with 195 samples including 38 abnormal samples were 94.9 %(185/195).Other hand, XN-9000 showed 98.5 %(192/195). Moreover, reexamination rate by microscopy were 26.1 % (4,592/17,597) for XE-2100, 15.0% for XN-9000 (3,636/24,245), respectively.

Conclusions: In this study ,our data suggested that new automated hematology analyzer XN-9000 showed good analytical performance and can contribute to improvement of the work efficiency in routine clinical laboratory.

E-15

Evaluation on the performance of ARKRAY ADAMS HA 8180 HbA1c analyzer

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Background: HbA1c is routinely used for the management of diabetes. In 2010, HbA1c was added to the diagnostic criteria by the American Diabetes Association.

Recently newly developed ARKRAY ADAMS HA-8180 HbA1c analyzer(ARKRAY KDK, Japan) was introduced. In this study, we evaluated the analytical performance of ARKRAY ADAMDS HA-8180 HbA1c analyzer and compared it with the NGSP certified Variant II Turbo(Bio-Rad Laboratories, USA).

Methods: According to CLSI EP5-A, Lyphochek Diabetes Controls(Bio-Rad

Laboratories, USA) were used for precision. Two(low and high) levels of QC materials were analyzed twice a day for 20 days. Total precision, Repeatability, Between-run and Between-day were calculated. ARKRAY ADAMDS HA-8180 HbA1c analyzer and Variant II Turbo were compared with 150 samples according to CLSI EP9-A2. The linearity and carry-over rate were also assessed.

Results: Coefficients of variation (CVs) of between-run imprecision for low and high level were 0.0% and 0.3%, respectively. CVs of between-day imprecision for low and high level were 0.3% and 0.2%, respectively. The linearity was excellent with $R^2 = 0.99$ in the range of 3.1~19.3%. A good correlation with Variant II Turbo was observed(R=0.9940). The carryover rate was 0.0%.

Conclusions: The ARKRAY ADAMDS HA-8180 HbA1c analyzer showed excellent precision, linearity and carryover rate. It also showed excellent correlation with NGSP certified variant II Turbo. In conclusion, it is reliable HPLC analyzer for HbA1c and could be very useful for the diagnosis, the treatment monitoring and risk assessment of diabetes.

E-16

Triclonality in immunofixation in plasma cell dyscrasias is not a mere artifact of treatment

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Background: Triclonal patterns (triclonality) in immunofixation (IF) are a very rare finding: our Centre has previously reported an incidence of 0.1% (representing 0.82% of all clonal patterns detected) in an 11-year series of 46 249 immunofixation assays.

We have also reported that in over half the patients (56.2%) triclonality was a transient feature, with patients acquiring triclonality and de-escalating to biclonality or monoclonality, and suggested that this transiency could be a feature of clonal evolution, involution and selection. Such clonal modifications could be induced by chemotherapy (CTx) or bone marrow transplant (BMT) or, alternatively, could represent an intrinsic behaviour of the neoplasm.

Aims: To clarify whether triclonality should be interpreted as merely an artifact of ongoing therapy, or if it could be deserving of attention by the clinician, we studied the relationship between the identification of triclonality in IF in multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) patients and the timing of treatment.

Methods: We analysed all 49 765 IF samples assayed in our Centre from January 2000 to February 24th 2012. We found 58 sera with triclonality, 44 of which were collected from 21 patients with MM and 5 with MGUS; the remaining 14 samples were not related to plasma cell dyscrasias, and were excluded.

Results: We identified triclonality at diagnosis in 7 patients (27%, 6 MM and 1 MGUS). One MM patient died before follow-up; the MGUS case maintained persistent triclonality, as did a MM patient who underwent therapy. All remaining MM patients lost triclonality, half spontaneously and half during treatment.

Two additional MM patients (8%) were diagnosed as "monoclonal" outside our Hospital and referred to us for treatment; triclonality was present upon admission to our Centre, and later lost with treatment.

Of the seventeen patients who were not triclonal at diagnosis, five (19%, 2 MM and 3 MGUS) spontaneously acquired triclonality without therapy. The three MGUS cases later spontaneously lost triclonality, while the two MM lost it with therapy.

The remaining 12 patients (43%, 11 MM and 1 MGUS) acquired triclonality after treatment (CTx or BMT for MM; the MGUS case was exposed to hydroxicarbamide due to concurrent essential thrombocythemia); 10 of the MM cases subsequently lost it.

Discussion and Conclusions: We found that 27 to 35% of patients with triclonality presented with this pattern at diagnosis, with a further 19 to 27% spontaneously acquiring it before treatment. Therefore, for over half the patients, triclonality was a feature of their disease and not a consequence of treatment.

On the other hand, for the remaining 46% of patients, the acquisition of triclonality was temporally associated with CTx or BMT; a causal relationship, however, is impossible to establish on the basis of this work.

We suggest that the appearance of a triclonal pattern on IF, though rare and in most cases transient, should not be discarded as a mere effect of treatment. The clinician should interpret results on a case-by-case basis, as the acquisition of triclonality could be a marker of clonal evolution and disease progression.

E-17

Assessment of Thomas plot in chronic hemodialysis patients contributes to lower iron but not erythropoietin supplementation and stable hemoglobin levels

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Background: Thomas plot (TP) is a recently introduced novel diagnostic method for monitoring erythropoietic responses and functional iron deficiency in order to guide therapeutic decisions regarding iron or erythropoietin supplementation. In chronic hemodialysis (CHD) patients adequate supplementation of iron and erythropoietin is essential for the management of renal anemia. In this study, we aimed to assess the therapeutic value of TP in the management of erythropoiesis in diurnal and nocturnal CHD patients, respectively.

Methods: In a single site hemodialysis center, the amount of iron and erythropoietin therapy was determined by TP (ferritin index: soluble transferrin receptor/log ferritin and the reticulocyte hemoglobin equivalent) in n=61 long-term hemodialysis patients (32 male, 29 female, median age 73 years) from April to December 2011. The therapeutic measure for erythropoietic response was hemoglobin level. The therapeutic consequences (iron, erythropoietin supplementation) due to TP assessment were compared in the same patients to the period from April to December 2010, when therapeutic decisions were based on serum iron, transferrin, ferritin and transferrin saturation (by means of box-and-whiskers-plot, Mann-Whitney-U test). Moreover, comparison of therapy decision from April to June 2011 assessing TP vs. serum iron, transferrin, ferritin and transferrin saturation was evaluated by the inter-rater agreement (kappa-coefficient). Stepwise multiple regression analysis was conducted to identify significant predictors of the erythropoietic management in hemodialysis patients.

Results: Therapeutic decision guiding by TP in 2011 significantly increased erythropoietin supplementation in comparison to conventional diagnostic procedures in 2010 ($P<0.0001$), whereas iron supplementation decreased significantly ($P<0.0001$). However, in nocturnal hemodialysis patients there was no change in the erythropoietin therapy ($P=0.3834$). Significant increases in erythropoietin and decreases in iron therapy between 2010 and 2011 were seen in all etiology subgroups of chronic renal diseases (P all <0.0076) except for diabetic nephropathy and iron supplementation ($P=0.0620$), respectively. Importantly, hemoglobin levels in CHD patients did not differ between 2011 (TP-based) and 2010 (conventional diagnostics). However, haemoglobin levels were significantly higher in nocturnal than in diurnal hemodialysis patients in both periods (medians: 12.1 g/dl vs. 11.5 g/dl; $P=0.0004$). To compare the therapy decision according to TP (B) vs. iron, ferritin and transferrin saturation (A) specifications of TP squares were defined as gold standard classified by CrP lower or higher than 5 mg/dl: true therapy decision (A): 34.4%; true therapy decision (B): 95.1%; weighted Kappa: -0.101 (CI: -0.214 to 0.0126). Thus, no agreement between the two therapy decisions was found. Stepwise multiple correlation analysis of therapy data between April 2011 and June 2011 identified iron therapy ($P=0.003$), serum ferritin ($P=0.0007$) and serum transferrin ($P=0.0083$) as significant predictors of erythropoietin therapy (R^2-adjusted: 0.3557; F-ratio: 12; $P<0.001$). Age ($P=0.0068$), ferritin-index ($P=0.0029$) and TP ($P=0.0001$) were significant predictors of iron therapy (R^2-adjusted: 0.4305; F-ratio: 16.1; $P<0.001$).

Conclusions: TP is a reliable therapeutic decision tool to identify patients for iron supplementation, whereas serum ferritin and transferrin saturation appear more sufficient predictors of erythropoietin management in CHD patients. The efficiency of the shown hemoglobin stabilization and the assessment of the erythropoietic status in end-stage renal disease should be analysed in subsequent prospective multicenter studies.

E-18

The effects of antimicrobial agents on red blood cell deformability in sepsis

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Backround: *Microcirculatory blood flow is altered in sepsis.* Decreased RBC deformability and increased viscosity have been extensively investigated in patients with sepsis. *Changes in hemorheological parameters may contribute to the alterations in microcirculatory blood flow. This study was conducted to observe the effects of antimicrobial agents in sepsis.*

Methods: *In this study, Wistar albino rats (n=36) were included. They were divided in 3 groups. Group 1 received ceftriaxone (50 mg/kg/day), Group 2 received meropenem (100 mg/kg/day), and Group 3 received normal saline solution intraperitoneally for 10 days.* Blood and plasma viscosity were determined in blood samples containing EDTA by Wells-Brookfield LUT cony-plate rotator viscosimeter (MA O20 2072 Engineering Laboratories, Stoughton, USA). The procedure was carried out at a shear rate of 60 rpm. Deformability was determined in blood samples containing EDTA by microfiltration technique in terms of pressure versus cell rigidity. RBC was prepared.

Results: *Erythrocyte rigidity was increased in the ceftriaxone group was increased in the meropenem group (p<0.05). Ceftriaxone resulted in an increase in plasma and blood viscosity.*

Conclusions: *We conclude that some antimicrobial agents may affect rigidity and viscosity. The effects of antibacterial drugs on hemorheologycal parameters may be distinguishing especially when the maintenance of microcirculation is important.*

E-19

Implementation of the SYSMEX XETM analyzers: significant impact on quality improvement of hematological results

M. Molina, E. A. Perobelli, D. R. B. Mansur. *DASA, Barueri, Brazil*

Objective: After many years using a specific Cell Blood Counter (CBC) instrument (called here “previous instrument”), a fully automated SYSMEX XE™ analyzers set was implemented in a core laboratory that performs approximately 10,000 blood counts per day seeking for improving productivity and the use of new parameters available only in this instrument.

Methodology: The %CVs were obtained daily derived from QCs performances. Parameters, such as WBC, RBC, HCT, HGB and PLT, were chosen to comparison and further address quality improvement after change of equipment. Data related to the previous instrument were obtained from January 2009 to December 2010. Sysmex XE™ instruments were installed in the core laboratory facility in late 2010 and data compiled from January 2011 to December 2011.

Results: Significant decrease of the %CV was evidenced after comparing the three years data related to WBC, RBC, HCT, HGB and PLT (Graphic1 - 5). The annual general mean of the %CV of those parameters showed a consequent decreased being PLT the most impacted one (from 4 and 4.21 in 2009 and 2010, respectively to 2.37 in 2011) - Table 1.

Conclusions: During 2011, after the implementation of the SYSMEX XE™ instruments for CBC, a imprecision decrease was observed with important reduction on the %CVs. These findings enabled the use of a lower number of quality control retesting increasing productivity.

E-20

Development and implementation of a method to measure hemoglobin in dried blood spots

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Background: Dried blood spots (DBS) are an increasingly popular alternative to liquid blood samples for a variety of applications, largely due to the small volume requirement and ease of storage and transportation. Recently, there has been demand from out-reach sites for a cost-effective means of hemoglobin determination from DBSs for use in screening for anemia.

Objectives: To develop a rapid and robust screening assay capable of determining hemoglobin concentration from a DBS.

Methods: Blood was collected by finger stick onto filter paper cards and allowed to dry overnight at room temperature. Two 3.2 mm disks were punched from a DBS and placed in a vial with 1 mL of Drabkin's reagent, which reacts with hemoglobin to form cyanmethemoglobin, which can be measured at an absorbance of 540 nm. The specimens were vortexed for 10 minutes to elute the blood and for the reaction of hemoglobin to cyanmethemoglobin to occur. After the specimens settled for 10 minutes, the supernatant was transferred to a cuvette and the absorbance measured at 540 nm. Hemoglobin concentration was calculated using a standard curve generated from patient samples with known hemoglobin concentrations that span the analytical measurement range (AMR). The AMR was set to a fairly narrow range of 8.0-15 g/dL, since the exact volume of blood with blood spot punches can be affected by extremes in hematocrit. Three levels of quality control (QC) materials (Sysmex e-check QC, spotted onto filter paper) are processed with each run; the patient results are accepted if QC values are within the acceptable range.

Results: Initial validation studies demonstrated acceptable inter-assay precision: QC level 1: mean=4.1, CV=9.2%; QC level 2: mean=9.45, CV=5.2%; QC level 3: mean=15.21, CV=5.5%. Patient and QC DBS samples demonstrate stability up to 10 days at room temperature and 60 days at 4 degrees C or -20 degrees C. No significant interference was observed from hemolysis, icterus, lipemia, or the presence of Hb S. Hemoglobin concentrations generated from DBSs were compared to whole blood hemoglobin measured on a Sysmex XE-2100 and a point-of-care HemoCue Hb 201. The comparison with the Sysmex (n=209) generated a slight negative bias (slope = 0.92) with weak correlation (R-squared=0.71). The DBS hemoglobin compared well to the HemoCue (n=20): slope = 0.97, R-squared=0.96. Using a population-based study, age- and gender-specific reference intervals were determined for this assay.

Conclusions: Overall, the presented method for hemoglobin determination from filter paper DBSs demonstrates acceptable analytical performance and stability, making this test both useful and practical, especially for samples that require extended transportation or are delayed in processing. This assay agrees well with the HemoCue method for hemoglobin determination. However, the correlation with the gold-standard hemoglobin assay (Sysmex) dictates that this assay only be used as a screening tool for anemia.

E-21

Standardization differences of the Ferritin method may cause that a large cohort is included or excluded in the detection of empty iron stores.

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Background: To demonstrate that unsatisfactory method standardization between providers may have great impact on the clinical decision making and treatment in the population. A widely perceived clinical standard is that empty iron stores are present when ferritin in plasma is less than 15 µg/L which also is a commonly used lower reference value for women (1).

Provided that the distribution of ferritin results are similar in neighbouring communities in samples from the primary health service, then the effect on the population of different standardizations could be estimated.

Methods: All plasma ferritin values on the patients' first contact with Primary Health Care in North Jutland, Denmark Oct. 2006 - Feb. 2012 were extracted from the Laboratory Information System (N=125 000, age 15-110 years, mean 53 years, women 62%). The samples from the Northern part of North Jutland, Site 1, were analyze on Centaur (TM), Siemens (direct chemiluminiscence immunoassay), from the Southern part of North Jutland, Site 2 on Modular (TM), Roche (electrochemiluminiscence immunoassay). Site 2 analyzed 60-70 % of the samples in the region of 474 000 inhabitants above 15 years.

Results: The practitioners requested ferritin on average in 5.5 of 100 inhabitants per year. The median value of plasma ferritin (µg/L) were, respectively for women and men, from Site 1 (Centaur) 56 and 134, and Site 2 (Modular) 65 and 169. Thus the median level from Modular was 16 % and 25 % higher compared to Centaur.

At the site using Centaur 14 % of women and 6 % of men, and at the site using Modular 10 % of women and 3 % of men had values below the reference values of 15 and 22 µg/L, respectively.

At site 1 using the Centaur-method 10 % had ferritin below 15 µg/L in contrast to only 7 % using the Modular method. Thus, if only one method was used in the whole region then the discrepant standardizations implied that 900 persons each year are classified wrongly, either diagnosing empty iron stores or the results being above the threshold.

Conclusions: • In the region of 474 000 inhabitants 900 persons could each year be wrongly classified to being iron deficient, or being above the threshold due to discrepant method standardization of ferritin.

• It is important to act on this very important issue to improve patient care. Improved standardization of methods in clinical chemistry is prerequisite to obtain effective medical decisions.

• Evidenced based medicine should be based on stringent standardization and transparent threshold values across studies and practices.

1. Milman N. Serum ferritin in Danes: studies of iron status from infancy to old age, during blood donation and pregnancy. Int. J. Hematol. 1996;63(2):103-135.

E-22

Stability of Activated Partial Thromboplastin Time Test at Room Temperature

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Objective: to evaluate the effect of time on activated partial thromboplastin time (APTT) test at room temperature.

Background: APTT is widely used to evaluate inherited and acquired bleeding disorders and monitor anticoagulant therapies. Current CLSI guidelines indicate that APTT testing for nonheparinized patients should be performed within 4 hours (hr) of specimen collection when samples are stored at room temperature. However, this time limit is not very practical for laboratories with outreach programs. In this study, we investigated the stability of APTT test at room temperature beyond the time limit recommended by CLSI.

Methods: The study included 120 samples obtained from hospitalized patients and patients seen at outpatient clinics, of which 102 samples showed normal APTT and 18 samples had abnormal APTT. None of the studied patients were on anticoagulant therapy at the time of testing. Blood samples were collected and immediately centrifuged. The plasma was maintained on the cell mass and APTT was determined using Sysmex CA- 1500 and CA-7000 coagulation analyzers (Sysmex Corporation, Japan) within 1 hr of collection (baseline) and at 4, 8, 12, 16, 20, and 24 hr. Tubes remained capped between time points. In a separate study, blood samples were collected from 10 healthy volunteers and either immediately centrifuged or maintained as whole blood at room temperature. Whole blood samples were centrifuged at the time intervals specified above and APTT was determined in parallel with plasma samples. The percentage changes in APTT were calculated relevant to baseline values and were considered clinically significant if they exceeded 15%, which is the APTT allowable total error (TEa) specified by CLIA. Statistical differences were evaluated using Student's t-test.

Results: APTT values increased over time and the greatest increases were observed at 24 hr. There were no overall statistically significant differences in APTT results at 4, 8, or 12 hr compared to baseline values and all differences were less than 15 %. At 12 hr, the average increase in APTT compared to baseline was 1.1 seconds for samples with normal baseline APTT and 1.6 seconds for samples with abnormal baseline APTT. Statistically and clinically significant changes in APTT were observed at time points beyond 12 hr. At 16 hr, APTT differences relevant to baseline exceeded 15 % for 2 samples. Changes in APTT were greatest at 24 hr with 20 samples showing clinically significant changes compared to baseline results. Samples from healthy volunteers maintained as whole blood and centrifuged at the time of testing showed no clinically or statistically significant differences at 4, 8, or 12 hr compared to those centrifuged at time 0 and maintained as plasma.

Conclusions: Our data demonstrate that samples for routine APTT testing from nonheparinized patients are stable for up to 12 hr at room temperature and these samples can be stored centrifuged or as whole blood.

E-23

Characterization of new real-time hemostasis monitoring device for multiplexed detection of PT/INR, Platelet Activity, Firbinolysis, and Hematocrit

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Background: We introduce a new methodology for rapid, simple, and multiplexed hemostasis determination by means of T2 Magnetic Resonance (T2MR). Multiplexed T2MR results generated by addition of a single, generic clotting agonist are presented. For example, through a single measurement a whole blood clot can be probed for clotting time, platelet activity, and hematocrit, which normally are obtained via three different assays and devices. We anticipate that this new approach will enable more effective decisions for anticoagulation and anti-platelet therapies, as well as selecting appropriate transfusion products during CABG, PCI, and other procedures.

Methods: T2MR of blood samples measures the water molecules diffusing throughout the blood and blood clot during coagulation processes. PT/INR measurements on human plasma and blood samples were obtained with two reference methods (Stago Start system, Hemochron Signature Elite) and T2MR using the same protocol and reagents. Platelet activity measurements from donor samples spiked with 0, 2 or 3 and 4 ug/mL Reopro (Abciximab) were obtained with T2MR using 34 uL citrated whole blood pre-mixed with kaolin (Haemonetics). T2MR and reference method fibrinolysis measurements following the citrated kaolin protocol and reagents of Thromboelastograph (Haemonetics) were performed on patient samples spiked with 50U/mL and 100U/mL of tissue plasminogen activator.

Results: We describe the basic principles of T2MR as it applies to Hemostasis measurements and demonstrate correlations with established methods for measuring PT/INR (n>40, R2>0.9), clotting time (n>20, R2>0.8), platelet activity (n>12, R2>0.90), fibrinolysis (n=12, R2>0.75), and hematocrit (n>20, R2>0.9). Additionally, we present data for the rapid and direct measurement of effectiveness of anti-platelet drugs such as Abciximab.

Conclusions: Prelimiary T2MR hemostasis measurements show great promise for rapid, real-time multiplexed monitoring with small sample volumes (<40 uL) of blood or plasma down to a fingerstick test on a low cost and compact device.

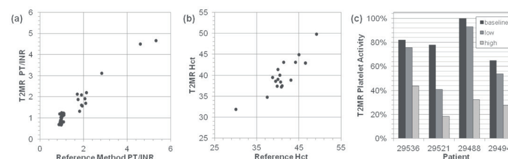

Figure 1. Demonstration of the multiplexing capability of T2MR Hemostasis measurements. (a) PT/INR method comparison, (b) Hematocrit method comparison, and (c) Platelet activity measurements of patients with different levels of Abciximab.

E-24

Reference intervals for early stage red blood cell parameters from physically active population

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Background: Red blood cell (RBC) parameters can be useful tool to diagnose sports anemia which can impair the athlete's performance or to identify possible doping methods such as enhancement of oxygen transport by recombinant erythropoietin abuse. However, to use the RBC parameters in sports medicine it is necessary to establish reference intervals (RI) in a trained population. The aim of this study was to establish RI for RBC parameters in a physically active population.

Methods: The reference population included male volunteers (n=128), with an average age of 18 ± 1 years. They participated for four months in a regular and strictly controlled exercise program, which consisted predominantly of aerobic activities (three hours daily and five days per week). Blood samples were collected under standardized conditions, after 12 h of fasting, in the morning, in tubes with K3-EDTA Vaccuete® (Greiner Bio-One). The samples were run on Sysmex XE-5000® and included: hemoglobin concentration (Hb), reticulocyte count (Ret), immature reticulocyte fraction (IRF) and reticulocyte hemoglobin content (Ret-H*e*). The RI were established following the International Federation of Clinical Chemistry (IFCC) rules using RefVal program 4.1 beta. The outliers were removed by Horn's algorithm. We calculated the non-parametric 2.5th and 97.5th percentiles, with their 90% confidence intervals (CI), by Bootstrap methodology.

Results: The Table 1 shows the RI for RBC parameters in a physically active population compared with non exercised population. IRF were slight higher in physically active population compared to non-exercised population. Hb, Ret and Ret-H*e* were similar in both populations.

Table 1. RI and CI for RBC parameters in physically active population compared with RI for Sysmex XE-5000 in healthy non-exercised population.

Analyte	Coefficient of analytical variation (CVA%)	Reference interval for physically active population	CI 2.5th	CI 97.5th	Reference interval for healthy non-exercised population
Hb (g/dL)	1.1	13.3 - 16.2	13.2 - 13.5	15.8 - 16.4	13.5 - 16.9
Ret (109/L)	3.8	28 - 75	24 - 30	66 - 83	23 - 70
Ret (%)	3.7	0.54 - 1.37	0.5 - 0.56	1.26 - 1.43	0.43 - 1.36
IRF (%)	10.0	3.0 - 14.3	2.7 - 3.6	11.5 - 14.8	1.6 - 10.5
Ret-He (pg)	2.5	30.8 - 39.2	29.1 - 32.1	38.2 - 39.8	32.1 - 38.8

Conclusions: Our results showed few differences in RBC parameters in a physically active population. However, to establish specific RI for exercised population help to improve the sensitivity in the athlete's RBC disorders.

E-25

Prediction of INR response based on CYP2C9 and VKOR C1 genotypes

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Background: Warfarin is the most widely used anticoagulant and its clinical management is difficult because of large inter-patient variability and narrow therapeutic window (INR 2-3). The connection between a measured INR value and a dosing regimen is often difficult to predict and manage for optimum patient safety. *CYP2C9* and *VKORC1* significantly influence the pharmacokinetics (PK) and pharmacodynamics (PD) of warfarin. These genetic factors have a major influence on the relationship between warfarin dosing and INR values. Several mathematical algorithms have been developed to estimate optimum warfarin maintenance dose based on genotyping. However, there is a need to develop simple-to-use computational methods to directly predict the INR response anticipated based on a particular dosing strategy. Using retrospective data from 25 patients undergoing warfarin therapy, we have developed a pharmacometric model based on dosing history, serial S-warfarin plasma concentration measurements, *CYP2C9* and *VKORC1* genotypes and INR responses measured over the first 30 days of initiating warfarin therapy.

Methods: We used a single compartment PK model and indirect response PD model to estimate PK/PD parameters: clearance rate, volume of distribution, IC_{50}, kin and kout using a nonlinear mixed-effect approach. We performed covariate analysis to investigate the relationship between *CYP2C9*, *VKORC1* genotypes and the PK/PD parameters. To assess the goodness-of-fit of the model, we performed five-fold cross-validation on the population predictions using Root Mean Square Error (RMSE) and R^2 as performance measures.

Results: *CYP2C9* was found to correlate with warfarin clearance rate ($R^2 = 0.32$, p = 0.003). Population estimations of warfarin clearance rate were: 0.24 L/hr for wild type, 0.18 L/hr for one variant allele carriers and 0.13 L/hr for two variant allele carriers. *S*-warfarin clearance rate determined for each *CYP2C9* genotype were consistent with those previously reported by our group and others. IC_{50} is correlated with *VKORC1* genotype ($R^2 = 0.31$, p = 0.004). On average 10 sequential INR results were measured for each individual patient and were compared to predicted values. This analysis demonstrated the ability of the model to capture the dose induced changes in INR values. Goodness of fit analysis on the population predictions including a total of 258 INR values revealed a RMSE of 0.73 and a R^2 of 0.33. The median RMSE and R^2 values for individual subjects were 0.61 (0.20 to 1.50) and 0.73 (0.02 to 0.91) respectively.

Conclusions: We conclude that our pharmacometric modeling approach when used during the first 30 days of warfarin therapy has potential for predicting anticipated INR changes based on combining warfarin doses with a patient's genotype information. This modeling approach has wide application and can be applied in conjunction with algorithms to estimate warfarin maintenance dose to refine initiation and maintenance dosing strategies. Diagnostic informatics approaches combining genetic information with dosing patterns to predict INR values may prove beneficial for managing warfarin therapy and assist in achieving and maintaining desired therapeutic responses in less time and with less time out of therapeutic range. (Supported in part by NHLBI R44HL090055).

E-26

Identification of Low Concentration of Hb H by Isoelectric Focusing in Alpha Thalassemia with One or Two Alpha Chain Deletions.

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Background: Alpha-thalassemia results from the deletion of one or more of the four alpha (α)-globin genes and is globally recognized as a common genetic disorder. Four genotypes are possible: silent carrier (αα/-α), α-thalassemia trait (-α/-α or --/αα), HbH disease (-α/--), and hydrops fetalis (--/--). Deletion of all four alpha-globin genes (Hb Barts) is incompatible with life, while Hb H disease usually presents as moderately severe hemolytic anemia. Carriers of α-thalassemia trait have only a mild hypochromic microcytic anemia, but couples with these genotypes are at risk for having a fetus with hydrops fetalis or a child with HbH disease. The most frequent single gene deletions are observed in the African population ($-\alpha^{37}$ and $-\alpha^{42}$); the most common double gene deletions are observed in Southeast Asia ($-^{SEA}$,$-^{FIL}$, and $-^{THAI}$) and the Mediterranean ($-^{MED}$ and-$(\alpha)^{20}$). Detection of HbH by high performance liquid chromatography (HPLC) has a limit of detection of approximately 3.5 percent. This is assumed to be sufficient, since it is thought that only patients with three mutant alleles produce detectable levels of HbH. Our data support that isoelectric focusing electrophoresis (IFE) can detect low levels of HbH otherwise not detected by HPLC alone. We sought to evaluate these patients using molecular analysis to look for alpha globin dene deletions.

Methods: Twenty whole blood patient samples (Nineteen with Hb H<2% and MCV<80 and one negative control) were selected using IFE. None of these specimens had detectable HbH using HPLC. IFE was performed under a pH gradient of 6.0-8.0 using a 1 mm precast agarose gel and reagents found in the RESOLVE Hb Neonatal Hemoglobin Screen Kit (Perkin Elmer, Waltham, MA). HPLC analysis was performed using the manufacturer's instructions for the BioRad Variant II alpha thalassemia Short Program (BioRad, Hercules, CA). Molecular analysis for seven most common alpha globin gene deletions was performed following PCR amplification of alpha globin gene cluster using genomic DNA. The scientist performing the genotype analysis was blinded to the hemoglobin phenotype.

Results: α-globin gene deletions were observed in 19/19 samples with the low HbH phenotype. The negative control (wildtype phenotype) showed a wildtype genotype. The most common deletion observed (10/19) was the South East Asian deletion ($-^{SEA}$) which is a two alpha gene deletion, followed by four patients (4/19) homozygous for a 4.2 kb deletion. Two patients (2/19) were heterozygous for the 4.2 kb single gene deletion, while heterozygous 3.7 kb, $-^{FIL}$ and compound heterozygous 3.7/,$-^{FIL}$ were observed in one of three patients respectively.

Conclusions: Currently it is thought that the only method to detect one or two alpha globin gene deletions is molecular analysis. However, our analysis indicates that low levels of HbH can be detected in a patient with one or two alpha gene deletions using IFE. This finding can offer a cost efficient approach for diagnosis and for genetic screening of patients with one or two alpha gene deletions.

E-27

Factor VIII Point Mutations with Higher Risk for Inhibitor Development in Mild/Moderate Hemophilia A: A Rapid, Specific Test Panel.

J. S. Buzby, M. McDaniel, J. C. Tucker, S. A. Williams, A. Soni, D. J. Nugent. *Children's Hospital of Orange County, Orange, CA*

Background: The conversion of mild or moderate Factor VIII-deficient patients into those with a severe bleeding disorder can be an alarming consequence of inhibitor development, which is not typically anticipated in these patients. Cross-reactivity of the inhibitor antibodies with the patient's own circulating FVIII can reduce their baseline FVIII activity from that of 5-10% to 0-1%, with severe bleeding diathesis. Immune tolerance can be effective, as with more severely deficient cases. But patients must undergo daily desensitization and use costly bypassing agents. While cross-reactivity of the inhibitor antibodies with the patient's circulating FVIII remains poorly understood, there is growing evidence that mild/moderate hemophilia A patients with specific FVIII point mutations are at greater risk than others for developing this life-threatening complication. Current hypotheses suggest that certain mutations result in greater conformational changes in the patient's circulating FVIII, so that the infused FVIII becomes immunogenic.

Methods: Based on review of previously published reports and the Haemophilia A Mutation, Structure, Test, and Resource Site (HAMSTeRS) database, we have selected nine point mutations, associated with multiple cases of mild/moderate hemophilia A, where at least 10% of those patients reported have developed inhibitors. Six of the nine are in the FVIII C-domain (Arg2150His, Trp2229Cys, Tyr2105Cys, Asn2286Lys, Pro2300Leu, and Arg2307Gln) and the other three are in the A-domain (Arg593Cys, Arg1997Trp, and Glu1999Gly).

As an alternative to complete DNA sequencing or heteroduplex analysis, we have developed a more straightforward panel to rapidly test for these point mutations directly using an RFLP-based strategy. Specific restriction endonucleases (New England BioLabs) were identified with overlapping recognition sites for each of the nine point mutations. For seven of the mutations, the restriction site is lost if a mutation occurs. For the other two, the restriction site is gained when the mutation is present.

Results: We evaluated this panel by digesting PCR amplicons from synthetic, control DNA templates (IDT), representing the wild-type and mutated FVIII exons encompassing each of the nine mutations. Differences between the resulting restriction

enzyme digestion patterns were successfully detected with a BioAnalyzer DNA 1000 Kit (Agilent). A pilot study has further tested genomic DNA from eleven pediatric hemophilia A patients, all of whom were negative for each of the nine mutations.

Conclusions: A more rapid, specific molecular screen that could be performed at the initial diagnosis of mild/moderate FVIII deficiency would allow providers to more readily identify those patients at risk for developing a FVIII inhibitor and adjust their treatment accordingly. This form of personalized medicine could help to prevent the potentially tragic outcome of inhibitor development, which can last from months to many years, in those patients that unexpectedly convert into a severe bleeding phenotype.

E-28

D-dimer for Exclusion of Venous Thromoembolism: a Multicenter Performance Evaluation of two Highly Sensitive Assays

E. Spanuth[1], T. Matsuya[2], Y. Okamura[2]. [1]*DIAneering GmbH, Heidelberg, Germany,* [2]*Mitsubishi Chemical Medience Corporation, Tokyo, Japan*

Background: Clinical sensitivities and negative predictive values (NPV) are major criteria for the exclusion of venous thromboembolism (VTE) using D-dimer assays. Aim of the study was to evaluate the performance of the PATHFAST D-dimer assay for the exclusion of VTE and to determine the best cut-off values.

Methods: We conducted a multicenter performance evaluation of the PATHFAST D-Dimer assay in comparison to the STRATUS CS D-Dimer assay. PATHFAST D-Dimer is a point-of-care (POC) chemiluminescence immunoassay. It does not require laboratory staff and the results are available within 17 min from plasma and whole blood samples. 212 frozen citrated plasma samples from patients who presented to the medical emergency room (ER) with symptoms of VTE were measured. The diagnosis of DVT and PE was established by duplex ultrasound, venography and spiral-CT in 41 and 10 patients, respectively. We also determined D-dimer in samples obtained from 82 healthy individuals and 79 in-patients in whom VTE was excluded and who served as control group.

Results: The correlation between PATHFAST and STRATUS results was particularly close in the patient group (r=0.9694), whereas slightly but significantly lower results were obtained with STRATUS in the control group. The cut-off values obtained by receiver-operating characteristic curve (ROC) analysis revealed unsuitable clinical sensitivities and NPVs. With the widely used cut-off value 0.5 µg/ml, PATHFAST demonstrated suitable sensitivity but not STRATUS. ROC analysis indicated that optimal cut-off values could be set at either 0.5 or 0.6 µg/ml and at 0.3 or 0.4 pg/ml for PATHFAST and STRATUS, respectively.

Tab. 1: Performance data of PATHFAST D-Dimer and STRATUS D-dimer for exclusion of venous thromboembo

	PATHFAST D-Dimer Mean (95% CI) (µg/ml FEU)	STRATUS D-dimer Mean (95% CI) (µg/ml FEU)	t-test
VTE patients (N=51)	3.32 (2.85-3.78)	3.20 (2.71-3.69)	p=0.05
Control group (N=161)	0.68 (0.57-0.79)	0.59 (0.48-0.71)	p<0.0001
ROC analysis AUC	0.947 (0.92-0.97)	0.932 (0.90-0.97)	p=0.044
ROC cut-off value(µg/ml FEU)	1.01	0.907	
SENS/SPEC (%)	94.1/82.6	86.3/83.9	
NPV/PPV (%)	97.8/62.3	95.1/62.5	
Selectes cut-off values (µg/ml FEU)	0.5 / 0.6	0.5 / 0.4	
SENS/SPEC (%)	100/60.3 / 100/67.7	94.1/68.3 / 98/61.5	
NPV/PPV (%)	100/44.3 / 100/49.5	97.3/48.5 / 99/44.6	
No. of false negative results	0 / 0	2 / 1	

Conclusion: The PATHFAST and STRATUS assays showed comparable performance and appeared to be suitable for the exclusion of VTE in the emergency room setting. PATHFAST demonstrated superior sensitivity for exclusion VTE, whereas our results for the STRATUS assay indicated that the optimal cut-off value needs to be further evaluated.

Thursday AM, July 19, 2012

Poster Session: 9:30 AM - 12:00 PM

Management

E-29

The Utility of an estimated Microalbumin/Creatinine Ratio (e-MACR).

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Background: Motivation: The current manufacturer of our microalbumin reagent (Ortho Diagnostics, Rahway, NJ) informed us in November, 2010 that because of unspecified manufacturing problems it would no longer be producing microalbumin reagent and could not give us a date when production and shipment might be resumed. Given our volume of microalbumin requests (approximately 1200/month) it was felt that send out to a referral lab was undesirable for both turn-around time and financial considerations.

Methods: We opted to derive an expression that would allow an estimated MACR [e-MACR] to be calculated from the urine total protein/creatinine ratio (UPCR) with both ratios being expressed as mgs protein/gram creatinine. All analytes were determined on our chemistry analyzers according to manufacturer's directions. Analytical precision was estimated using QC data with commercial control material (BioRad: Hercules, CA). Estimates for the analytical precision of the ratio were calculated from the SD and means of the components (PR Bevington and DK Robinson. Data Reduction and Error Analysis for the Physical Sciences]. An equation was developed with data from 31 samples using non-linear regression software (Table Curve 2D: Systat; San jose, CA.]. Validation used an additional 23 samples with data analyzed using Deming regression (EP Evaluator: Data Innovations; South Burlington, VT).

Results: Analytical precision for the ratios were estimated to be 6.75 and 4.4 percent for mean MACRs of 23.5 and 49.1 mgs/gram respectively and for UPCR were estimated to be 4.35 and 4.4 percent for mean values of 462.7 and 618.6 mgs/gram respectively. This analysis based on 31 samples that were simultaneously measured for MACR and UPCR yielded the following expression: $MACR = 7.71 + [.05735 * (UPCR)^{1.33}]$ (r-sq = .956)

The eMACR compared to the measured MACR on the 23 validation samples gave the following result: Calculated MACR = .74 Direct MACR + 7.02 (r=.995)

The slope was significantly different from 1.0 (95% confidence interval: .704-.777). Discussion: A negative bias at values of the MACR above100 mgs/gram was noted but this was of doubtful clinical significance as the upper limit of the reference interval is 30 mg/g. We adopted the following selection rules: 1. Results <= 60 mgs/g were accepted. 2. Results >60 mg/g but consistent with previous data in the patient's electronic medical record were accepted. 3. If e-MACR appeared discrepant compared to past results or there was no previous result the specimen was sent to a referral lab. Over a two week period, while a new source of MA reagent was being validated, 507/547 (92.7%) values of the e-MACR were used without complaint from medical staff.

Conclusions: Although it is likely that individual institutions would have to derive their own estimating equation because of differences in population served as well as in analytical system used an estimated MACR may be useful as a fall back approach in unexpected situations.

E-30

Evaluation of business process redesign methodology and PEEM maturity model approach in a laboratory

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Background: The strategy of any organization is implemented by their processes. In the laboratory, to provide the best possible service level to customers, there is a need for processes with high added value, with minimal rework, maximizing flexibility and agility. Several approaches have been proposed to optimize processes in laboratories, such as Lean Six Sigma and Business Process Mapping and Redesign (BPR). Process maturity models, such as Process and Enterprise Maturity Model (PEEM Model; M. Hammer, 2007) has been proposed to align business management with a process-oriented strategy. The aim of this study was to evaluate a BPR project associated with the PEEM model in a laboratory.

Methods: The project was developed during a three months period, from February to April 2011. The BPR traditional methodology was customized to the laboratory needs, being implemented by a workflow with five main steps: (a) Identification and mapping of pre-existing processes ("AS IS" phase), with context maps (connections between different processes) and process maps (workflow charts), and with a registration of disconnections and opportunities for processes improvement; (b) identification of customer needs and comparative market information analysis; (c) Diagnosis of processes and organization maturity using the PEEM model; (d) Planning of new processes ("TO BE" phase), aligning the findings identified in previous phases of the project; (e) Implementation of the new process.

Results: Were identified 26 different processes in the laboratory (including technical and administrative processes) and registered 52 processes improvement opportunities. Improvement actions were proposed for the new processes, including issues of process, people management and IT. Assessing the processes maturity by PEEM model, critical gaps were identified related to organizational structure and processes performance monitoring (performance indicators management). Evaluating the organization maturity were identified gaps related to knowledge about process management and process governance. The new processes were defined and implemented, providing a better use of the available resources, including people and IT resources, and reducing the total process time (TAT). Were observed with the new processes, a defects and rework reducing, including sample redraws. Additionally, the new processes brought a greater level of agreement to ISO/IEC 17025 normative, a quality standard in which our laboratory is accredited.

Conclusions: The BPR approach and PEEM maturity model were considered fully adaptable, useful and effective to the laboratory needs for process improvement, providing a significant increase in process added-value level, which benefits for the company and its customers. In addition to these immediate results, gains are expected in a medium to long term, related to the alignment of laboratory management for a process-based strategy.

E-33

Miles to Go: Application of Lean-Six Sigma Protocol in a Hospital Laboratory

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Background: There has been a continuous challenge in the health care sector to provide reports in clinically relevant turn around time (TAT), while maintaining highest level of quality credentials. Our aim is to pursue Lean-Six sigma in a hospital laboratory, in order to improve TAT through process improvement and eliminate error in process design through validation of tests.

Methods: Our current state value stream maps (VSM) identified opportunities to use Lean-Six Sigma strategies in our process flow. Therefore, we designed the laboratory process flow according to DMAIC (Define, Measure, Analyze, Improve and Control) flow. In the define phase, the tools we used were project charter, SIPOC, CTQ tree. In the measure phase, we used prioritization matrix, VSM, data plots and patterns, process capability. Here we calculated DPMO (defects per million opportunities) and expressed the value as a sigma rating. In the analyze phase, tools used were VSM, gap analysis, root cause analysis. In the improve phase, we used brainstorming, decision analysis matrix. In the control phase, the tools were control charts, audits etc. In our laboratory, Lean- six sigma strategies were applied in process improvement (TAT compliance) and in process design (validation of all tests).

Results: In our process improvement case study, we found that after receiving the STAT sample (with particular reference to Troponin I), both the non value added (NVA) times and value added (VA) times were around 45 minutes. So we eliminated NVA steps and our current TAT came down to 45 minutes from 1.5 hrs. For Troponin I TAT, in August, 2010, we had 111 Troponin I samples, 28 noncompliance (i. e., not reported within 45 minutes), 74.7% compliance, 50450 DPMO and 3.14 sigma. In July, 2011 for 257 Troponin I samples, it improved to 5 noncompliance, 98.8% compliance, 3891 DPMO and 4.16 sigma. In the last one year period from August, 2010 to July, 2011, our monthly sigma values were 3.14, 3.11, 3.20, 3.53, 3.61, 3.72, 3.85, 3.87, 3.89, 4.43, 4.33 and 4.16, respectively.

In our process design case study, validation of tests [with particular reference to prenatal screening tests, i.e., Free Beta-Human Chorionic Gonadotropin (Free Beta-hCG) and Pregnancy Associated Plasma Protein A (PAPP-A) in Cobas e 411] were done by verifying reference intervals, analytical accuracy and precision, inter-assay

and intra-assay variations, limit of detection, linearity and reportable range, i.e., analytical measurement range and clinically reportable range. Our obtained average Multiple of Medians (MOM) values for Free Beta-hCG were 1.13 at 11-12 weeks of period of gestation (POG), 1.25 at 12-13 weeks POG and 1.85 at 13-13^{+5} weeks POG. Our obtained average MOM values for PAPPA were 1.21 at 11-12 weeks of POG, 1.32 at 12-13 weeks POG and 1.55 at 13-13^{+5} weeks POG.

Conclusions: Lean-six sigma helped us in elimination of NVA steps and in focusing on the value added VA steps in process design and process improvement. It ensures that accurate and precise results are reported in a clinically relevant TAT.

E-34

The preanalytical stage of laboratory investigations; Analysis of the status of phlebotomy training and skills in three regions of the Russian federation

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Background: Three distinct steps can be identified in the measurement procedure : preanalytical, analytical and postanalytical. It has been reported that a majority of errors (60 - 70 %) occurs during the preanalytical step, which is heavily depending on human factors. Phlebotomy is a most important part of the preanalytical step. In the Russian Federation blood samples are collected by medical nurses employed by clinical divisions of the Health Care System. The aim of the present study was to analyze skills and knowledge of the nurses.

Methods: A questionnaire comprising 24 questions was developed and distributed to 153 nurses in State and private outpatient clinics and hospitals in 3 cities in different regions of the Russian Federation; St.-Petersburg (North-West Region), Khanty-Mansiysk (Siberia) and Noyabrsk (Siberia). The questionnaire addressed the demographics of respondents, their professional experience and particularly phlebotomy training using evacuated blood collection systems (EBCS), dealing with difficulties encountered during phlebotomy, transport/transfer of blood samples and safety issues/information support. Answers were de-identified and then evaluated using general statistical methods.

Results: The investigation revealed that about 20 % of nursing staff was not trained in EBCS systems. Of those that were trained 55% attended courses given by manufactures of EBCS and 45 % were trained by their colleagues. Half of respondents (50 %) had difficulties to answer to the questions concerning method for obtaining samples with EBCS. 86 % of phlebotomists answered questions about rules for application of a tourniquet incorrectly and 75 % were unaware of the volumes of blood required for different types of evacuated tubes. More than 50 % of the respondents reported difficulties during venipuncture. The most common problems were repeat sampling (80 %), difficulties to locate a vein (40 %), needle-stick injury (28 %), venipucture of neonates and children less than one year old (10 %). The most frequent reasons for repeated sample collection were: hemolysis (80 %), errors in sample labeling (16 %) and broken evacuated tubes (4 %). The survey revealed that 7 % of nurses refused to use EBCS and preferred to work with traditional methods.

Conclusions: The study indicates that there is an insufficient training of phlebotomists in using EBCS. This creates a potential risk for patients and needs attention in formulating the curriculum for medical nurses within the Russian Federation Health Care System.

E-35

Designing QC Rules in the Presence of Laboratory Bias: Should a QC Rule be Centered on the Instrument's Mean or the Reference Mean?

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Background: Objective - Evaluate and compare performance of QC strategies centered on the individual instrument mean versus a reference mean.

Relevance - Using a reference mean rather than the individual instrument mean for QC rules appears to be a common practice when multiple analytic units evaluate the same analyte. The comparative efficacy of this approach has not been formally evaluated. We compare the expected number of unreliable results reported due to the occurrence of an out-of-control condition, $E(N_U)$, when the QC rule mean is centered on the instrument mean versus the reference mean.

Methods: We assume an allowable total error (TE_a) of 10%, 100 patient examinations between QC events, 1:3s/2:2s/R:4s QC Rule, 2 QC levels, and instrument CV and bias combinations that give sigma values, (TE_a-bias)/CV, ranging from 3.5 to 5.9. We compute $E(N_U)$ using published equations (Clin Chem 2008, page 2051) over a range of systematic error out-of-control conditions when the QC rule mean is set to the instrument's mean and the reference mean. In each case we determine the maximum value for $E(N_U)$ and area under the $E(N_U)$ curve.

Validation - The mathematically derived $E(N_U)$ curves were recomputed using simulation. The simulation results matched the direct computations.

Results: Results are shown in the table.

Efficacy of Using Instrument Mean vs. Reference Mean

Instrument			Instrument Mean E(NU)		Reference Mean E(NU)	
CV(%)	Bias(%)	Sigma	Max	AUC	Max	AUC
1.6	0.5	5.9	0.015	0.053	0.008	0.046
1.6	1.0	5.6	0.038	0.123	0.014	0.088
1.6	1.5	5.3	0.091	0.302	0.032	0.192
2.0	0.5	4.8	0.404	2.237	0.244	2.107
2.0	1.0	4.5	0.762	3.670	0.355	3.049
2.0	1.5	4.3	1.420	6.627	0.587	4.441
2.4	0.5	4.0	2.898	21.694	1.919	21.020
2.4	1.0	3.8	4.804	29.398	2.489	25.816
2.4	1.5	3.5	7.965	43.597	3.583	29.196

Conclusions: Using the reference mean for the QC rule reduces the risk of reporting unreliable patient results. The reduction in the worst-case E(NU) ranges from 34% to 65% when the QC rule mean is set to the reference mean instead of the instrument's mean for the cases studied. The reduction across all out-of-control conditions characterized by the area under the E(NU) curves ranges from 3% to 37% when the reference mean is used. In general, the improvement in QC rule performance using the reference mean increases as the ratio of instrument bias to imprecision increases.

E-36

Control containing ten analytes for the quality assessment of specialised clinical immunoassays.

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Background. Assayed human quality control material with an extended range of analytes applicable to complex clinical immunoassays are beneficial for the monitoring of the accuracy and precision of an increased number of tests . As such, multi-analyte control material containing procalcitonin, 25 hydroxy vitamin D and its physiological active form 1-25 hydroxy vitamin D, osteocalcin, parathyroid hormone (PTH), C-peptide, insulin, anti-thyroglobulin antibodies (anti-TG), anti-thyroid peroxidase antibodies (anti-TPO) and insulin-like growth factor (IGF-1) can be used in a variety of clinical fields related to sepsis, bone and mineral metabolism, diabetes, autoimmune disorders and growth related disorders. This study reports the evaluation of a developed human multi-analyte control material including these ten analytes, which is of interest for the quality control of an expanded scope of specialised clinical immunoassays addressed to a variety of clinical conditions.

Methods. Multi-analyte human immunoassay sera, containing 10 analytes, were generated at three levels covering normal and pathological ranges. Each level of control material was dispensed in 2ml vials and freeze dried. Values were assigned using different immunoassay systems from up to 170 laboratories following manufacturers' instructions. The frozen stability was tested for each analyte by reconstituting and storing for 28 days at -20 degrees Celsius a vial of each level of multi-analyte control before thawing and calculating percentage recovery from a freshly reconstituted vial. The open vial stability was determined for each analyte as the percentage recovery of each level stored at +2-+8 degrees Celsius at time points of 4 hours to 5 days related to freshly reconstituted material. The shelf life stability was tested by storing each freeze dried multi-analyte control level for 24 months and testing at approximately 6 months intervals over this period and calculating the percentage recovery from freshly reconstituted material which was stored at -80 degrees Celsius for the same period of time.

Results. The frozen stability data showed percentage recovery in the range 91-108% after 28 days. The percentage recovery of reconstituted control material stored at +2-+8 degrees Celsius at time points of 4 hours to 5 days compared to freshly reconstituted material was in the range 90-108% for 25 hydroxy vitamin D, 1-25 hydroxy vitamin D, anti-TPO, insulin after 5 days; 93-102% for osteocalcin and PTH after 4 hours; 95-109% for anti-TG after 3 days; 91-105% for C-peptide, IGF-1 and procalcitonin after 1 day. The shelf life data showed a percentage recovery of control material in the range 90-110% after 24 months for all the analytes at each concentration level.

Conclusion. Data indicate that the developed tri-level multi-analyte immunoassay control includes ten analytes at three levels in a stable lyophilised form for use in specialised clinical immunoassays.

E-37

Errors in Laboratory Medicine: an Assessment by a Decade of External Quality Assurance

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Background: Reducing medical errors is a goal of health systems on a global basis. Identifying sources of laboratory error contributes to overall quality improvement efforts. Failure rates derived from proficiency testing data provide an objective source of acceptability of laboratory results and contribute to the quality-improvement cycle. They can also serve as basis for revision of criteria of acceptability in proficiency testing.

Methods: Results submitted in the proficiency testing programs in the categories clinical chemistry, endocrinology, toxicology, and hematology carried out by the NY State Department of Health over the 10-year period 2000-2009 were reviewed for acceptability. Failure rates for 87 analytes were determined. An in-depth analysis of a single year (2009) was undertaken to further characterize the nature of unsatisfactory results for systematic errors, random errors, specimen mix-up, data entry errors, and method-selection errors.

Results: Over 3.1 million proficiency test results were reviewed encompassing 33893 unacceptable results and an overall defect rate of 10.8 parts per thousand (ppt) was found. Defect rates among CLIA disciplines were: clinical chemistry (8.0 ppt); endocrinology (20.0 ppt); toxicology (9.4 ppt); and hematology (11.1 ppt). Among the highest defect rates were: sodium (14.9 ppt), free triiodothyronine (44.8 ppt), digoxin (17.4 ppt), and cell identification/morphology (24.7 ppt). Among the lowest defect rates were: potassium (2.6 ppt), qualitative hCG (2.4 ppt), theophylline (4.8 ppt), and leukocyte count (3.5 ppt). Unsuccessful performance, as defined by CLIA'88 regulations, was observed at ~30 per 70,100 opportunities per year (0.39 ppt) for the above categories, Even in cases where "peer" evaluation was used, systematic errors were found to exceed random errors.

Conclusions: Unacceptable results were largely attributable to analytical error and not due to other sources (Table). CLIA-unsuccessful performance (two failures/three consecutive events) was relatively infrequent (0.39 ppt or ~5-Sigma), while the error rate of any unsatisfactory result was considerably larger (3-10 ppt or ~3-Sigma).

Table: Error rates (ppt) for 2009

Error type	Fibrinogen	Glucose	Urea	TSH	Digoxin	Leukocyte Count
Analytical: Systematic	13.4	5.2	2.8	7.8	5.6	1.7
Analytical: Random	0.9	2.7	2.3	3.2	1.7	1.0
Specimen Mix-up	0.6	1.4	1.8	0.5	2.3	0.6
Data Entry	0.3	0.0	0.0	0.0	0.0	0.0
Incorrect Method Selection	0.0	0.0	0.0	0.0	0.0	0.0
Total	15.2	9.3	6.9	11.5	9.6	3.3

E-38

Differences in laboratory requesting patterns in emergency department in Spain

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Background: Compare laboratory requiring patterns in patients admitted to emergency department (ED) during 2010, in 36 Hospitals in Spain.

Methods:15 chemistry tests ordered by ED physicians during 2010 were examined in a cross-sectional study. Data were collected from laboratory databases and indicators that measured every test request per 1000 ED admissions or related test requesting ratios were calculated.

Results: Figure shows mean, median, range and variability index (Percentil90/Percentil10) of every indicator result. Each group was sorted according variability index. The mean/median analysis show that the frequency of ordering any of the stat tests ranged from 5 to 41 per 100 ED patient's admissions.

Conclusions: Indicator results, a simple way of detecting inappropriate use of pathology tests, show that considerable variability exists in the use of every stat laboratory test by physicians in 36 ED. Variability between centers is extremely high, especially in the less requested tests, despite clear indications of such request in emergency setting, indicating that can be often determined as a matter of routine or out of habit in some areas. These large variations of test requesting included stat tests that are clearly redundant, as urea/creatinine and AST/ALT.

The high variability of indicator results shows a probable stat abuse and misuse, a dangerous issue in Emergency setting. Requests that are not justified may lead to delays in testing for patients who have truly life-threatening conditions.

Appropriateness indicators can be applied across a spectrum of clinical laboratories, being useful for comparing requesting patterns. The study highlighted the need to unify demand by optimizing the use of appropriate tests, through interdepartmental communication to achieve a good use of diagnostic testing, on which many emergency clinical decisions are based.

	Mean	Median	Range	Variability Index
Tests requesting per 1000 ED admissions				
Creatinine	412,1	373,6	218,4-790,9	2,40
Glucose	408,7	372,7	217,9-788,3	2,41
Sodium	408,8	370,5	221,2-798,8	2,41
Potassium	406,2	364,5	221,2-755,9	2,43
Troponin T	98,3	93,4	25,3-193,4	2,52
Urea	380,5	366,5	87,3-709,5	2,63
Urinalysis	155,2	148,7	27,1-511,8	3,55
Amilase	89,0	84,6	0,1-292,7	5,20
Creatine Kinase (CK)	113,2	106,7	6,5-309,1	13,58
C-Reactive protein (CRP)	203,4	217,7	1,7-467,3	14,41
Calcium	44,0	26,4	1,7-183,3	30,98
Alanine transaminase (ALT)	112,3	92,6	1,3-336,6	53,39
Total bilirubin	89,5	74,7	0,2-267,0	60,42
Protein, total	52,0	24,5	0,3-309,3	79,72
Aspartate transaminase (AST)	123,3	80,0	0-489,6	∞
Related test requesting ratio				
Urea/Creatinine	0,93	0,99	0,25-1,12	1,40
CK/Troponin T	1,27	1,09	0,13-7,36	12,33
AST/ALT	5,53	0,99	0-122	∞

E-42

Evaluation of Non-conformances Identified in Point of Care Section of Ontario Laboratory Accreditation

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Introduction: Quality management of point-of-care testing (POCT) in hospitals carries distinctive challenges. In Ontario, Quality Management Program - Laboratory Services (QMP-LS) conducts a mandatory accreditation program for licensed laboratories in the province. In addition to ISO 15189:2007, Ontario Laboratory Accreditation (OLA)'s 15189Plus™ accreditation requirements are based on ISO 22870:2006 *Point-of-Care testing*. The aim of this study was to determine quality management challenges for POCT in health-care institutions.

Methods: Non-conformances identified in the POCT section of OLA requirements were evaluated by the aggregate data observed in accreditation visits of 136 laboratories. POCT section of OLA requirements consists of 10 subsections: Responsibility, Personnel Policies and Training, Document Control, Purchasing and Inventory, Process Improvement, Equipment, Pre-analytical Processes, Analytical Processes, Quality Assurance, and Post-analytical Processing. In this study, top ten non-conformances were calculated based on number of conformances divided by number of applied requirements evaluated. To investigate the effect of defining responsibility and the scope of POCT on non-conformance rates outside the Responsibility section, laboratories were divided into two groups (Group 1 and 2). Group 1 experienced non-conformances in the Responsibility section, but Group 2 did not.

Results: Top three causes of non-conformances were: 1. Lack of definition of the

scope of the POCT in the facility (28.3 %), 2. Absence of clearly defined units, reference intervals, date, and/or time associated with POCT results in patient records (27 %), and 3. Lack of resource personnel (24.8 %). Other non-conformances in the top ten lists were: unauthorized persons' performing tests (23 %), issues related to application (21.2%) and absence of training programs (21.2 %), lack of correlation studies between POCT and laboratory methods (21.2 %), and verification of manufacturers' analytical performance claims (17.7 %), not generating or recording quality control data (17.1 %), and lack of internal audits for POCT section (16.7 %).

There were 43 laboratories with and 70 without non-conformances in the Responsibility section of POCT requirements (Group 1 and 2, respectively). In Group 1, 79 % of the laboratories also had non-conformances in the Other sections, while this rate was 46 % in Group 2.

Conclusions: When institutions implement a quality management system, the laboratory director is responsibile for policies, procedures and appropriate measures to monitor POCT within the institution, regardless of where the examinations are performed. If roles of authority, responsibility and accountability are clearly defined, the occurrences of non-conformances in the Other sections were found to be decreased. However, in spite of the mandatory accreditation requirement that POCT services shall be managed by the laboratory regardless of location in the institution, some POCT devices used were not included in the scope of quality management systems.

Inaccuracy in the reporting of patient results, and training of the personnel ensuring that tests are performed by authorized personnel are also important challenges that caused non-conformances at 1 out of 5 institutions, at minimal.

E-43

A Multinational, Multisite Study of Suggestions for Assessing Method Bias and Reproducibility with Some Recommendations

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Background: In seeking guidance to update our protocols for assessing method bias and imprecision, we found that four organizations (CLSI, AACC, CLIA and the Province of Ontario (OLA)) offer a diversity of suggestions regarding the number of points (20 - 200) for measuring bias, the number of runs (no suggestion up to 5 runs), the number of days for reproducibility data (no suggestion to 20), and statistics to assess the data (no suggestions in one case). There is no consensus on any of the variables mentioned in the suggestions; more than 20% of the slots for reproducibility are empty. None of these groups makes a recommendation dealing with changes of reagent or calibration lots. Our study was undertaken to provide robust answers to these questions.

Methods: Method bias data from 6 analytes (cyclosporine, BNP, TSH, vitamin B-12, free phenytoin and creatinine) from four laboratories were studied. We also studied data from protime and APTT using two coagulation instruments. The number of paired patient samples ranged from 23 to 80. We simulated data (20 to 100 points) to extend the laboratory data in 50 additional experiments. The data were evaluated using slope-intercept, t-tests (paired and unpaired), the correlation coefficient (r), the standard error of the estimate (Sxy), and per cent bias. The difference plots were studied. Reproducibility was measured using 30 replicates within a run and for run-to-run and across reagent/calibration changes. ANOVA, the F-ratio and the t-tests were used to assess these data. The analysis of means plots aided in assessing these data.

Results and Conclusions: 1) The paired t-test is the most sensitive statistic to measure a bias between methods. A bias (between methods or between lots of reagents or calibrations) of $\geq$2% can be detected with as few as 20 points using the paired t-test. 2) The difference plots and 3) the analysis of means plots are visual tools that supplement the t-test. 4) Slope and intercept data are sometimes difficult to interpret and should be used cautiously. 5) Because the correlation coefficient and Sxy are measures of random error, they are not useful in bias studies. 6) Reproducibility can be determined with as few as 8 replicates when the CV is less than 10%. 7) The F-ratio should be used to measure differences in precision. 8) The t-test is the most useful to detect lot-to-lot variation. As with bias, a variation of 2% can be detected. 9) Using both patient samples and control material are recommended for assessing changes in lots of calibrator and reagent(s).

E-44

A Door Not Closed: A Systematic Review of Unacknowledged Send-out Results

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Background: Send-out testing volumes have increased in the past decade due to an increased number of tests, including genetic and other esoteric tests. Send out tests are associated with error and patient harm because of their longer turnaround times, complex logistics involving multiple reference labs, difficult result interpretations, and the lack of systems for guaranteeing that the tests are acknowledged.

Methods: This study aimed to evaluate the state of unacknowledged abnormal send-out results at a pediatric hospital in the western United States. Send-out tests that were excluded from the study were nutritional and allergy testing. Adult (>18 yr) patients and deceased patients were also excluded. All send-out tests that met the criteria were evaluated prospectively during a one month convenient sample, and characterized by total volume, test ordered, normal/abnormal results, reference lab, and order-to-result turn-around time. We defined the Time to Acknowledge and Abnormal Send-out Result (TAASR) as the time it took for the provider to acknowledge a result after it was available for viewing in the electronic medical record. Unacknowledged abnormal results were further analyzed by sending a questionnaire to the ordering provider and assessing possible patient harm.

Results: During this time, 1254 send-out tests that met the above criteria were sent to 59 reference laboratories. The median order to result turn-around time was 3 days (mean 7 days). Seventeen percent of the results took longer than one week to return, with a maximum of 286 days. Of these results, 22% (281) were abnormal. Of these abnormal results, 55% (154) were acknowledged in the chart, and 45% were not. The TAASR had a median of 7 days (mean 13 days). Forty-eight percent of acknowledged results took greater than one week to acknowledge, with a maximum acknowledgement time of 60 days. Preliminary data from the questionnaires included 17 responses. Three cases resulted in patient harm classified as failure to treat due to delayed diagnosis. Of the 12 cases that were classified as causing no harm, three involved presumptive treatments, two were acknowledged without a note in the EMR, and four results were not clinically significant.

Conclusions: We found that abnormal send-out test results are unacknowledged 45% of the time and preliminary data suggest a harm rate of 1%. Unacknowledged results can lead to patient harm by delaying diagnosis and failing to treat, receiving inappropriate treatment, increasing length of hospital stay, transient or prolonged morbidities, and death. Estimating error rates is not trivial, and future work will include further characterization of harm in a larger number of cases. This evaluation will enable us to identify appropriate interventions to target the areas that cause the most harm defined by frequency of occurrence and severity.

E-45

A Medium Sized Lab Solving Complex Problems by Collaborating with a Local Academic Industrial Engineering Group that Emphasizes Math Modeling.

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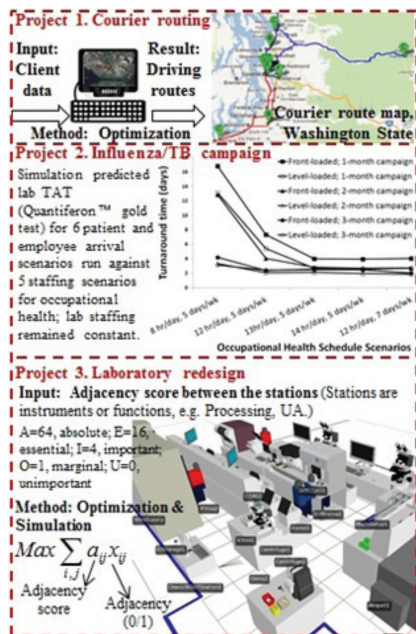
Background: Our hospital uses Continuous Performance Improvement (CPI), emphasizing Lean, as its problem-solving approach. CPI has improved our lab's performance, but we struggle with complex problems, including lab coordination with other departments, specimen transportation, and lab design.

Methods: Following the lead of larger labs, we sought help from industrial engineers. Our approach is collaboration with the University of Washington Department of Industrial and Systems Engineering, an academic group focusing on healthcare. UW collaborators consist of 1 faculty, 1 postdoctoral fellow, 1 graduate student and 8 undergraduates. They interact with lab staff (N=165; 7 doctoral level laboratorians) through numerous ad hoc meetings and a weekly management meeting. The collaboration's goals are solving complex problems, publishing applied research, and educating UW students and our lab staff. The collaboration has focused on problems amenable to math modeling as a solution.

Results: Two projects are completed and one is in progress. The completed projects are: 1) optimization modeling to establish efficient routes for transporting specimens between two labs and several clinics in Seattle; 2) simulation modeling to inform

resource planning for a TB-testing, influenza vaccination campaign in which > 4000 employees had concurrent TB testing and flu vaccination over 90 days. This model linked activity in 2 departments, lab and occupational health. The project in progress is redesign of a 24/7 core lab with 35 FTE. An analytic model determines potentially usable lab layouts, and the results feed into a simulation model which estimates layout performance regarding turnaround times. Figure 1 illustrates the 3 projects.

Conclusions: We are collaborating with an academic industrial engineering group to solve complex problems, using mathematical modeling. Such collaboration can help labs unable to hire engineers or engineering consultants. The projects are challenging, meaningful, and produce research scholarship, while providing novel educational opportunities for students and lab staff.



E-46

Economic analysis of the randomised assessment of treatment using panel assay of cardiac markers - contemporary biomarker evaluation study (RATPAC CBE)

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Objective: To estimate the potential cost-effectiveness of using highly sensitive troponin assays (at presentation alone or presentation and 90 minutes later) and new cardiac biomarkers instead of 10-12 hour troponin measurement.

Methods: A decision tree model was developed to explore the costs and health outcomes associated with different diagnostic strategies. The model took an economic perspective of the NHS in England and Wales and a lifetime horizon with mean life expectancy based on UK interim life tables and applied different testing strategies for myocardial infarction (MI) to a hypothetical cohort of patients presenting to hospital with symptoms suggestive of MI, but with no diagnostic ECG changes. Diagnostic data was taken from RATPAC-CBE trial (Randomised Assessment of Treatment using Panel Assay of Cardiac markers Contemporary Biomarker Evaluation) for high sensitive cardiac troponin T (cTnT) and cardiac troponin I (cTnI) measured on the Stratus CS plus heart fatty acid binding protein (hFABP).

The following diagnostic strategies were applied to each patient:

No testing: Discharge all patients without treatment.

High sensitivity troponin at presentation: Discharge if negative or admit for troponin testing at 10-12 hours if positive. (Sensitivity cTnT 0.78, cTnI 0.79).

High sensitivity troponin and hFABP at presentation: Discharge if negative or admit for troponin testing at 10-12 hours if either test is positive. (Sensitivity cTnT plus hFABP 0.86, cTnI plus hFABP 0.92).

High sensitivity troponin at presentation and 90 minutes: Discharge if both tests negative or admit to hospital for troponin testing at 10-12 hours if either test is positive.(Sensitivity cTnT 0.83, cTnI 0.95)

Standard troponin testing at 10-12 hours. (Sensitivity 1.00)

It was assumed blood tests performed at presentation were undertaken in the emergency department (ED) and that results would be available and a decision made within two hours of sampling. Troponin measurement at 10-12 hours was the reference standard for MI.

Results: At the £20,000/Quality Adjusted Life Year (QALY) threshold, ten hour troponin testing is cost-effective (£12,090/QALY) when immediate discharge occurs but not in the other scenarios, where the ICER for ten hour troponin, compared to high sensitivity cTnT or cTnI alone or plus hFABP at presentation, exceeds £20,000 per QALY, so it is unlikely to be considered cost-effective. In the other two scenarios (once daily ward and twice daily ward rounds), the analysis shows that the strategies based on high sensitivity cTnT or cTnI and hFABP at presentation are likely to be considered cost-effective compared to the next most effective alternative using a £20,000/QALY threshold.

Conclusions: The results showed that, as expected, effectiveness (QALYs) increased with increasing sensitivity and costs increased with decreasing specificity. Measurement of high sensitivity troponin alone at presentation was the most cost effective. At £20,000/QALY threshold, in all but one scenario a strategy of measuring high sensitivity cTnT or cTnI and hFABP at presentation (with admission or a ten hour troponin if positive and discharge home if negative) was the optimal strategy.

E-49

Suggestion of Selection Criteria for Delta Check Methods for Clinical Chemistry Test Items Based on the Ratio of Delta Difference to Width of Reference Interval

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Backgrounds: Many laboratories have commonly used 4 delta check Methods delta difference, delta percent change, rate difference, and rate percent change. However, well-defined guidelines and research related to the selection criteria for delta check methods have not yet been provided. We performed large-scale analysis to suggest selection criteria for delta check methods for each clinical chemistry test item.

Methods: We collected a total of 811,920 and 669,750 paired (present and previous) clinical chemistry test results for inpatients and outpatients, respectively. The coefficient of variation (CV%) of absolute delta difference (ADD) and Pearson correlation coefficient between ADD and time difference were used as decision criteria for delta check methods on 27 items. Also, we categorized delta check methods based on the ratio of delta difference to width of reference interval (DD/RI) and compared them with both those based on the CV% of ADD and those reported in two previous studies.

Results: The suggested delta check methods based on the DD/RI corresponded well with those based on the CV% of ADD except for only two items both in the inpatients and outpatients. Compared with delta check methods suggested in the two previous studies, the new delta check methods based on the DD/RI also corresponded with them except for one and seven items in the inpatients and outpatients, respectively.

Conclusions: DD/RI seems to be more feasible, intuitive selection criteria and can easily explain the changes in results by reflecting both the biological variation of test item and clinical characteristics of patients in each laboratory. We suggest this rule as a measure to determine delta check methods of clinical chemistry test items.

E-50

Preanalytical automation: a good tool to guarantee the diagnostic blood specimens integrity?

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Background and Objective: Several studies demonstrate that automation reduces laboratory turnaround time. Our aim was to evaluate if preanalytical automation improves quality and patient care safety.

Methods: 7mL of whole blood from 100 fasting volunteers, was collected into two (3.5mL) gel separator vacuum tubes with 52.5 USP lithium heparin to obtain plasma; needles and vacuum tubes were of the same lot. Before analysis, all samples were left in upright position for 10 min at room temperature(20ºC) to allow complete *in vitro* anticoagulation; then one sample from each volunteer was respectively assigned to: (G1) *Traditional processing,* starting with centrifugation

at 1200*g* for 10min at room temperature; (G2) *Preanalytical laboratory automation* with MODULAR® PRE-ANALYTICS EVO - MPA system (Roche Diagnostics GmbH). The panel of tests included: glucose(GLU), total cholesterol(COL), HDL-cholesterol(HDL), triglycerides(TG), total protein(TP), albumin(ALB), blood urea nitrogen(BUN), creatinine(CRE), uric acid(UA), alkaline phosphatase(ALP), amylase(AMYL), aspartate aminotransferase(AST), alanine aminotransferase(ALT), g-glutamyltransferase(GGT), lactate dehydrogenase(LDH), creatine kinase(CK), total bilirubin(BT), direct bilirubin(BD), phosphate(P), calcium(CA), magnesium(MG), iron(FE), sodium(NA), potassium(K) and haemolysis index were performed in duplicate on the same instrument cobas® 6000 <c501> module (Roche Diagnostics GmbH). G1 samples were uncapped manually and immediately placed into the instrument. G2 samples were directly fed from MPA to instrument without further staff intervention. At the end: i) the G1 samples were stored for 6 h at +4°C as prescribed in our accredited laboratory; ii) the G2 samples were stored for 6 h into the MPA *output buffer*. The clinical chemistry results from G1 and G2, before and after storage were compared by paired Student's t-test after checking for normality. As non-normal distribution was found for some test results, Wilcoxon ranked-pairs test was employed. The level of statistical significance was set at $P < 0.05$.

Results: Significant increases were observed in G1 vs G2 samples as follows: a) before storage for: ALP, LDH, FE and haemolysis index; b) after storage for: COL, TG, TP, ALB, BUN, CRE, AU, ALP, AMY-P, AST, ALT, GGT, LDH, CK, CA, FE, NA, K and hemolysis index. Moreover significant increases were observed in: c) G1-after vs G1-before storage samples for: COL, HDL, TG, TP, ALB, BUN, CRE, AU, AST, ALT, LDH, P, CA, MG, FE, NA, K and hemolysis index; d) G2-after vs G2-before storage only for: LDH, P and CA.

Discussion and Conclusions: we explain our results as follows: reduced evaporation time due to much faster recapping of primary tube in MPA system than in traditional system; in fact on cobas® 6000 <c501> module with MPA system, clinical chemistry tests are performed immediately after aliquoting plasma in barcoded cups. In traditional processing the primary tubes remain open inside instrument during all the testing process (like the majority of clinical chemistry analysers), and are routinely recapped and stored manually only when the rack tray is full. In conclusion our results show that the MODULAR® PRE-ANALYTICS EVO - MPA system (Roche Diagnostics GmbH) improves the quality of laboratory testing and adds safety to the following patient treatments.

E-51

Under the Microscope: A Global View of Medical Error Disclosure

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Background: In any health care process, adverse events resulting from errors are inevitable (Institute of Medicine, 2001). Disclosure of an adverse event is an important element in managing the consequences of a medical error. We have previously reported a non-punitive, "no-fault" model for reporting medical and clinical errors. Although attempts have been made to minimize adverse events and medical errors, a dichotomy has developed between medical errors occurring and the disclosure of these errors by medical professionals.

Methods: We reviewed and compared the various medical error disclosure initiatives across Canada and the globe (USA, Australia, New Zealand, and United Kingdom) to analyze the progress made in this key area. The designing of an error disclosure policy requires integration of various aspects including bioethics, physician-patient communication, quality of care, and team-based care delivery.

Results: The United States Joint Commission on Accreditation of Healthcare Organizations mandated an open disclosure of any critical event during care to the patient or their families. This was deemed as an essential accreditation standard for the institution. In Australia, the Australian Council for Safety and Quality in Health Care offered an approach that addresses the unique interests of patients, health care professionals, administrators and managements. The Australian policy integrates the disclosure process with a risk management analysis towards investigating the critical event. In New Zealand, the patients suffering a medical error are rehabilitated and compensated through a no-fault, state-funded compensation scheme. This model aims to encourage the health care providers towards an honest disclosure of a medical error and effectively bars medical malpractice claims. The National Health Services (NHS) of the United Kingdom declared a 'duty of candour' that directs the doctors and managers to inform a patient of an act of negligence or omission that causes harm. The NHS scheme offers a remedial package to the patient that includes an apology and financial compensation in return for the patients waiving their right to litigate. In Canada majority of provinces have adopted some form of a disclosure policy while others are in the process of developing such policies. These Canadian provincial initiatives, though similar in content, remain isolated because of their non-mandatory nature and absence of federal or provincial laws on disclosure.

Conclusions: We suggest that a balance should be achieved in dealing with errors in a non-punitive manner and respecting the patients' right to an honest disclosure. Effective communication between health care providers, patients and their families throughout the disclosure process is integral in sustaining and developing the physician patient relationship. This approach would be very beneficial in terms of developing a culture of safety and enhancing overall quality of care.

E-52

The business case for optimized quality control practices

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Objectives:

1.To assess the business case for investment in software and education to improve QC practice

2.To assess the current state of

a.Patients at risk due to possible failure to detect clinically important change, and

b.Time and cost spent on false positive QC flags

Methods:

1.We examined Nov 2011 QC data, patient volumes, cost per test and labor costs for CA, CHOL, NA, BHCG, DIG, TSH, PT and APTT from 13 instruments across 6 Laboratories.

2.We set True Values for QC samples based on peer review

3.We set Total Allowable Error Limits (TEa) based on the Stockholm Hierarchy, IFCC, 1999

4.Optimized Quality software created reports that included, for each QC sample:

a.current Total Error and Margin for Error (TEa - TE)

b.recommendations for assigned chart values and QC rules

c.number of runs to flag a Failing Change (a shift that would cause 5% of results to exceed TEa limits)

d.number of patients at risk

e.clinical costs associated with patient risk, and value of risks avoided

i.based on nominal clinical values of $1 per patient at risk and $10 for patients who actually receive results that exceed TEa limits and are therefore potentially clinically misleading

f.number of false positives and value or cost to the laboratory budget

g.comparisons of Effectiveness and efficiency of recommended and actual QC processes

5.We examined the data to determine

a.the impact of optimized quality processes on QC Effectiveness (ability to flag a Failing Change in the first run to minimize patient risk) and Efficiency (ability to minimize wasted time and resources for false positive QC Flags)

Results:

Effectiveness and Efficiency of Current QC Practices compared to Optimized Quality Practices			
	Optimized Quality Recommended Action Flags (1-2s to 1-3.5s)	Current Process If staff react only to Reject Flags (1-2.5s to 1-11.2s)	Current Process If staff react to single Warning Flags (1.7 s to 7.5s)
Maximum number of runs with patients at risk	134	425	163
Maximum number of patients at risk	6,435	21,924	10,836
Maximum clinical cost	$15,140	$41,396	$21,514
Maximum number of false positive runs	389	83	909
Maximum cost for false positive runs	$12,518	$2,901	$20,122

Conclusion: 1.There is a valid business case for optimizing QC Practice

a.There are potential savings for reduced patient risk and false positive QC flags

2.Current practice was less effective and efficient than QC processes recommended by Optimized Quality software.

E-53

Development and verification of a new statistical model for external quality assessment using linear mixed model

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Background: External proficiency testing program is an important part of quality control and is currently being implemented in many countries. These programs calculate the standard deviation index (SDI) and use it to compare a laboratory's results to its peer group. In South Korea, total and peer group SDIs are used for evaluation. However, the total SDI and peer group SDI do not give the reasons for the outliers such as institution itself, analytical method or instrument. Furthermore when the total SDI and the peer group SDI show contradictory results, it is difficult to interpret them and if the peer group is too small, it is hard to calculate the peer group SDI. In this study, we developed a new statistical model using linear mixed model and applied it to real and randomly generated data for verification.

Methods: The SDI is based on results from each laboratory but we assumed those results are affected by different factors, such as analytical methods, instruments, manufacturers and institutions. We calculated SDIs of those four factors using linear mixed model (. . Thus SDIs for analytical methods, instruments, manufacturers and institutions were calculated and evaluated in the same way. If one of those four SDIs shows outlier, the result is considered inappropriate and the reason might be the one whose SDI is over +/-2. We used the raw data of WBC count from all institutions reported in the first trial of 2011.

Results: With a new statistical model, we found outliers for analytical methods, instruments, manufacturers, and institutions. Many institutions with outliers in total SDI regardless of peer group SDI were found to have outliers in SDI for institutions, which means abnormally measured values came from institution itself. A few institutions with outliers in total SDI were found to have outliers in instrument or manufacturer SDIs, which means specific instrument or manufacturer can be the reasons for those abnormal results. Several institutions with acceptable total SDI but unacceptable peer group SDI didn't show outliers in SDIs for four factors. The reason for unacceptable peer group SDI is the cutoff of +/- 2 is not appropriate in peer group of less than 30 institutions because small group do not follow normal distribution but t-distribution.

According to simulation with randomly generated data with normal distribution, biased data (1SD, 2SD and 3SD) were better detected in our new model.

Conclusions: A new statistic model using linear mixed model can detect outliers and also specific reasons for them, which gives insight into the institution when outliers are found. It also gives consistent evaluation without false positivity seen in peer group SDI when peer group comprise of less than 30 institutions which follows t-distribution and consequently cutoffs need to be readjusted. And finally institutions without peer group SDI because peer group number is too small can also be evaluated with this model. It is more sensitive than current total SDI because it can better detect biased data. In conclusion, our new model can be successfully used in external proficiency testing for evaluation.

E-55

Cost Savings By Applying Six Sigma Metrics for Internal Quality Control

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Background: Internal Quality Control (IQC) is the middle piece of the laboratory quality asurance and plays an important role in monitoring accuracy and precision of all tests. However, quality is not free and often comes with a price. In this era of cost awareness and the constant reminder that time equals money, SunMed laboratory is urged to perform better by reducing the costs without compromising quality of results. Central part of every laboratory is, its obligation to assure that the whole testing process is accurate, reliable and ultimately, useful to the clinicians. The objective of the study is to investigate whether applying six-sigma metrics for internal quality control could actually reduce the running costs of quality control (QC) materials and costs of failures without compromising patient outcomes.

Methods: A retrospective comparative study of costs of QC materials, frequency of QC runs and costs of failures in Chemistry department performed between pre new IQC strategy implementation in 2009 and post new IQC strategy implementation in 2010 and 2011 were reviewed and analyzed. We followed the recommended guideline in setting up our QC system as stated in CLSI (C24-A2, section 5). Firstly, we determine the quality requirements of each assay, in which we used the biological variation information from Ricos *et al*.After determining the quality required for the assays, we evaluated the test performance of the 22 assays. The method performance was assessed on monthly IQC data transmitted online from Architect *ci* 8200 into Unity Real Time Software. Our test accuracy or bias was obtained from the peer group mean. We then utilized the Westgard Advisor available tool in Unity Real Time to calculate and display the sigma-metric of each assay. This helps us in implementation of the proper rejection criteria and selection of control rules that enables us to decide on the frequency of QC runs for each assay.

Results: The results showed 17 out of 22 assays that were evaluated have a six sigma metric performance of 4 and above; therefore it is only required to perform QC runs once a day for two levels instead of twice a day for two levels.The costs of QC materials and costs of failures spent in 2009 before six - sigma implementation were USD 31,819 and after six sigma implementation in 2010 and 2011 were USD 19,314 and USD 19,038 respectively.

Conclusions: The application of six-sigma metrics for internal quality control appears to significantly reduce the cost of QC materials and cost of failures by 39 % or USD 12,500 annually with improved performance characteristics on most assays thus patient safety is not compromised at all. The new QC strategy has been hailed as an "evolution in quality management" leading to improvement in process reliability, operating costs go down and customer satisfaction increasing.

E-59

Differences in laboratory requesting patterns in general practice in Spain

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Background: Compare requiring patterns by General Practitioners (GPs) of 37 laboratories providing services to 37 Health Areas (all over Spain, 8.130.334 inhabitants).

Methods: 28 chemistry tests, ordered by GPs during 2010, were examined in a cross-sectional study. Data were collected from laboratory databases and indicators that measured every test request per 1000 inhabitants or related test requesting ratios were calculated.

Results: Figure shows, for every indicator result, the mean, median, range and variability index (Percentil90/Percentil10). Test requesting per 1000 inhabitants results were sorted by the variability index. It is observed that the most requested tests are the least variable, except uric acid and urinalysis.

Conclusions: An increased requesting of triglycerides, cholesterol, glucose, creatinine, ALT, glycosylated hemoglobin, ferritin, TSH and calcium is recommended to discover treatable diseases in an early stage. The question that arises is what the reference amount should be for a given test. Study results show that calcium is under- and uric acid is over-requested. Urinalysis continues to be frequently requested as screening. It is difficult to analyse PSA requesting, because usefulness as screening purposes is controversial. The high variability in CRP and Vitamin B 12 shows the inappropriate requesting, at least in some of the areas.

Low values are recommended in every related test requesting ratio. That occurs in FT4/TSH, BIL D/BILT and FPSA/PSA ratios. The AST/ALT, BILT/ALT, LDH/ALT and GGT/ALT ratios must be reduced. Urea is over requested and folic acid is generally required at 1:1 proportion as compared with vitamin B12 without being necessary when neurological symptoms. Isolated serum iron has a limited clinical value, and phosphate must be requested after abnormal calcium results.

Considerable variability exists. This is the first step before creating interventions to improve appropriate laboratory use to be monitored through showed indicators.

	Mean	Median	Range	Variability index
Tests requesting per 1000 inhabitants				
Glucosa	372,6	366,4	227,9-486,3	1,53
Triglycerides	342,4	333,2	226,7-456,2	1,53
Cholesterol	357,1	353,3	226,3-470,9	1,54
Creatinine	351,7	344,5	184,1-481,9	1,59
Alanine transaminase (ALT)	332	329	179,0-490,1	1,69
Cholesterol, HDL	275	279	131,5-407,0	1,81
Glycosylated hemoglobin	85	88,8	53,0-134,5	1,99
Thyrotropin (TSH)	174,4	178,6	108,1-285,4	2,09
Prostate-specific antigen (PSA)	51,1	51	19,1-85,2	2,14
Uric acid	298,5	302,1	106,4-434,0	2,17
Ferritin	130,9	127	74,7-202,7	2,18
C-Reactive protein (CRP)	60,6	53,1	21,2-239,8	3,53
Urinalysis	196,7	211,2	18,2-345,2	3,77
Vitamin B 12	31,5	27,8	4,0-88,0	6,42
Calcium (Ca)	88,2	80,3	14,9-291,4	7,01
Alkaline phosphatase (ALP)	139,1	125,5	9,3-306,5	7,4
Related test requesting ratio				
Folic acid/Vitamin B 12	0,93	0,96	0,46-1,23	1,24
Gamma-Glutamyltransferase (GGT)/ALT	0,78	0,87	0,23-1,00	2,06
Iron/Ferritin	1,02	0,96	0,31-1,91	2,36
Aspartate transaminase (AST)/ALT	0,78	0,94	0,09-1,0	2,81
Phosphate/Ca	0,66	0,66	0,05-0,99	3,31
Total bilirubin (BILT)/ALP	1,21	1,01	0,27-6,39	4,31
Urea/Creatinine	0,63	0,64	0,02-1,06	5,62
Thyroxine (FT4)/TSH	0,37	0,31	0,07-1,0	6,07
Free PSA (FPSA)/PSA	0,15	0,13	0,00-0,53	9,42
Transferrin/Ferritin	0,42	0,34	0,03-1,05	12,35
Direct bilirubin/BILT	0,12	0,05	0,01-0,91	23,2
Lactate dehydrogenase (LDH)/ALT	0,14	0,06	0,01-0,73	26,38

E-61

Carryover - Comparison of Three Methods Calculation

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Background: As described on the 2002 NCCLS document, carryover is *"the discrete amount of analyte carried by the measuring system from one sample reaction into subsequent sample reactions, thereby erroneously affecting the apparent amounts in subsequent samples"*. The calculation of carryover has been a challenging over the years and new methods have been proposed. The knowledge of the real percentage of carryover, along with other error rates, can lead to a better management of the analytical quality of specific analytes.

Objective: Use three different methods for carryover assessment. The objective of the present study is to evaluate the actual method in order to assess the correct values for carryover.

Methodology: Three different methods were used to calculate carryover: (A) Broughton *et al* (1969) propose a formula that uses results obtained from three measurements of two specimens - high and low values - sequentially analyzed - Formula 1, (B) Levin *et al.* (1985) consider that the best calculation is provided by the analysis of a high and low value specimens that should be tested 10 and 3 times, respectively showing that using this later approach underestimated carryover values were obtained with the former - Formula 2 and (C) NCCLS EP10-P document proposal that uses only two measurements of the high value specimen followed by three of the low value one. The following analytes were analyzed herein: Alanine Aminotransferase (ALT), Creatinine, Glucose, Creatine Kinase (CK), Cholesterol (total), Triglycerides, White Blood Cells (WBC) and Platelets. In order to allow comparison, values obtained with method A (Broughton *et al,* 1969) were turned in percentage.

Results: No significant differences were found after the comparison of the three proposed methods. When comparing these carryover values with the 2011 results (obtained from the modified Method A), clinical chemistry tests showed higher absolute values for carryover than the previous study (exceptionally for glucose and triglycerides the values were lower in the present study) while hematology results showed an opposite situation. The hematology department used specimens with lower difference than the ones used in 2011 while clinical chemistry chose specimens with a greater difference between the high and low specimen. This difference should be related to the better choice of the specimen than to the used method itself. In other words, carryover underestimation is more related to the specimen choice than to the calculation method.

Conclusion: In conclusion, a laboratory may use any one of the three methods. But, to avoid unnecessary specimen measurements, one can implement method C; the important is the choice of the specimen. The importance of this study relies on the reliability that correct carryover measure brings to laboratory tests results.

E-62

Optimizing the use of collection tubes in a large core laboratory.

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Background: One of the great challenges for clinical laboratories is to produce more with less resources due to the competitiveness and customers' high requirements: less sample volume, lower consumption of reagents, workforce optimization, greater productivity at short and long term, all of this together while maintaining superior quality standards, recognized and perceived by customers.

The direct cost of the material used in the collection process grows progressively without a corresponding increase in the reimbursement value, reflecting directly in the business profitability. In order to overcome the aforementioned challenges, DASA decided to optimize the number of collection tubes used in the tests performed in its main core laboratory that produces about 160,000 tests per day. This site has a production area with approximately 300 equipment and 180 of them are considered high throughput ones.

Methods: The challenge proposed was to collect most of the tests that use the same biological sample (serum) in just one tube, even if different platforms were used to process them.

After the definition of a maximum number of tests per tube, each test was grouped in specialties of biochemistry, hormones, and infectious diseases, according to the execution workflow in three groups of platform : 6 Advias 2400, 13 Advias Centaur XP and 18 modules Modular E.

This study of 3,000 analytes resulted in the design of a new flow for a group of about 200 analytes. This project had the direct participation of a multidisciplinary team of 12 people and took six months to be developed and validated.

This project was only possible due to the implementation of pre-analytical sorter RSD PRO (PVT), sorting and addressing the tubes to the right instrument without loss of productivity.

Results: The premise of the tube reduction brought benefits to the patient - due to the smaller volume of sample collected - in addition to financial benefit and productivity.

The number of serum tubes with separating gel decreased 13.33% - which represented an economy of USD 10,000.00 per month - without increasing the turnaround time of the results or loss of the stability of the sample.

In the post-analytical phase, the benefit was the reduction of storage space occupied by samples and consequently reduction of infectious waste.

Conclusions: This project succeeded in one of the core laboratories and was also implemented in others DASA laboratories, bringing significant financial and nonfinancial gains for the company and customers.

E-63

Automation gains - the use of RSD PRO sorter in managing sample flow in a large Brazilian Laboratory

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Background: The increasing of production complexity, combined with the market competition, has driven organizations to seek ways to remain competitive and present a superior performance in terms of quality, productivity and cost compared to its own history and also the other competitors in their own segment. Currently, mergers and acquisitions have created larger corporations as one way of pursuing these goals.

In healthcare market segment, the clinical analysis laboratories have followed this worldwide trend and in Brazil, DASA was the pioneer in this kind of endeavor .

Specifically when it comes to production scale, automation plays an important role in this scenario, combined with several improvements of information technology and robotics.

Objectives The relevance of the use of automation equipment for Pre and Post-analytic is directly related to the volume of samples being processed in analytical platforms. This work intends to show in a large laboratory:

a) applications of the sorter RSD PRO (PVT) for Pre-analytical, Analytical and Post-analytical areas;

b) integration of sample flows;

c) benefits of this automation.

Methodology: The studied lab processes between 40,000 to 45,000 tubes / day and performs between 130,000 to 150,000 tests / day. Because of this high volume of

daily samples, implementation, improving of automation and optimization of sample flow was important to increase the productivity of the laboratory, adding safety to the process and maintaining the traceability of the samples.

The study compared the process of manual sorting with the automated process performed by six equipments, in the following phases:

i) reading the bar code, recording in the Laboratory Information System (LIS);

ii) decapping tubes;

iii) driving tubes in equipment racks for subsequent execution of tests;

iv) storage of samples.

Results: The results obtained with the sorter were:

a) 66% reduction of labor in the sorting areas of biological samples, reducing repetitive strain injury (RSI);

b) improving the management of the sample flow for the different execution platforms and equipment essential to saving approximately 52,000 tubes / month. This created a positive impact for customers and environmental aspects related to reduction of infectious waste;

c) bringing forward 3 hours at the beginning of daily processing;

d) reduction of 6 hours in the process of daily storage of the samples.

Conclusion: In addition to the gains already exposed, the role of the mentioned equipment is not limited to distributing samples, but to serve as a manager of the production flow, distributing quickly and properly, running a high volume of samples, identifying errors in bar codes, analyzing the needs for repeating tests, supporting the process of traceability of samples and subsequent archiving.

Finally, in the near future the connection of some types of sorters to conveyors, with the consequent elimination of transportation steps, reducing the risk of handling, will improve even more the TAT and increasing the productivity to a higher level.

E-64

Comparison of the SST ™ II Advance (Standard Gel Tube) with RST (Rapid Sample Tube) in Emergency Laboratory in Bakirkoy Sadi Konuk Research and Training Hospital

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Background: Upon admittance to our hospital emergency department we aim to provide fast and accurate test results. With the permission of the patients who had been admitted to our emergency department requiring laboratory test results, we collected their blood samples in two different tubes (SST and RST) and then compared the results.

Methods: Blood samples have been collected from 75 adult patients by routine phlebotomy into BD RST and BD SST™ II Advance tubes. The BD SST™ II Advance tubes require a minimum clotting time of 30 minutes prior to centrifugation to obtain serum, and are then centrifuged with 2000g for 10 minutes (Used by Hettich Rotofix 32 A). The thrombin additive in the tube promotes rapid clotting of the blood. Because of that, the BD RST tubes (The thrombin additive in) do not need to wait for clotting more than 5 minutes, and then are centrifuged with 1800g for 3 minutes (used by Stat Spin). They also prevent the blood from being hemolyzed. Which means reduction in the TAT (Turn Around Time) especially for emergency tests. Serum from each tube was then tested for Alanine aminotransferase, Alkaline Phosphatase, Amylase, Aspartate Aminotransferase, Direct Bilirubin, Total Bilirubin, Blood Urea Nitrogen, Calcium, Chloride, Creatinine, Total Creatinine Kinase, Creatinine Kinase-MB, Gamma-Glutamyl transferase, Glucose, Lactate Dehydrogenase, Lipase, Potassium, Sodium, Total Protein, on an Abbott Architect® 16000.

Results: When compared with the BD SST™ II *Advance* tube samples results BD RST tube samples results were clinically equivalent or clinically acceptable. Statistical calculations were performed with NCSS 2007 program for Windows. Besides standard descriptive statistical calculations (mean,standard deviation), paired t test was usedin the comparison of two blood tube groups, Intraclass Correlation Coefficient was used reliability measurements blood results. The results were evaluated within a 95% confidence interval. Statistical significance level was established at $p<0,05$.

Conclusions: Considering the meaning of emergency it is very important to provide fast and accurate test results. BD RST tubes do not require a waiting period for clotting, thereby decreasing TAT by nearly 30 min. This can lead to increased laboratory efficiency and improved patient management in Emergency Department.

E-65

A Web-based Route Planning Tool for Transporting Laboratory Specimens

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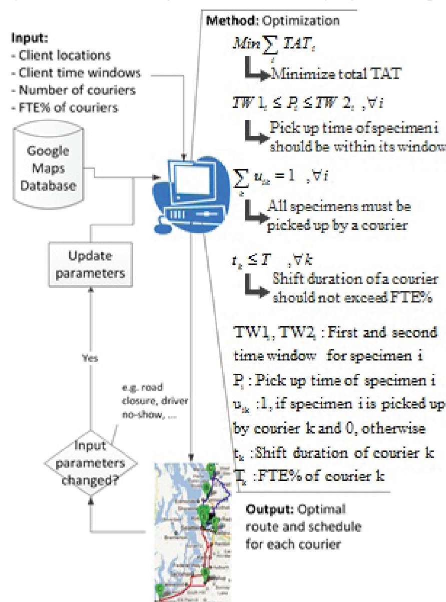
Objective: Planning courier routes for efficient transport of specimens is important for geographically dispersed laboratory networks. Our objective was to develop an optimization tool to design courier routes and schedules.

Methodology: The inputs, method, and outputs illustrated below, include clinically-important parameters such as windows of time during which specimen pickup is acceptable and turn-around time (TAT). We developed algorithms which provide optimal solutions in an efficient manner. Because driving time is influenced by traffic intensity, we incorporated traffic data from the Google Maps database. We also developed a web-based user interface to make the optimization tool accessible to laboratory managers.

Results: For a network of 30 clients and 7 couriers, there are 6,300 alternative ways to manually assign client pickups to couriers. This is a highly complex decision-making process. Computation time for the optimization algorithm was only 3 seconds for this network using a conventional desktop computer. For two larger networks of 100 clients and 7 couriers, and 250 clients and 12 couriers, the number of alternative ways to assign pickups to couriers was 70,000 and 750,000, which is extremely complex to handle manually. The optimization algorithm efficiently designed optimal routes for these networks in 45 and 1,664 seconds, respectively. In a 3-week pilot period, we used the optimization method to assess the timing and sequence of previous courier routes that had been developed by empiric methods. For example, a route that had required frequent unscheduled driver overtime could be modified and additional pick-ups added to the route using computerized modeling, with successful and rapid optimization that would have been difficult and time-consuming to achieve by conventional trial and error manual methods.

Conclusions: Planning courier routes is critical in laboratory operations. We developed an easily used optimization tool to design courier routes and schedules where the objective is minimizing total TAT and satisfying client requirements.



E-66

Process is important in Clinical Chemistry Total Lab Automation

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Background: Raffles Hospital (RH) is a 100-bed acute care hospital with onsite outpatient clinics and a health screening service in addition to a network of offsite physician office practices. In 2009 it introduced the Roche total lab automation (TLA)

system. The TLA comprised the Modular Pre-Analytics unit (sample loader, two centrifuges, decapper, aliquoter, bar-code labeler, re-capper, and stocker for post-processing) connected to three inter-linked Modular E170 immunoassay analyzers in one line, and two inter-linked Cobas C501 chemistry analyzers in another line. An additional off-line standalone Cobas C501 and Elecsys2010 served STAT and after-office hour samples. This 24-h service is crammed into a space 512 sq.ft with an additional 500 sq.ft for sample reception and administrative support.

By 2011, the general chemistry workload had increased by 35% to 1.0 million tests and immunoassays by 25% to 400 thousand tests. Turnaround time (TAT) outliers (> 1h) for urgent samples was mounting - 21% renal panel , 23% liver panel, 15% lipid panel, 27% CRP, 22% cardiac enzymes.

Objective: A back to basics process improvement exercise was undertaken to improve TAT.

Methods: The entire lab testing cycle was analyzed for improvement opportunities.

Observations: Phlebotomy is done by nurses at the care sites and then transported by porters to the lab for onsite tests. Offsite tests are received in the late afternoon from a courier service. On receiving the samples orders are then entered by lab staff into the LIS (Foxpro) for downloading to the analyzers. The time taken from sample loading to the analysis station is 20 minutes on average. After testing the results are sent back to the LIS. In the LIS, the lab staff verifies the results and a senior staff approves them prior to transmittal to the LIS. When a lab report is requested, the LIS will generate a PDF report for on-screen for viewing and printing. For routine reports, the LIS retrieves the PDF report and prints to the care site printer. One-third of the samples are from the RH wards and outpatient clinics; a further third is from the health screening service requiring a 3-h TAT. The offsite physician office clinics only need their results the following day. Immunoassays account for 35-40% of the testing.

Improvements: The TLA was re-configured to have two standalone Cobas 6000 (C501 & E601) and the MPA connected to a single Cobas 6000. Five small (12 place) rapid (5-min) centrifuges (3000g) were used to process ward and outpatient samples. The MPA dealt with the health screening and offsite samples.

After results are verified, the LIS was re-designed to auto-generate PDF reports for viewing or printing. HbA1c has been added to the Cobas 6000 from the semi-automated BioRad D-10.

Results: Besides automating the HbA1c and syphilis testing, TAT outliers for urgent samples have been reduced - 10% renal panel , 11% liver panel, 10% lipid panel, 11% CRP, 7% cardiac enzymes, HbA1c 25% (from 39%).

Conclusion: Users over-expect and vendors over-promise the benefits of TLA. Process is as important as TLA in delivering good TAT.

E-67

Racial and Gender Disparities in cTnI Testing: Findings From Two Hospitals

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Background: Acute myocardial infarction (MI) is a major cause of death worldwide. Cardiac troponins I and T (cTnI and cTnT) are established as preferred biomarkers for early diagnosis of MI in patients with a recent onset of chest pain. The guidelines from the Global Task Force for the Universal Definition of Myocardial Infarction, the National Academy of Clinical Biochemistry, and American College of Cardiology/American Heart Association, recommend at least two serial measurement of cTnI or cTnT within the first 24 h after onset of symptoms of myocardial necrosis.

Objective: The specific objective of this study was to determine whether there were racial and gender disparities in cTnI testing at two Atlanta hospitals (*A* and *B*).

Methods: We performed a retrospective analysis of the data obtained for all patients tested for cTnI for a period of 10 years from 2002 to 2011 for both hospitals: 116259 patients from hospital *A*, and 75535 patients from hospital *B*. All analyses were performed with SAS version 9.2. (SAS Institute) for the following groups: blacks (*B*), whites (*W*), females (*F*), males (*M*), black females (*BF*), black males (*BM*), white females (*WF*), and white males (*WM*).

Results: On average each patient had 2.09 cTnI tests done per admission in both hospitals in the span of 10 years. Interestingly, in 2009 there was an increase of cTnI tests per admission in both hospitals: 2.27 in *A* and 2.19 in *B*. In 2011 in hospital *A* cTnI measurements per admission were the following: *B* - 1.97; *W* - 1.99; *F* - 1.89; *M* - 2.08; *BF* - 1.89; *BM* - 2.09; *WF* - 1.89; *WM* - 2.07. In 2011 in hospital *B* measurements per admission were the following: *B* - 2; *W* - 2.25; *F* - 2.02; *M* - 2.15; *BF* - 1.99; *BM* - 2.09; *WF* - 2.17; *WM* - 2.3. In 2011 in hospital *A*, for patients tested positive on cTnI upon admission the percent of patients receiving subsequent cTnI tests (≥2 tests) was the following: *B* - 15.4%; *W* - 16.5%; *F* - 13.4%; *M* - 18.9%; *BF* - 12.2%; *BM* - 20.4%; *WF* - 14.8%; *WM* - 18%. In 2011 in hospital *B*, for patients tested positive upon admission the percent of patients receiving subsequent cTnI tests (≥2 tests) was the following: *B* - 17.6%; *W* - 20.2%; *F* - 15.5%; *M* - 21.3%; *BF* - 14.7%; *BM* - 21.5%; *WF* - 19.2%; *WM* - 20.8%.

Conclusions: Our study demonstrates both racial and gender discrepancies among patients receiving cTnI testing in these 2 hospitals. Significantly less cTnI testing was done for females compared to males ($P < .0001$). Belonging to a specific race/gender group also determined a distinct volume of cTnI testing per each admission ($P = .0033$). The same trend continued for same groups of patients tested cTnI positive upon admission. A multicenter study is being conducted in order to include additional data from other hospitals located in various geographic regions of the country in an effort to reduce the selection biases present in this initial retrospective analysis.

E-68

Standardization of Network Reference Ranges

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Background: Our laboratory is the managing partner for an integrated network of laboratories providing services to physicians and hospitals throughout the northwest. Our network is standardized for many aspects of laboratory practice, including reference intervals for a core group of chemistry and hematology analytes. All testing sites utilize the same reference intervals established by a network-wide study conducted in 1997. Changes in instrumentation and the addition of new partners necessitated a new study to either confirm our current ranges or establish new ones. The core analytes in our reference range study are listed in the table below.

Methods: Seventeen network testing sites employing a variety of instrument platforms participated in the study. CLSI document C28-A3 guidelines were followed. Each site recruited healthy adult volunteers, obtained consent for the study, gathered personal health information on a standard questionnaire, collected blood specimens and assayed all the core analytes. Data forms with results were then submitted to us, along with the completed health questionnaires. Before any data were included in the study, the health questionnaires were reviewed to ensure that the subjects did not meet a predetermined set of exclusion criteria. Seven hundred forty three healthy subjects were included in the study. Data analysis was done using EP Evaluator®, Release 9. Department Directors then reviewed the data analysis to establish the central 95% range and establish reference intervals based on the study.

Results: See table

Conclusions: This study enabled our network to update our current ranges and establish new network-wide reference ranges applicable for current analytical methodology at all testing sites. A sufficient number of subjects were included for rigorous statistical analysis of the data, giving a high level of confidence in the results. The large number of subjects also allowed us to establish gender-related ranges, where appropriate.

Network Reference Intervals – Chemistry & Hematology

Analyte	Units	n	Reference Interval	Analyte	Units	n	Reference Interval
Albumin	g/dL	735	3.6 - 5.0	WBC	K/uL	658	3.8 - 11.0
Alkaline Phosphatase	U/L	735	35 – 115	RBC – Female	M/uL	368	3.70 – 5.10
ALT	U/L	734	10 – 65	RBC – Male	M/uL	289	4.20 – 5.70
AST	U/L	735	10 – 45	Hemoglobin – Female	g/dL	369	11.3 – 15.5
Bilirubin, Total	mg/dL	735	0.1 – 1.5	Hemoglobin – Male	g/dL	289	13.2 – 17.0
BUN	mg/dL	742	8 – 25	Hematocrit – Female	%	369	34.0 – 46.0
Calcium	mg/dL	742	8.5 – 10.2	Hematocrit – Male	%	289	39.0 – 50.0
Chloride	mmol/L	743	99 – 109	MCV	fL	658	80.0 – 100.0
CK – Female	U/L	371	30 – 240	MCH	pg	658	27.0 – 34.0
CK – Male	U/L	316	55 – 400	MCHC	g/dL	658	32.0 – 35.5
Creatinine, IDMS – Female	mg/dL	360	0.50 – 1.00	RDW	%	622	11.0 – 15.5
Creatinine, IDMS – Male	mg/dL	299	0.70 – 1.30	Platelet Count	K/uL	658	150 - 400
Ferritin - Female	ng/mL	318	6 - 170	Neutrophils (Auto Diff)	%	654	40.0 - 75.0
Ferritin - Male	ng/mL	268	11 - 450	Neutrophils (Auto Diff)	K/uL	654	1.9 - 7.4
GGT	U/L	684	5 - 75	Lymphocytes (Auto Diff)	%	654	15.0 - 48.0
Iron - Female	ug/dL	344	30 - 180	Lymphocytes (Auto Diff)	K/uL	654	1.0 - 3.9
LD	U/L	635	115 - 225				
Magnesium	mg/dL	689	1.7 - 2.4				
Phosphorus	mg/dL	689	2.3 - 4.8				
Potassium	mmol/L	743	3.5 - 5.0				
Protein, Total	g/dL	735	6.3 - 8.2				
Sodium	mmol/L	743	135 - 143				
T4, Total	ug/dL	549	4.7 - 11.3				
TIBC - Female	ug/dL	345	260 - 490				
TIBC - Male	ug/dL	287	250 - 450				
TSH	uIU/mL	662	0.45 - 5.10				
Uric Acid - Female	mg/dL	662	2.2 - 7.1				
Uric Acid - Male	mg/dL	315	3.2 - 8.6				

Data reviewed and reference intervals proposed by William A. Dittman, Jr., M.D.

Data reviewed and reference intervals proposed by Lawrence M. Killingsworth, Ph.D., DABCC, FACB and Carmen L. Wiley, Ph.D., DABCC, FACB.

E-69

Application of Statistical Process Control (SPC) in Manufacturing of In Vitro Diagnostic Reagents

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Objective: Statistical Process Control (SPC) is a statistical tool for the differentiation of assignable variations from random ones in a process. In this study, SPC was preliminarily applied to the monitoring of internal quality controls for a product as a part of efforts in managing product manufacturing processes in a science-driven and objective evidence-based manner.

Methods: The output of SPC monitoring used descriptive statistical parameters - means, standard deviations, and process capability Indexes. These values were used to construct control charts. Control limits were calculated for the charts based upon the random variations. X-bar, Range, Run Chart, Cusum, Process Capability (Cpk) and Probability Plot control charts were constructed using measurements from the process output, and data generated outside of the upper and lower control limits (UCL and LCL) was identified for the investigation of assignable variations. The control charts will be re-evaluated, and the refined control limits applied. This process will be reiterated as needed prior to our implementation of real-time process monitoring.

Results: The QC data range used for analysis included measurements collected over 19 months of a manufacturing process. The Cpk and Probability plot both established that the process exceeded minimal capability (Cp = 1.181, Cpk = 1.095). The Xbar chart displayed only 1 data set out of control (OOC) and the range chart showed no range points were out of control (OOC). Additionally, Xbar (average) and Cusum charts demonstrated the variation and mean shift. A trend was found below the center line for eight consecutive time points on the Xbar control chart, and an investigation has been initiated to identify this probably assignable variation.

Conclusions: SPC allows for detection of shift in means and variability of the output of a reagent manufacturing process. The statistical representations may provide improved characterization of product performance and manufacturing process control independent of Quality Control functional specifications. Identification of the OOC data set and Cusum mean trending provided a statistical tool for the early detection of potential process trends and identified areas for pro-active, continuous improvement opportunities to support our commitment to Total Quality Management (TQM).

E-70

Evaluation of serial patient ALT and AST demonstrates 50% more biologic variability in AST: Another reason to stop ordering AST and ALT in tandem

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Background: When diagnosing or monitoring liver disease, physicians very often order AST in tandem with ALT. ALT is far more specific for liver disease but its biologic variability is putatively twice that of AST (24.3% vs. 11.9%, from Ricos). We used a unique methodology to derive biologic variability (s_b) from consecutive patient data and demonstrate that s_b of ALT is significantly lower than that of AST.

Methods: A laboratory data repository provided all of the AST and ALT results measured together over a two year period at University of Alberta Hospital in Edmonton. These measurements (on mostly inpatients) were made on the Beckman LX-20 (Brea, CA). After removing patient results that exceeded the 95 percentile limits (82 and 159 for ALT and AST, respectively) 31282 and 48519 different patients had 98971 and 192161 ALT and AST ordered at least twice within 4.5 days, respectively (median ALT and AST = 24 and 28, average ALT and AST = 30 and 38 IU/L). We tabulated the pairs of intra-patient ALT and AST that were separated by 0- 12, 12 to 24, 24 to 36, 36 to 48, 48 to 60, 60 to 72, 72 to 84, 84 to 96, and 96 to 108 hr. The standard deviations of differences (SDD) of the paired ALT's and AST's were calculated for each time interval. The graphs of SDD vs. time interval were linear; the y intercept provided by the linear regression equation represents the sum of s_b and short term analytic variation (s_a): $y_0=(s_a^2+s_b^2)^{1/2}$. The short term analytic variation (s_a) was determined with the same type of consecutive pairs SDD analysis of 2 levels of Bio-Rad quality control specimens analyzed 5 to 7 times daily over 2 months.

Results: A minimum of 2400 differences were obtained for each time interval. The regression equation of the intra-patient SDD vs. time yields y intercepts of 11.7 and 4.8 IU/L for AST and ALT, respectively. Substitution of the QC derived s_a results in a s_b of 11.6 for AST at 58 IU/L (20.1%) and a s_b of 4.7 for ALT at 36 IU/L (13.2%). The significantly higher s_b for AST may be explained since AST is derived from many non-liver tissues, including red blood cells, skeletal muscle, heart, lungs, intestine and skin.

Conclusions: The significant difference in the biological variation between the ALT and AST makes ALT the preferred test for tracking changes in liver function since ALT's critical difference is much smaller than that of AST.

E-71

Implementation of a quantitative hCG assay to screen for pregnancy adversely affected the predictive value of the screen.

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Background: Rapid testing of human chorionic gonadotropin (hCG) in serum is frequently desired in healthcare settings to identify a possible pregnancy and allow for clinical decisions in favor of protecting a fetus. While the gold standard for pregnancy testing is the serum quantitative hCG test, it has a longer turnaround time (TAT) than most of the point-of-care qualitative serum or urine tests. In order to provide more rapid TAT, some laboratories offer a serum qualitative assay in addition to the serum quantitative test. However, the qualitative assays are less sensitive for diagnosing a pregnancy compared to serum quantitative methods. Additionally, we determined that using automation and auto-verification, our TAT for negative hCG serum quantitative tests was similar to the TAT for our qualitative manual method. Positives were reported in about twice the amount of time (limited AMR and dilution).

Objective: The goal of this study is to determine whether implementation of a more sensitive hCG screening test improved detection of pregnancies, especially early pregnancies, thus improving patient care and clinical decision making in this population.

Methods: In July 2011, we eliminated the serum qualitative Wampole Clearview hCG point-of-care test kit in favor of the more sensitive serum total β-hCG on the Beckman DxI, and utilized qualitative reporting to maintain the rapid TAT (ie. did not dilute the positives). The quantitative screen is performed by measurement of β-hCG but is reported only as negative (<5mIU/mL) or positive (≥5mIU/mL).

Results: When we compared data from the six months prior to the switch (Jan-Jun 2011) with the six months after (Jul-Dec 2011), we found that the percentage of positive results increased from a mean of 4% to 12%. Upon chart review of patients that had tested positive (n=136), we found that our false positive rate increased from 20% to 50% and positive predictive value decreased from 85% to 48%. Positive qualitative screens that also had quantitative β-hCG measurements (n=23) had a 9% false positive rate based on the quantitative result. Positive screens had also had quantitative β-hCG measurements ordered (n=72) had a 28% false positive rate upon retesting. However, an additional 26% were true positives below the detection limit of the qualitative Wampole kit, which suggests that these pregnancies may have gone undetected using the previous method.

Conclusions: These data indicate that while the quantitative assay is more sensitive, it is not as specific for diagnosing pregnancy as the qualitative test and a dramatic increase in the false positive rate appears to be leading to increased costs of confirmatory testing (including ultrasounds in some cases).

E-72

Reduction of Hemolysed Specimens Following Placement of Phlebotomy Personnel in the Emergency Department

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Background: Hemolysis is the major reason for rejecting urgent specimens received from the emergency department. This leads to sample recollection causing delay in patient care and in patient dissatisfaction. Several factors such as collection site and technique as well as specimens handling have been attributed to cause hemolysis. Establishing and maintaining standardized blood collection among the various caregivers (nurses, physicians, emergency medical technicians, and medical and nursing students) in a busy emergency department is difficult. This study examines the impact on hemolysis following placement of laboratory phlebotomy personnel dedicated to blood collection in a busy emergency department.

Methods: Laboratory phlebotomy personnel were assigned to blood collection in the emergency department. The percentage of hemolysed specimens collected by caregivers prior to placement of phlebotomy personnel was calculated. Percentage of blood specimens collected by phlebotomists and by patient caregivers as well as

those rejected for hemolysis by each group were recorded and calculated respectively.

Results: Percentage of hemolysed specimens collected by caregivers prior to placement of phlebotomy personnel ranged from 3.0% to 4.0 % (mean 3.7 %). During the study period, caregivers collected 20% of patients' specimens with percentage hemolysis ranging from 4% to 12% (mean 7.6%). Percentage of hemolysed specimens collected by phlebotomy personnel ranged from 0.1% to 0.4% (mean 0.25%). Although phlebotomy personnel collected 80% of specimens during the study, their percentage of hemolysed specimens did not change significantly compared with those obtained by caregivers which declined significantly (P<0.05).

Discussion: There was a marked reduction in hemolysed specimens when collected by dedicated phlebotomy personnel in the emergency department. Although the percentage of hemolysed specimens collected by caregivers remained relatively high, this declined during the study which indicates transfer of knowledge and assistance by phlebotomists. This overall decline in hemolysis resulted in reduction in number of repeat blood draws, improved patient care and patient satisfaction, as well as reduced laboratory involvement with such poor quality specimens.

E-73

Prevalence and type of preanalytical recall: knowing the cause to eliminate the problem

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Background and Objective: Laboratory testing became very important in clinical decisions regards to diagnosis and therapies of serveral pathologies. Preanalytical errors were associated to more than 70% of laborory error. Therefore, this phase enfolds the greatest potential for quality improvement. The classification and quantification of the recall of blood specimen in clinical laboratory is a quality indicator. The aim of this study was identify all the preanalytic reasons for the recall of diagnostic blood specimen in a clinical laboratory.

Methods: The sources for all recall for diagnostic specimen in a period of 12 moths was recorded and stratified in a clinical laboratory that performed ~ 796.000 tests/year.

Results: 1552 recall/year of diagnostic specimen attributed to pre-analytical problems was recorded. The stratification causes of recall are showed in Table 1.

evalence and type of pre-analytical problems

	Hematology n (%)	Coagulation n (%)	Immunochemystry n (%)	Parasitology n (%)	Urinalysis n (%)	Microbiology n (%)	Molecular Biology n (%)	Genetics n (%)	Toxicology n (%)	Cytology n (%)	All samples n (%)
t received	12 (0.8%)	6 (0.4%)	31 (2.0%)	3 (0.2%)	2 (0.1%)	4 (0.3%)	- -	- -	1 (0.1%)	- -	59 (3.8%)
ate container	40 (2.6%)	56 (3.6%)	150 (9.7%)	- -	- -	5 (0.3%)	9 (0.6%)	3 (0.2%)	12 (0.8%)	- -	275 (17.7%)
ate volume	46 (3.0%)	54 (3.5%)	366 (23.6%)	22 (1.4%)	119 (7.7%)	53 (3.4%)	26 (1.7%)	- -	22 (1.4%)	- -	708 (45.6%)
s	5 (0.3%)	80 (5.2%)	99 (6.4%)	- -	- -	- -	5 (0.3%)	- -	3 (0.2%)	- -	192 (12.4%)
	101 (6.5%)	20 (1.3%)	25 (1.6%)	- -	- -	- -	- -	5 (0.3%)	- -	- -	151 (9.7%)
	- -	2 (0.1%)	17 (1.1%)	2 (0.1%)	5 (0.3%)	12 (0.8%)	2 (0.1%)	- -	2 (0.1%)	1 (0.1%)	43 (2.8%)
	3 (0.2%)	4 (0.3%)	108 (7.0%)	2 (0.1%)	1 (0.1%)	3 (0.2%)	- -	- -	- -	3 (0.2%)	124 (8.1%)
nalytical problems	207 (13.3%)	222 (14.3%)	796 (51.3%)	29 (1.9%)	127 (8.2%)	77 (5.0%)	42 (2.7%)	8 (0.5%)	40 (2.6%)	4 (0.3%)	1552 (100%)

s of non-conformities were showed as n (%) considering non-conformity in each sector and total pre-analytical errors (1552 sample recall)

emistry section combines clinical chemistry, clinical immunology and hormones laboratory testing)

Table 1. Pr

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Discussion and Conclusions: Our frequencies of recollect diagnostic specimen during 12 moths represent 0.25% of total number of specimens collected in the period, and were similar to other studies. Inappropriate sample volume (frequency 45.6%) and inappropriate sample container (frequency 17.7%) were the most frequent cause of recall. Very few studies reported similar data, so comparison with other laboratories performances were difficult. In conclusion, our results showed that the appropriated identification and quantification of blood sample recall could be relevant for laboratory quality management and the first action to minimize this source of error. Moreover we encourage others laboratory quality managers to implement the same described procedure as a quality indicator and improve future benchmarking.

E-74

Are Volumetric Pipettes Required for Preparing Quality Control Materials?

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Background: Manufacturers of lyophilized quality control (QC) and calibrator materials typically state in the product package insert to reconstitute their products using a volumetric pipette. This requirement is presumably due to inconsistencies between technicians using traditional plunger style pipettes which often have different "stop" positions for aspiration versus dispense causing erroneous delivery in an untrained or incautious hand. Digital pipettes may not display the same differences between technicians since there are no "stops". The goal of this work is to determine whether volumetric pipettes are required for preparing QC.

Objective: Evaluate the precision and accuracy of volumetric and digital pipettes.

Methods: Five technicians were selected from among our laboratory with the following years of clinical laboratory experience in years: <1, 5, 9, 14, and >30. Each of these technicians was asked to dispense distilled water into an aliquot cup on a laboratory balance for 5 replicates of 1, 3, and 5mL volumetric and digital Fisherbrand pipettes. Two different digital pipettes of the same model were used to verify that any differences seen would be consistent and not related to time elapsed since pipette calibration. Careful observation of each tech was performed while the experiment was conducted and differences in pipetting techniques were recorded. The volume was calculated using the mass measurements and density (0.9970 g/mL) for water at 25.0°C. The average standard deviation (SD) and accuracy (% recovery = measured/expected x 100%) was calculated for all 5 participants and their 5 replicates.

Results: Precision of the 1mL digital pipettes show approximately a 5 fold improvement in the SD. The average SD for the 1mL volumetric was 0.014mL whereas the SD for the digital pipettes was 0.002mL and 0.003mL. Accuracy of the 1mL digital pipettes also showed improvement over the volumetric pipettes. Average % recovery for the 1mL volumetric was 98.59% whereas the recovery for the digital pipettes was 99.14% and 99.60%. Precision of the 3mL digital pipettes show improvement over the volumetric but not quite as dramatically as the 1mL. The average SD was 0.015mL whereas the SD for the digital pipettes was 0.010mL and 0.006mL. Accuracy with the 3mL digital pipettes was equivalent with the volumetric pipettes having average recoveries 99.14%, 99.62% and 99.42%, respectively. Precision and accuracy with the 5mL volumetric and digital pipettes are equivalent with an average SD of 0.011mL for all three and average recovery of 99.60%, 99.38% and 99.70%, respectively. All five technicians had a slightly different technique with the volumetric pipette which did not seem to affect the overall results. Years of experience did not seem to correlate with an improvement in precision since the 5 year and 14 year technicians had the highest SD with 0.017mL and 0.022mL, respectively.

Conclusions: The 1 mL digital pipettes had improved accuracy and precision. The larger volume pipettes had equivalent performance. This data suggests the manufacturers' recommendation to use volumetric pipettes to reconstitute calibration and QC material may not be required when digital pipettes are available.

E-75

The role of FMEA tool in the management of the risk and quality improvement in a clinical laboratory of a public medical school hospital

M. E. Mendes, P. A. Ebner, P. Romano, L. L. A. Cazoti, A. Sant Anna, L. C. Barcos, V. R. Morsoleto, G. G. Fragoso, M. Pacheco Neto, N. M. Sumita. *Central Laboratory Division & Laboratories of Medical Investigation (LIM-03) of Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil*

Background: The risk management process in the clinical laboratory concerns the actions taken to recognize or identify risks, to measure the likelihood that something will happen, due to a potentially hazardous situation and evaluate the severity of its impact. The prevention minimizes the possibility of occurrence of the errors and to increase the patient safety. An useful tool to study this condition is the FMEA (Failure Mode Effect Analysis). The aim of this work is to present the use of FMEA tool, demonstrating its applicability in the improvement process in a laboratory of medical school hospital.

Methods: The team of analysts developed the concept for application during introduction of new blood gas analyzers. The proposed goals were to increase the production, to change the workflow and to expand the level of patient safety. The criteria for assessment of the risks were defined as follows: the nature and types of the failures, the modes of the failure and the effects of these failures, the likelihood of the failure and the risk level. The analysis of the effects took into account the consequences of failures, the impacts on its upper level, the final effect of failure, the function and the situation in the analyzed system. The sources of risk, the areas of impact, the events and their causes, the potential consequences and possible loss of opportunities were identified in a comprehensive manner. This risk analysis included the activities that constitute the process, the types of failure which could affect the patient, the severity, the probability of the failure occurrence, the most critical effects of each failure, the potential causes of these effects, the positive and negative consequences. The risk quantification aimed the decision making based on

the prioritization and its implementation. The score was called Risk Priority Score (RPS) obtained by multiplying three factors: severity(1 to 4), occurrence(1 to 5) and detection (1 to 4). The RPS result to trigger the preventive action was equal or greater than 40 points.

Results: The first action: This action referred to administrative procedures related to the tender and its follow up, aiming to increase the efficiency. The initial score of the RPS was 48 points and after the improvement actions the score decreased to 4 points.

The second action: The TAT for carrying out the ionized calcium in serum samples required an interval of three times when compared with the equipments currently in use. After a workload study we decided to increase the number of equipments. The patient risk was reduced from 40 to 12 points.

The third action was directed to clinical staff. To avoid complains a POCT was introduced inside the emergency room. The RPS was 60 and dropped to 4 points.

The last action referred to increase the number of technician and RPS dropped from 40 to 20 points.

Conclusions: The detailed study of the laboratory process allowed the identification of potential error. The study of its effects and risk analysis demonstrated to be a useful tool in the improvement of the process reducing the patient risk.

Thursday AM, July 19, 2012

Poster Session: 9:30 AM - 12:00 PM

Infectious Disease

E-78

A new gold electroplating-modified sandwich amperometric immunosensor to detect HIV p24

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Background: Acquired immune deficiency syndrome (AIDS) is a severe communicable immune deficiency disease caused by human immune deficiency virus (HIV).The laboratorial diagnosis of HIV infection is a crucial composition of controlling AIDS. P24 antigen, the HIV-1 capsid protein, is of considerable diagnostic interest because it is detectable several days earlier than host-generated HIV antibodies following HIV exposure.

Methods: Here, we present a new sandwich HIV p24 immunosensor based on directly electroplating the electrode surface with nano-gold using current time curve method, which greatly increased the conductivity and reversibility of electrodes.

Results: Under optimum conditions, the electrochemical signal had a linear relationship with the concentration of the p24 ranging from 0.01ng/ml to 100ng/ml (R>0.99) and the detection limit was 0.008ng/ml. Compared with ELISA, this method increased the sensitivity by above two orders of magnitude (the sensitivity of ELISA: about 1ng).

Conclusions: This immunosensor may be broadly applied in clinical simple as it was distinct in its ease of application, mild reaction conditions, guaranting reproducibility and anti-inference ability.

E-79

The utility of a single tube Widal test in evaluation of typhoid fever in south eastern Nigeria and the possible cross-interference with malaria co-infection.

J. Okoye. *Department of Laboratory science, Nnamdi Azikiwe University, NNEWI, Nigeria*

Background - Malaria and Typhoid fever remain endemic in sub-Saharan Africa especially in Nigeria. Due to financial constraints, most people in resource limited countries rely on the microbiology lab for diagnosis and decision about therapy .The Widal-Felix test is commonly used in the region to make a diagnosis of typhoid fever and patients usually start treatment based on the results.

Purpose - We decided to report on our experience using the Widal test from January 2008 to July 2011 and to emphasize the need for more affordable point of care diagnostic test for typhoid fever. We also evaluated the cross interference with malaria infection .We used the thin and thick smear for malaria diagnosis.

Method - We did a retrospective chart review of 275 patients who presented at the Guinness Eye Center, Onitsha for evaluation of possible typhoid fever or malaria between January 2008 and July 2011.We used a single cut-off titer of > 200 for the O agglutinin or >100 for the H agglutinin for possible typhoid infection.

Results - A total of 275 patients (134 females and 141 males) were evaluated during the time period. The age range was 22 to 55 years. In 2008, there were 76 patients, 42(55%) were females, 34(45%) were males.48 (63%) had positive titers .44(58%) had positive smear for malaria parasite .16(21%) had both plasmodium spp on the thin smear and positive widal titers .In 2009,there were 79 patients, 32(41%) were females, 45(57%) were males.33 (42%) had positive titers. 46(58%) had plasmodium spp on the thin smear .22(28%) had both malaria parasite and positive titers. In 2010,there were 82 patients, 38(46%) were female, 44(54%) were males.29 (35%)had positive titers.42 (51%) had positive malaria parasite and 16(20%) had both malaria parasite and positive titers. In 2011, there were 38 patients (from Jan 2011 till July 2011). 22(59%) were females and 16(42%) were males.17 (45%)had positive titers .28(74%) had malaria parasite and 11(29 %) had both malaria parasite and positive titers.

Conclusion - Although a single tube agglutination test is not diagnostic of typhoid fever; high titers are suggestive of either active infection or prior exposure. In resource poor countries where patients have to pay out of pocket for all their medical expense, better point of care diagnostic testing are urgently needed. The high prevalence rate (35 % to 63%) of positive widal titers in our cohort is of clinical concern given that most of these patients can at least be advised to seek medical care .The issue of false positive results and cross interference with other febrile illnesses are also of concern. We found a cross interference rate of 21-28% with positive malaria smear in our cohort. Prospective studies using affordable and accurate diagnostic point of care testing are urgently needed in these countries.

E-83

CRP And LDH In CSF And Serum : Promising Markers For Early Diagnosis of Pyogenic Meningitis In Adults : A Pilot Study

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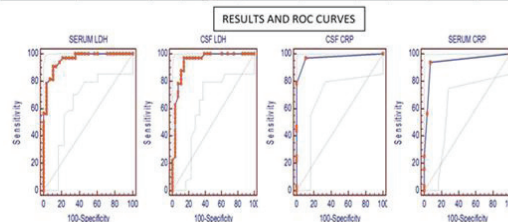
Objectives: Meningitis is one of the commonest neurological diseases in children and adults in India with high mortality and morbidity. Standard routine analysis of CSF in meningitis does not always provide rapid definite information about the causative agent and many hospital laboratories lack facilities for culture and sophisticated equipment. At the basic laboratory setup with a colorimeter or spectrophotometer, estimation of CSF LDH and CRP and comparing it with the levels of Serum LDH and CRP levels may help in early diagnosis of pyogenic meningitis.

Sampling And Data Collection: Study was conducted in Government Hospital, Vijayawada. Study included 32 cases of pyogenic meningitis between 18 to 65 years and 28 age and sex matched controls. CSF was collected on admission by lumbar puncture in sterile bottles and blood was drawn within one hour of lumbar puncture and sent for biochemical, microbiology and cytological studies. Serum LDH was estimated by Wroblewski and LaDue spectrophotometric method. CRP was estimated by using Latex Slide Agglutination semi quantitative method (Accurex).Gram staining, culture and cytological studies were used to confirm diagnosis. Results were analyzed using Analysis of variance, Chi-square and Z Test. ROC curves, Odds ratio and analysis of logistic regression were done to establish the best diagnostic marker. SSPS 17 and Met cal statistical software were used for statistical analysis.

Result: Data collected is tabulated and represented as ROC curves. CSF LDH and CRP show equal sensitivity but CRP has better specificity. Regression analysis showed CSF CRP as the better marker.

Conclusion: LDH, CRP and CSF:serum ratio of CRP are sensitive parameters with high degree of sensitivity and specificity and can be used along with CSF glucose and protein for early diagnosis of pyogenic meningitis in labs where resources are limited and facilities for culture are obscure. A study with larger sample size is required to confirm.

	INCREASED IN CASES	%	MEAN	Sensitivity %	Specificity %	PPV %	NPV %	Z VALUE	P VALUE
CSF LDH	31	96.8	159.062 ± 124.562 IU/L	96.8	85.7	88.6	96.0	13.564	<0.0001
SERUM LDH	29	91	274.25 ± 131.347 IU/L	90.6	89.2	90.6	89.2	20.473	<0.0001
CSF CRP	31	96.8	2.25 ± 2.021 mg/dl	96.8	89.2	91.1	96.1	25.464	<0.0001
SERUM CRP	30	93.7	1.725 ± 2.008 mg/dl	93.7	92.8	93.7	92.8	4.64	<0.001
CSF/SERUM CRP	30	93.7	1.73 ± 1.097 mg/dl	93.7	92.8	93.7	92.8	8.5	<0.001



E-85

Robustness studies and ease of use of a new automated molecular Assay for the detection of MRSA, the BD MAX™ MRSA assay*.

D. Roy, S. Tremblay, H. Galarneau, M. Tremblay, C. Roger-Dalbert. *BD Diagnostics, Quebec city, QC, Canada*

Background: Hospital acquired infection (HAI) is a growing problem in most of the industrialized world. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the leading candidates for these infections in the US as well as in some European

countries. Indeed, even though the rate of infection has generally decreased over the past decades, its rate remains high and is still a major risk for the health care system and its patient population. The objective of this study is to evaluate a novel assay developed on the BD MAX(tm) system for MRSA detection and mainly focuses on its robustness, hands on time (HOT) and overall turn around time (TAT).

Methods: The robustness studies performed on BD MAX(tm) MRSA Assay focused on inadequate use of the assay and the system. Potential failure modes were identified and tested to evaluate their effects on the assay performance. MRSA negative specimens and MRSA positive specimens at 2-3 x LoD95% (Limit of Detection) were tested in presence of negative nasal specimens. The effect analysis was based on status analysis of positive and negative specimens as well as statistical equivalency on one selected PCR metric. In a clinical laboratory environment, time is of the essence. HOT and TAT were measured as an average of three independent runs performed by three technicians. Finally, carry-over and cross-contamination studies were performed.

Results: The robustness tests revealed that the assay performances were not impacted by inadequate vortexing (duration or speed), swab length, and a 4 hour delay between the sample preparation and start of the assay runs. All expected results were obtained and no statistical difference between conditions were observed for cycle threshold values. No carry-over contamination was observed, HOT is about 30 seconds per sample and overall TAT, including sample preparation and complete PCR, was under 180 minutes.

Conclusions: The present study confirms that the BD MAX(tm) MRSA assay is a robust diagnostic test. Its ease of use and automation reduces technician time and ensures fast and reliable results for use by clinicians and health professionals.

*The BD MAX(tm) MRSA Assay is not available for sale or use in the U.S.

E-86

Robustness studies and ease of use of a new automated molecular Assay for the detection of toxigenic Clostridium difficile, the BD MAX™ Cdiff assay*.

D. Roy, J. Hudon, M. Boutin, N. Paquette, C. Roger-Dalbert. *BD Diagnostics, Quebec city, QC, Canada*

Background: In antibiotic treated patients, some toxin-producing *Clostridium* difficile can colonize the gut, causing mild to life threatening diarrhea. This infection is aggravated by the fact that *C. difficile* is able to sporulate and easily spread to other patients. For these reasons, *C. difficile* outbreaks remain a major risk for the health care system and its patient population. The objective of this study is to evaluate a novel assay developed on the BD MAX(tm) system for *C. difficile* toxin B gene detection and mainly focuses on its robustness, hands on time (HOT) and overall turn around time (TAT).

Methods: The robustness studies performed on BD MAX(tm) Cdiff Assay focused on inadequate use of the assay and the system. Potential failure modes were identified and tested to evaluate the effect on the assay performance. Negative specimens and negative stool specimens spiked with *C. difficile* at 2-3 x LoD95% (Limit of Detection) were tested. The effect analysis was based on status analysis of positive and negative specimens as well as statistical equivalency on one selected PCR metric. In a clinical laboratory environment, time is of the essence. HOT and TAT were measured as an average of three independent runs performed by three technicians. Finally, carry-over and cross-contamination studies were performed.

Results: The test performed on the Failure Mode Effect Analysis (FMEA) revealed that the assay performance was not adversely impacted by inadequate vortexing (duration or speed) or by a 4 hour delay between the sample preparation and start of the assay runs. Moreover, a loss of as much as 50% of the sample buffer in the sample buffer tube had no impact on the assay performance. All expected results were obtained and no statistical difference between conditions were observed for cycle threshold values. No carry-over contamination was observed, HOT is less than 1 min per sample and overall assay TAT, including preparation and complete PCR analysis of 24 samples, was under 180 minutes.

Conclusions: Overall, the present study confirms that the BD MAX(tm) Cdiff assay is a robust diagnostic test. Its ease of use and automation reduces technician time and ensures fast, reliable results for use by clinicians and health professionals.

*The BD MAX(tm) Cdiff Assay is not available for sale or use in the U.S.

E-87

Comparison of Soluble Transferrin Receptor (sTfR) Level to the other Anemia Parameters in Patients with Chronic Viral Hepatitis

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Background: We aimed to observe if there is a correlation between sTfR level and the other anemia panel tests; such as serum iron, ferritin, total iron binding capacity(TIBC), transferrin saturation, some CBC parameters(Hb,Hct,MCV) to see whether or not sTfR is useful as a new marker to determine iron deficiency anemia existence of chronic viral hepatitis disease.

Methods: We estimated sTfR levels in thirty(30) chronic hepatitis B patients and twenty(20) chronic hepatitis C patients and compared the results towards the control group consist of twenty(20) seronegative non-anemic healthy people. We calculated sTfR-F index by dividing sTfR levels to the logarithm of serum ferritin levels. sTfR data measured with a particle-enhanced immunoturbidimetric assay (Roche Diagnostics) on Roche Cobas Integra 800 autoanalyzer.

Results: We found no statistical difference between sTfR, sTfR-F index, serum ferritin,TIBC and CBC parameters among the groups(AI-II-III). In the control group and hepatitis B group, there was a weak linear correlation between sTfR and serum iron, ferritin and hemoglobin. In hepatitis C group , there was no correlation between sTfR and serum iron, ferritin and hemoglobin. We made a re-grouping by hemoglobin levels under 13 g/dL and over 13 g/dL disregarding if a patient is chronic hepatitis B or C. We found a significant statistical difference among this groups(AIII-BI-BII). The group hemoglobin under 13 g/dL has higher sTfR and sTfR-F index values, and elevated TIBC despite normal serum iron and ferritin concentrations.

Conclusions: sTfR is a quantitative measurement of tissue iron deficiency, not affected by the infection, more sensitive test to demonstrate iron status than ferritin in infected patients, best correlated with hemoglobin in the present of infection, not increased in anemia of chronic infection, only high in iron deficiency; so it is thought to be useful to assess the status of iron deficiency in chronic viral hepatitis.

Comparison of Anemia Parameters in Chronic Viral Hepatitis

	Group A-I. Hepatitis B n=30 (mean ± SD)	Group A-II. Hepatitis C n=20 (mean ± SD)	Group A-III. Healthy Control n=20 (mean ± SD)	(OneWay ANOVA) AI-II-III p=	Group B-I Hb≤ 13g/dL n=19 (mean ± SD)	(Post-Hoc2s) BI-AIII p=	Group B-II Hb≥ 13g/dL n=31 (mean ± SD)	(Post-Hoc2s) BII-AIII p=
sTfR (mg/dL)	0.42±0.25	0.36±0.12	0.31 ± 0.08	0.095	0.53 ± 0.27	0.000	0.32 ± 0.08	0.291
Ferritin (ng/dL)	96.15±111.52	86±79.34	59.27 ± 26.37	0.330	51.75 ± 78.09	0.892	116.81 ± 103.58	0.053
Serum Iron (µg/dL)	95 ± 42.56	85.25 ± 27.15	65.45 ± 26.67	0.016	64.15 ± 26.63	0.905	107.61 ± 33	0.000
TIBC (µg/dL)	326.4±64.65	352.85 ± 71.49	312.8 ± 65.28	0.161	360.52 ± 72.58	0.03	322.54 ± 61.88	0.634
Hemoglobin (g/dL)	13.5 ± 2	13.48 ± 1.45	13.95 ± 1.27	0.515	11.64 ± 1.07	0.000	14.62 ± 1.03	0.291
Hematocrit (%)	40.6 ± 5.9	40.17 ± 4.29	41.98 ± 4.13	0.459	35.05 ± 2.99	0.000	43.72 ± 3.25	0.405
MCV (fl)	89.23 ± 5.05	88.5 ± 3.63	88.74 ± 3.29	0.823	86.83 ± 5.15	0.220	90.23 ± 3.58	0.407
sTfR-F index	0.3 ± 0.28	0.23 ± 0.13	0.18 ± 0.06	>0.05	0.45 ± 0.31	0.000	0.17 ± 0.05	0.990
TSAT (%)	30.84 ± 16.27	25.28 ± 9.89	24.44 ± 18.04	>0.05	19.23 ± 13	0.271	34.37 ± 11.79	0.080

E-88

Evaluation of a Rapid Antigen Test for the Detection of Norovirus in Stool Samples

H. Kim[1], J. Hyun[1], S. Lee[2], K. Lee[1]. [1]*Hallym University College of Medicine, Seoul, Korea, Republic of,* [2]*Neodin Medical Institute, Seoul, Korea, Republic of*

Background: Norovirus is a leading cause of epidemic and sporadic acute gastroenteritis worldwide. Because of the rapid transmission of the virus, early detection is important to prevent outbreak of norovirus infection. We evaluated the analytical and clinical performance of the newly developed rapid antigen test (SD BIOLINE norovirus test) using immunochromatographic technique for detecting human norovirus group I and II in stool specimens.

Methods: The limit of detection of this rapid antigen test was determined by 2-fold serially diluted positive samples of pooled fecal sample and the concentration of norovirus in each sample was determined by quantitative real time RT-PCR technique

(qRT-PCR). Repeatability/reproducibility were studied using the same 2-fold serially diluted positive samples near the detection limit and negative sample run in triplicates on 10 consecutive days by using 3 three different lots. Cross-reactivity was assessed for 22 virus, 24 bacteria, and 2 fungi. Interference study was performed using blood, hemoglobin, triglyceride, bilirubin, barium sulfate (contrast agent), loperamide (anti-diarrhea drug), metronidazole, and vancomycin (antibiotics). Ninety-two norovirus qRT-PCR positive stool samples and 126 norovirus qRT-PCR negative samples were tested by rapid antigen test for comparison study. Percent positive agreement (PPA) and percent negative agreement (NPA), overall percent agreement of rapid antigen test compared to qRT-PCR were obtained.

Results: Rapid antigen test could detect norovirus equivalent to 4.48 x 10^6 copies/mL norovirus genome in stool samples. Samples above this concentration were all positive (100%), and samples below this concentration (2.45 x 10^6 copies/mL) were 97.8% negative by repeatability/reproducibility test. There were no cross-reactivity for tested 22 viruses, 24 bacteria, and 2 fungi, and no interference for tested substances (blood, hemoglobin, triglyceride, bilirubin, barium sulfate, loperamide, and metronidazole, vancomycin). Eighty-three samples of 92 qRT-PCR positive samples (90.2%) were positive by rapid antigen test and all of 126 qRT-PCR negative samples were negative by rapid antigen test. Therefore, PPA, and NPA, and overall percent agreement of rapid antigen test compared with qRT-PCR were 90.2% (95% confidence interval: 82.2-95.4%) 100% (97.1-100%), and 95.9% (92.3-87.1%), respectively. Including 15 minutes-waiting time for reading, total procedure of rapid antigen test was finished within 20 minutes.

Conclusions: SD BIOLINE norovirus test was easier and quicker to perform, and showed excellent reproducibility, no cross-reactivity, no interference, and high agreement rate with qRT-PCR (especially, 100% negative percent agreement). This test seems to be a useful tool for rapid screening of norovirus infection.

E-89

Developing and Validating Robust Genotyping Assays for IL28B and ITPA SNPs for Global Clinical Trials and Epidemiologic Studies in Hepatitis C Virus (HCV) Infected Subjects

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Background: HCV infects up to 3% of human population and is a common cause of chronic liver diseases. Genome-wide association studies identified genetic variations in *IL28B* region as the strongest predictors for control of HCV infection, and medically benign variants in *ITPA* gene conferring protection to standard of care drug ribavirin-induced anemia. To add to the knowledge on HCV viral characteristics, host-genetics, and their relationship in HCV infected populations, global clinical trials in HCV treatment and epidemiologic studies in HCV infection are ongoing at Merck to evaluate treatment responses associated with *IL28B* and *ITPA* genotypes, and to compare the distributions of these genotypes between ethnic populations from different regions of the world.

Methods: Due to regulatory policies in several participating countries, *IL28B* and *ITPA* genotyping assays must be performed by local accredited laboratories. This requires robust assays that can be easily transferred, executed and analyzed across different laboratories in different countries. TaqMan SNP genotyping technology was chosen due to the availability of ABI assay for four of the seven interested SNPs, and wide adoption of ABI real-time PCR instrumentations. For three *IL28B* SNPs, no commercial ABI assay was available, likely due to high degree of sequence similarity among several chromosomal regions. Custom TaqMan SNP genotyping assays for these three SNPs were designed at Merck, using sequence knowledge obtained from 96 DNA samples with different ancestries. The validated and optimized assays were then transferred to a Contract Research Organization (CRO) and validated again with more DNA samples with different ancestries.

Results: High levels of genetic polymorphisms around the three *IL28B* SNPs among different ethnic groups discovered during assay development forced an iterative design process in order to achieve 100% concordance between results obtained from designed TaqMan SNP assays and Sanger sequencing. PCR parameters had to be modified and DNA input masses had to be increased to ensure all assays can be run with the same PCR conditions. A template for TaqMan Genotyper software was designed to ensure consistent data analysis. All seven genotyping assays were validated at Merck for precision, accuracy, specificity, sensitivity, and analyte stability before transferring to the CRO, where the majority of samples from participating countries will be analyzed. At the CRO, an additional 100 DNA samples with African, Chinese, South Asian, or Caucasian ancestry were analyzed with both Sanger sequencing and the transferred assays to confirm 100% concordance and thus accuracy of the transferred assays. Intra- and inter-run precision were tested on 10 DNA samples extracted from whole blood samples collected in EDTA tubes and another 10 from whole blood collected with PAXgene method and 100% precision was achieved. Assay efficiency expressed in the percentage of tests producing valid genotype calls were 99.8% in two assays and 100% in the rest of five assays.

Conclusions: Robust *IL28B* and *ITPA* TaqMan SNP genotyping assays were developed and validated at Merck and transferred to the CRO for global studies that will provide critical information on the likelihood of HCV treatment response based on *IL28B* genotype distribution and of ribavirin-induced anemia based on *ITPA* genotypes.

E-90

Syphilis on the VITROS® 5600 Integrated System Ready for the Routine Laboratory Testing

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Today syphilis screening (treponema pallidum) plays a major role in clinical testing as well as blood and transplant donor screening. Laboratories wishing to facilitate a random access solution for this service must ensure an excellent performance to maximize sensitivity in clinical subjects to ensure no undue patient stress and no rejection of very precious blood or organ donations. This must also be balanced with equally good specificity. Ortho-Clinical Diagnostics have recently introduced a VITROS® Immunodiagnostic Products Syphilis TPA Assay which we evaluated for use in this laboratory.

Our study comprised a precision study using three pools of patient serum and analysed in duplicate twice a day for twenty days. A clinical comparison using two hundred samples previously tested on Abbott Architect i4000. Cross reactivity study tested cohorts of samples from potentially cross reacting conditions including positive hepatitis B antibody and antigen, anti-nuclear antigen, anti-dsDNA, anti-mitochondrial antibody, anti-parietal antibody, high tumour marker samples and antenatal samples. An interference study was conducted to determine whether haemolysis, icterus or lipaemia affected the interpretation of the result.

Total precision for the three levels produced a coefficient of variation of <3.0%. One hundred and forty six samples screened non-reactive by both methods. Forty three samples screened reactive by both methods (confirmed TPPA reactive). Eight cases were reactive on Architect and non-reactive on VITROS; all cases were TPPA non-reactive. One case of grey zone on Architect which was both VITROS and TPPA reactive. Two cases of both Architect and VITROS reactive, one TPPA non-reactive and one indeterminate. Using TPPA as confirmation the percent positive agreement was 100% and the percent negative agreement was 99.3% with an overall accuracy of 99.5%. The cross reactivity study did not produce any reactive syphilis results. Neither lipaemia nor icterus showed any significant interference at an S/CO of 0.8 or 1.6. However haemoglobin (2000mg/dL) showed over recovery of the S/CO in both samples potentially producing a reactive screening result.

An excellent performance was seen for the syphilis assay on the VITROS 5600. No cross reactivity was detected in any of the potential interferents tested in this study. VITROS correctly identified eight samples previously screened reactive, but confirmed negative and one case of grey zone on Architect which was reactive on VITROS. Haemolytic interference, although causing over recovery can be flagged and auto-invalidated with the simultaneous use of the serum indices on the analyser ensuring no incorrect results are reported.

In conclusion, a good option for a laboratory looking for a random access system for both clinical sample and donor screening testing of syphilis.

E-91

Distribution of genotypes/species of HPV in penile lesions biopsies

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Introduction: Investigations on the human papillomavirus (HPV) are mostly focused on women. However, HPV infection in men and its consequences are not completely understood. The limited studies available suggest that the virus prevalence in men is high, and that they are the spreader of the infection. Thus, the aim of this study was to describe the HPV infection in penile lesions by comparing the results of biopsies with viral genotyping.

Methods: Through retrospective analysis of our laboratory results database, we assessed patients who had undergone biopsies and HPV genotyping in penile injury on the same day, between February 2009 and May 2011. Forty-three samples met this condition. Next, we evaluated the prevalence of HPV genotypes/species, and

concordance between the two methodologies. In addition, we correlated the different cytological abnormalities evidenced by biopsy (exophytic lesion, hyperkeratosis, parakeratosis, irregular acanthosis, papillomatosis, hypergranulosis, coilocitotic atypia, keratinization and hyperplasia and loss of basal polarity) with the presence of the virus, the type of infection (simple and multiple) and its oncogenicity (high and low risk). The average age of patients was 30.3 ± 7.9 years, and genotyping was performed by PapilloCheck (Greiner Bio-One), which evaluates 24 different HPV types (18 high risk and 6 low risk).

Results: Genotyping demonstrated that 72.1% of penile lesions were positive for HPV. Among these, 38.7% were single infections and 61.3% multiple infections. Of 68 found viruses, 57.3% were low risk, 42.6% high risk, and the five most prevalent genotypes were 6 (25%), 11 (13.2%), 42 (10.3%), 39 (5.9%) and 44/55 (5.9%). When we grouped the genotypes in species, A10 was the most prevalent (44.1%), consisting of low-risk genotypes, followed by A7 (14.7%) consisting of high-risk genotypes. However the biopsy demonstrated that 65.1% of the penile lesions showed cellular changes suggestive of viral infection. Thus, the agreement among the two methods was 69.6% (P = 0.078). The cellular changes associated with HPV were hyperkeratosis (P = 0.003), irregular acanthosis (P = 0.003) and papillomatosis (P = 0.037). The Koilocitótic atypia was close to significance (P = 0.09). In addition, we observed that parakeratosis (P = 0.046), koilocitótic atypia (P <0.0001), hyperplasia (P = 0.024) and loss of basal polarity (P = 0.024) are more prevalent in multiple infections than in simple. However, we found no association between virus oncogenicity and cytological alterations.

Conclusion: In penile lesions for HPV positivity is high, multiple infections surpass the simple, and the genotypes/species of low risk are the most prevalent. The correlation between biopsy and genotyping is not complete, suggesting the presence of HPV types not covered by the genotyping test run, and the presence of the virus in the form of subclinical lesions. On the other hand, the association among the multiple infections and some types of cellular changes indicate the need for the presence of more than one type of viruses for its appearance in men. Thus, it is noted that important information about HPV infection in men could be extracted by analyzing a database of a clinical laboratory.

E-92

Changes in HPV infection pattern with advancing of age in men and women

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Introduction: Human papilomavirus (HPV) is the most common sexually transmitted infection. Since age influences the sexual behavior, it can also affect the HPV infection patterns in men and women. In a previous study, we observed that positivity, multiple infections, and low-risk HPV genotypes and species are more prevalent in men than in women. Now, the aim of this work was to describe the influence of age on these aspects by assessing our clinical laboratory results database.

Methods: Through retrospective analysis of our HPV genotyping database, we assessed the sample results between February 2009 and May 2011. 4,251 samples were included, all from HPV genotyping test of anogenital region. The genotype and species prevalences, single or multiple infection, high and low oncogenic risk were identified and presented by age groups ≤30 years, 31-44 years, ≥45 years, and gender. Overall, 1,991, 1,693, 567 samples were included in each age groups, respectively. Of these, 1,507, 1,299, and 395 were women, and 484, 394, and 172 were men. HPV genotyping was performed using PapilloCheck (Greiner Bio-One), which evaluates 24 different HPV genotypes (18 high-risk and 6 low risk).

Results: The database analysis demonstrates that men had proportionately more positive results than women in all age groups, 63.8% vs 49.1% (p<0.0001) for ≤30 years, 54,1% vs 33,9% (p<0.0001) for 31-44 years, and 43% vs 26,8% (p<0.0001) for ≥45 years. In addition, the positivity declines with age in both genders (Ptrend<0,0001). Moreover, multiple infections are more frequent in young men, 64.1% vs 47.9% (p<0.0001), and middle-aged men, 48.8% vs 36.4% (p=0.0029) than women in the same age group. However, this difference was not found between older men and women, 35.1% vs 31.1% (p=0.62). Also, multiple infections decline and simple infections increase with age in both genders (Ptrend<0,0001). Besides that, in women, the occurrence of some genotypes vary with age group, HPV31 - 5,15%, 4,02%, and 1,88% (Ptrend=0.04), HPV39 - 5,94%, 2,91%, and 1,25% (Ptrend=0.0002), HPV42 - 5,87%, 4,16%, and 3,13% (Ptrend=0.04), HPV44 - 4,36%, 7,48%, and 14,38% (Ptrend<0.0001), HPV45 - 1,29%, 2,63%, and 3,13% (Ptrend=0.01). This was not observed in men. Moreover, the proportion of high and low risk genotypes and HPV species distribution did not vary in and with age.

Conclusion: In this study, we observed that some patterns of HPV infection vary significantly in the assessed age groups. The positivity and multiple infections decrease, and single infections increase with advancing age in men and women. Also, the frequency of HPV31, HPV39, HPV42 decrease, and HPV44, HPV45 increase with age only in women. Moreover, younger and middle-aged men have more multiple infections than younger and middle-aged women, but this was not observed for the elders. The proportion of high and low risk genotypes and species did not vary among age groups. Thus, we conclude that some HPV infection patterns are influenced by age in men and women.

E-96

Comparison of the qualitative results of the Venereal Disease Research Laboratory test with those of an automated rapid plasma reagin test

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Background: Syphilis has several clinical manifestations, and this makes laboratory testing a very important aspect of diagnosis. Traditionally, testing for syphilis includes an initial screening with an inexpensive nontreponemal test (e.g., the Venereal Disease Research Laboratory [VDRL] test and the rapid plasma reagin [RPR] test), followed by the retesting of reactive samples with a more specific and expensive treponemal test. We compared the efficacy of an automated rapid plasma reagin test (HiSens Auto RPR, HBi) with that of the manual VDRL test for diagnosing syphilis.

Methods: We collected 231 sera samples that were reactive in the VDRL test from the Seoul Paik Hospital, Seoul, Korea, between May 2010 and Oct 2010. All the samples were analyzed using the HiSens Auto RPR test, and the results were compared with those of the VDRL test. Additional treponemal tests (i.e., treponema pallidum haemagglutination, FTA-ABS, and HiSens Auto TP Ab test) were performed for samples that showed discrepant results with the VDRL test and HiSens Auto RPR test.

Results: The overall concordance rate between the VDRL test and HiSens Auto RPR test was 49.4% (114 of 231 samples). The remaining 50.6% (117 of 231 samples) of the samples showed negative results with the HiSens Auto RPR test. Of these 117 samples, 77 were reactive in 1 or more treponemal tests, 32 were nonreactive in these tests, and the remaining 8 were not tested. Of the 77 samples that were nonreactive in the HiSens Auto RPR test, 24 were collected from clinically diagnosed patients who had begun treatment for syphilis. These samples would have been missed if the HiSens Auto RPR test was used as the initial screening test.

Conclusions: The automated RPR test (HiSens Auto RPR) shows a relatively low concordance rate with the manual VDRL test and may yield false-negative results in patients who need treatment, especially those with latent syphilis.

E-97

Performance Characteristics of a DNA Sequencing-Based HIV-1 Coreceptor Tropism Assay for Use in a Prospective Double-Blind Randomized Phase 3 Study (A4001095, MODERN)

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Background: Following submission of analytical and preliminary clinical performance data, the Siemens HIV-1 Coreceptor Tropism laboratory-developed test (LDT)* received regulatory approval (IDE) for use in the study titled "Maraviroc (MVC) Once-daily with Darunavir Enhanced by Ritonavir (DRV/r) in a New regimen (MODERN)." The MODERN study is a prospective double-blind study with two-stage randomization of treatment-naive individuals to genotypic or phenotypic testing followed by randomization to treatment arms (MVC QD + DRV/r; TDF/FTC QD + DRV/r). This is the first prospective application of sequencing-based viral tropism testing to select patients for entry inhibitor therapy in a large registrational clinical trial.

Methods: The assay input is viral RNA extracted from plasma. Triplicate reverse transcription and PCR amplification reactions are performed, followed by dye-terminator DNA sequencing of the V3 loop of HIV-1 glycoprotein 120 to identify CCR5 coreceptor-utilizing viruses (R5). Assay analytical parameters were tested with panels of in vitro-transcribed *env* gene clones, subtype B HIV-1 (8E5), and non-B subtype HIV-1 isolates. Clinical performance was evaluated using treatment-experienced patient samples from the MOTIVATE and A4001029 studies (N =

315), including equal numbers of R5 and non-R5 patients at screening based on phenotyping using Trofile™(Monogram Biosciences, Inc.). Of these patients, 94% were previously determined to be infected with clade B HIV-1. Two-sided 95% confidence intervals (95% CI) were calculated for comparison of results between genotyping and phenotyping.

Results: All analytical performance specifications were met or exceeded, including viral load sensitivity for B and non-B subtypes (300 and 1,000 RNA copies/mL), sequencing accuracy (99.99%), and clonal sequence mixture sensitivity (20%). The assay's reportable result rate in this retrospective study was 95.4%. Substantial concordance (77.8%) with enhanced sensitivity phenotyping was achieved using the Geno2Pheno [coreceptor] algorithm (MPI, Saarbruecken, Germany) with false-positive rate threshold of 10%. Clinical sensitivity to predict positive virological outcome (defined as week 8 viral load decrease of $\geq 2 \log_{10}$ or undetectable viral load) was 65.7% (95% CI: 58.5%-72.8%). Clinical specificity, prediction of negative virological outcome, was 53.4% (95% CI: 45.3%-61.5%). These results were comparable to phenotyping, with a clinical sensitivity of 64.5% (95% CI: 57.3%-71.7%) and specificity of 60.3% (95% CI: 52.3%-68.2%).

Conclusions: Assay analytical performance was consistent with that of other population sequencing methods, and clinical performance was comparable with phenotyping. This assay is being further examined in the ongoing MODERN study. Results from this clinical trial may provide additional support for the routine clinical use of genotypic tropism testing.

* Under development. Not available for sale.

E-104

Kinetics of Toxo Seroconversion in a Swiss Pregnant Women Population

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Objective: Retrospective evaluation of the kinetics of Toxo antibody production in pregnant women undergoing Toxo seroconversion.

Relevance: Whereas a high Toxo IgG avidity result excludes an acute toxoplasmosis a low avidity result does not diagnose a recent toxoplasmosis. The goal of this evaluation was to determine if ARCHITECT Toxo IgG antibody ratios using paired sera can assist in the detection of a recent toxoplasmosis in patient samples containing Toxo IgM antibody and low avidity Toxo IgG.

Methods: Archived patient samples (n = 284) selected from pregnant women (n = 53) with documented recent seroconversion for toxoplasmosis were tested by the ARCHITECT Toxo IgG, IgM and IgG avidity assays and by the AxSYM Toxo IgG and IgM assays. ARCHITECT and AxSYM Toxo IgG titer ratios from paired sera were calculated as follows: ARCHITECT or AxSYM Toxo IgG titer sample 2/ ARCHITECT or AxSYM Toxo IgG titer sample 1, where sample 2 is ideally drawn 2-4 weeks after sample 1. Both samples were tested on the same instrument on the same run on the same day.

Results: In samples from pregnant women undergoing seroconversion for toxoplasmosis detection of Toxo-specific IgM before IgG occurred in 6/53 patients (11%). Analysis of ARCHITECT and AxSYM Toxo IgG titer ratios were possible in 36 patient cases: 18 cases were untreated and 18 cases were treated with Rovamycine (spiramycin). The untreated and treated cases were analyzed separately and the data were sorted in descending order of ARCHITECT Toxo IgG titer ratio. A significant increase in ARCHITECT or AxSYM Toxo IgG antibody titer was defined as a ratio > 2.0 and was considered the titer ratio cutoff. In untreated cases, sensitivity of detection of a recent toxoplasmosis, defined as Toxo IgG seroconversion within the past 3 months, was 94% (17/18) by the ARCHITECT assay. In the detected untreated case group, median time from the last seronegative bleed to sample 1 was 57 days and the median ARCHITECT and AxSYM titer ratios were both 6.5. In treated cases, the sensitivity of detection of a recent toxoplasmosis drops to 61% (11/18) by the ARCHITECT assay. In the detected treated case group, median time from the last seronegative bleed to sample 1 was 44 days and the median ARCHITECT and AxSYM titer ratios were 5.6 and 6.2, respectively. The median time interval between bleeds in both detected untreated and treated cases was 23 days.

Conclusions: In cases of pregnant women not treated with spiramycin, where Toxo IgM and low avidity IgG are present, if the ARCHITECT Toxo IgG titer ratio is > 2.0 in paired sera ideally drawn 2-4 weeks apart, the infection likely occurred within the past 3 months from the first serial bleed draw. In untreated cases where the ARCHITECT Toxo IgG ratio < 2.0, no conclusion can be drawn and the samples should be sent to a toxoplasmosis reference laboratory for further analysis. The sensitivity of detection of a recent toxoplasmosis in treated cases is less due to the presumed attenuation of the Toxo IgG response by spiramycin therapy.

E-105

Performance Evaluation of a Prototype HBsAgII Assay on the ADVIA Centaur System

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Objectives: HBV surface antigen (HBsAg) is the established serological marker used routinely for the diagnosis of acute or chronic HBV infection. Beginning in the 1980s, mutant forms of HBV were recognized that evade the host's immune response and in some cases fail immunoassay detection, particularly when using tests based on monoclonal antibody reagents. We report the development of an enhanced assay* for the detection of HBsAg and commonly found HBsAg mutations, and the results of an evaluation of this assay.

Methods: Specificity and sensitivity were determined by testing approximately 2790 paid donor samples presumably negative for HBsAg and approximately 370 patient samples. Each sample was tested in singlicate. Analytical sensitivity was determined using serial dilutions (WHO Second International HBsAg Standard) run in duplicate using two assay kit lots. Mutation sensitivity was evaluated with 27 HBsAg mutant samples with single or multiple amino acid substitutions.[†] Seroconversion sensitivity was evaluated with 30 commercial seroconversion panels. Precision was determined according to the CLSI EP5-A2 protocol: two runs/day for 20 days. Samples from HAMA, rubella, VZV, SLE, ANA, EBV, HSV, HBcT, HCV, HIV, HTLV, HAVT, CMV, ToxM, RF, syphilis, nonviral liver disease, and flu vaccine recipient samples were tested for cross-reactivity. Interference was evaluated according to the CLSI EP7-A2 protocol. High-dose hook effect was evaluated by testing serially diluted HBsAg ad and ay samples.

Results: Sensitivity and specificity were 100% and >99.9%, respectively. Calculated analytical sensitivities for each kit lot were 0.037 IU/mL and 0.035 IU/mL. This assay also detected a variety of mutations in amino acids 122 to 145 of the main antigenic determinant of HBsAg that were tested, including the most commonly reported G145R mutation. Mutant sensitivity was 100%. The overall seroconversion sensitivity of the assay was equivalent to that of other commercial methods. The HBsAgII assay had within-run and total %CVs of less than 7% and 11%, respectively, over the assay range in a 20-day precision study. This assay was evaluated for potential cross-reactivity with other viral infections and disease state specimens, and no change in clinical interpretation was observed. No clinically significant changes were observed for the common interferents tested. The assay did not hook below the cutoff range (Index: <1.0) at HBsAg concentrations as high as approximately 3 mg/mL.

Conclusions: The results of this study show that the prototype ADVIA Centaur® HBsAgII assay is sensitive and specific for detecting HBsAg. The assay performs comparably to currently marketed assays and additionally detects wild-type HBsAg and HBsAg mutants within the critical "a" determinant regions.

* Under FDA review. Not available for sale in the U.S. This assay is CE marked.

[†] In this study, 26 recombinant mutants and one native mutant were tested.

E-106

Implementation of FilmArray respiratory viral panel in a core laboratory improves testing turn-around-time and patient care

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Background: FilmArray respiratory virus panel (Idaho Technologies; Salt Lake City, UT) uses multiplex PCR technology to detect 15 viral agents in respiratory specimens. The test requires 5 minutes hands-on time, 65-min of analysis time, and general medical technologists with proper training are able to perform the test. In a survey of 10 clinical labs in the United States, only one lab performs the test in the core lab, with the rest performing the test in the microbiology, virology, or molecular lab.

Methods: To provide timely diagnostic service for the emergency department, we performed FilmArray in the core lab which provides 24/7 service with ~35 FTEs. All the tests in core lab are based on Lean, single-piece flow principles without batching. Since this is the first molecular test in the core lab, multiple continuous education sessions were given to all core staff regarding technical procedures and the principles. Prior to this molecular test, we sent respiratory viral testing to a reference lab for direct immunofluorescence assay (DFA) testing which detects 8 viral agents. DFA testing involved multiple processing steps in our lab and specimen transport to the reference lab. DFA specimens were batched and run 3 or 4 times per day depending on the

volume during respiratory virus season.

Results: Respiratory virus testing with FilmArray was initiated in December, 2011. During the first 6 weeks, 738 tests were performed. The average and median turn-around-time (TAT) were both 1.5 hours with 80% completed in 2 hours and 95% in 3 hours. During the same period last year 255 DFA tests were performed. The average and median TAT for DFA were 7 and 6.5 hours, respectively, with 2% completed in less than 3 hours. Rhinovirus was detected in 25% and coronavirus in 6% of samples using FilmArray (viruses not detected by DFA). Thus, an additional 223/738 (31%) specimens had viruses detected by FilmArray that would have been missed by DFA. The first 10 cases of positive influenza A were evaluated. Test results were available in less than 2.5 hours for 4 patients, who received oseltamivir in less than 3 hours after tests were ordered. Two inpatients received oseltamivir the next morning after test results were released during the night. Three additional patients were discharged and positive results were communicated to parents or primary care physicians within a few hours or the next morning. One of the 10 patients who had live attenuated influenza vaccine given <7 days prior to testing was tested positive for both influenza A and B.

Conclusions: The implementation of FilmArray in core lab has decreased the TAT to detect respiratory viruses. Although current treatment for respiratory viral infection is limited to Influenza A and B, detection of other viral agents is valuable because clinical suspicion of respiratory tract infections may be confirmed, unnecessary additional work-up and therapy can be avoided, and clinicians and parents are reassured. In addition, we implemented a molecular-based diagnostic test in our core lab for the first time, marking a new era in pediatric clinical laboratory services.

E-107

Comparison of LIAISON® XL MUREX automated immunoassays to ADVIA Centaur for the detection of HBsAg, anti-HCV and HIV markers

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Background: The aim of the study was to compare the immunoassays analyzer LIAISON® XL (XL) (DiaSorin Saluggia- Italy) to ADVIA Centaur (Siemens Healthcare Diagnostics USA) (ADVIA) currently used in our laboratory for the detection of HBsAg, anti-HCV and HIV markers.

Methods: XL HCVAb is an indirect test with recombinant antigens of HCV regions Core, NS3 and NS4 for the qualitative detection of antibodies. XL HBsAg Quantitative is a sandwich test employing a set of anti-HBs monoclonal antibodies directed against highly conserved epitopes of the superficial antigen internal region to ensure high sensitivity in the detection of different mutants and genotypes. XL HIV Ab/Ag assay employs HIV-1, HIV-1 group O, and HIV-2 antigens and anti-p24 monoclonal antibodies in two coupled reagents cartridges. We tested samples from routine activity and from selected samples with values slightly above the Centaur cut-off: 380 samples for HBsAg, 463 for HCVAb and 333 for HIVAb+Ag.

We added Ortho Clinical Diagnostics VITROS® HBsAg Assay and VIDAS HBs Ag Ultra bioMérieux Clinical Diagnostics for discordant results for HBsAg test;: Ortho Clinical Diagnostics VITROS® Anti-HCV Assay and Chiron® RIBA™ HCV 3.0 SIA for HCVAb test; VIDAS® HIV DUO QUICK bioMérieux Clinical Diagnostics and Chiron RIBA HIV1/HIV-2 Strip Immunoblot Assay (RIBA HIV-1/2 SIA) for HIV test.

Results: Sensitivity (Sens) and specificity (Spec) were: XL HBsAg Quantitative Sens= 100%, Spec=100%; HBsAg ADVIA Sens= 100%, Spec=98.0%; XL HCVAb: Sens= 100%, Spec=99.7%; HCV ADVIA Sens= 100%, Spec=96.1%; XL HIV Sens=100%, Spec=99.1%; HIV Combo ADVIA Sens= 100%, Spec=82.7% (See Figure; R=Reactant; NO R=No Reactant). We examined a "panel of recDNA-HBsAg mutant antigens" (supplied by DiaSorin) (TAB) to evaluate the sensitivity against HBsAg mutants.

Conclusions: Sens and Spec of LIAISON® XL HBsAg Quant, anti-HCV and HIV Ab/Ag are amenable to routine screening assays and LIAISON XL HBsAg Quant mutants detection was particularly satisfying.

Figure

#	LSN	VITROS	VIDAS	HEPANOSTICA	ELECSYS	CENTAUR
1	R	R	R	R	R	R
2	R	NR	NR	R	NR	R
3	R	NR	NR	R	NR	NR
4	R	NR	NR	R	NR	NR
5	R	NR	R	R	NR	R
6	R	NR	R	R	NR	NR
7	R	R	R	R	R	R
8	R	R	R	R	R	R
9	R	R	R	R	R	R
10	R	R	R	R	R	R
MATRIX	NR	NR	NR	NR	NR	NR

E-108

Serum Cytokine Levels in Chronic Hepatitis B Patients during the Course of Peginterferon Alpha-2a Therapy

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Background: The relationship between cytokines and responses to peginterferon α-2a treatment in chronic hepatitis B (CHB) patients has not yet been elucidated. We analyzed the serum levels of interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor, interferon-γ (IFN-γ), tumor necrosis factor-α, monocyte chemotactic protein-1 (MCP1) and epidermal growth factor (EGF) during the course of peginterferon α-2a.

Methods: A total of 93 sera from 20 CHB patients were prospectively collected before, during and after 48 weeks of peginterferon therapy and were assayed for 12 kinds of cytokines. The patients were categorized as either virologic responders (VR) or non-responders (NR) according to serum HBV DNA levels taken at 24 weeks after initiation of treatment. VRs were subdivided into remission and recurrence groups according to serum HBV loads taken at 6 to 12 months after the therapy had finished. The Evidence Investigator (Randox, Antrim, UK), a protein chip analyzer, was used to quantify cytokines.

Results: Among 12 cytokines, levels of MCP1, IL-6 and IFN-γ were increased during treatment in VRs, while EGF and IL-4 levels were decreased during treatment in the same group (Table 1). MCP1 levels were higher in VRs than in NRs, and EGF concentrations were increased in NRs after the treatment finished. Levels of those cytokines also showed similar changes according to treatment phases in the remission group.

Conclusions: Serum cytokine levels reflected the pathological differences of individual treatment phases and could also be useful indices for monitoring responses to peginterferon treatment in CHB patients.

Table 1 Serum levels of cytokines according to responses and treatment phases in chronic hepatitis B

Cytokines (pg/mL)	VR			NR			P-value
	Before Tx (n=11)	During Txa (n=25)	After Txb (n=17)	During Txa (n=19)	After Txb (n=13)	After Txb (n=13)	
EGF	114.90 (28.35 - 123.62)	37.85 (17.15 - 48.70)	57.95 (38.48 - 87.13)	66.62 (43.59 - 99.03)	43.45 (31.65 - 74.57)	109.80 (80.78 - 131.90)	0.0001
IFN-γ	0.00 (0.00 - 0.74)	1.20 (0.00 - 1.51)	1.02 (0.00 - 1.53)	0.00 (0.00 - 0.54)	0.93 (0.00 - 1.30)	0.00 (0.00 - 0.92)	0.0151
IL-1α	0.00 (0.00 - 0.48)	0.00 (0.00 - 0.27)	0.00 (0.00 - 0.00)	0.12 (0.00 - 0.38)	0.00 (0.00 - 0.29)	0.00 (0.00 - 0.32)	0.4628
IL-1β	0.97 (0.75 - 1.23)	0.87 (0.00 - 1.22)	0.81 (0.00 - 2.50)	1.17 (0.98 - 6.43)	1.20 (0.13 - 3.17)	1.77 (0.88 - 5.14)	0.0939
IL-2	3.41 (0.55 - 7.49)	3.28 (0.00 - 3.93)	3.80 (3.37 - 4.97)	0.00 (0.00 - 18.51)	0.00 (0.00 - 4.93)	3.54 (0.00 - 8.79)	0.5231
IL-4	2.77 (0.00 - 7.44)	0.00 (0.00 - 0.00)	0.00 (0.00 - 2.09)	2.09 (0.87 - 2.57)	0.00 (0.00 - 2.23)	2.17 (0.00 - 2.60)	**0.0009**
IL-6	0.69 (0.59 - 0.96)	1.50 (1.15 - 2.48)	0.97 (0.75 - 1.43)	0.57 (0.52 - 1.39)	0.93 (0.64 - 1.82)	0.84 (0.64 - 1.56)	**0.0041**
IL-8	72.15 (45.70 - 112.83)	28.85 (17.96 - 49.76)	43.13 (21.08 - 316.30)	42.01 (31.72 - 115.55)	16.22 (11.52 - 38.66)	273.20 (21.10 - 609.17)	0.0810
IL-10	0.97 (0.64 - 1.49)	0.82 (0.53 - 1.07)	0.60 (0.40 - 0.75)	1.28 (1.05 - 1.60)	0.95 (0.59 - 1.17)	0.62 (0.42 - 0.98)	**0.0112**
MCP1	260.40 (220.33 - 325.92)	370.20 (337.80 - 457.60)	363.50 (300.73 - 389.30)	190.45 (174.38 - 245.28)	263.60 (229.20 - 301.37)	218.60 (170.13 - 260.20)	**<0.0001**
TNF-α	2.32 (1.82 - 2.94)	2.65 (2.11 - 3.57)	2.32 (1.53 - 3.10)	3.40 (1.78 - 4.93)	2.69 (1.73 - 4.33)	2.03 (1.48 - 3.48)	0.2795
VEGF	72.33 (46.57 - 162.10)	88.19 (37.15 - 127.83)	50.46 (38.37 - 160.50)	98.73 (67.11 - 174.36)	66.65 (58.98 - 97.82)	72.33 (45.86 - 138.67)	0.7009

Abbreviations: VR, virologic responder; NR, virologic non-responder; Tx, peginterferon α-2a therapy for 48 weeks; EGF, epidermal growth factor; IL, interleukin; IFN, interferon; MCP1, monocyte chemotactic protein-1; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.
Data are shown as median (1st to 3rd quartiles).
a Samples were obtained at 12 to 36 weeks from peginterferon α-2a therapy initiation.
b Specimens were collected at 6 to 12 months after 48 weeks of peginterferon α-2a therapy had finished.

E-109

Clinical Performances of the Abbott RealTime High Risk HPV, Roche Cobas HPV and Hybrid Capture 2 Assays Compared to Direct Sequencing and Genotyping of HPV DNA

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Background: Infection of high-risk (HR) human papillomavirus (HPV) genotypes is an important risk factor for developing cervical cancers. We evaluated the clinical performances of two new real-time PCR assays for detecting HR HPVs compared to the Hybrid Capture 2 test (HC2) (QIAGEN GmbH, Hilden, Germany).

Methods: A total of 356 cervical swab specimens were assayed by HC2 as well as two real-time PCR assays: Abbott RealTime HR (Abbott Molecular Inc., Abbott Park, IL, USA) and Roche Cobas HPV (Roche Molecular Diagnostics, Pleasanton, CA, USA). Discrepant results among the three assays were assessed by genotyping using liquid bead microarray and direct sequencing.

Results: The overall concordance rate among results for the three assays was 82.6%, and the results of the Abbott RealTime HR and Roche Cobas HPV assays agreed with those of HC2 in 86.1% and 89.9% of cases, respectively. The two real-time PCR assays agreed with each other for 89.6% of the samples, and the concordant rate between them was equal or greater than 98.0% for detecting HPV 16 and 18. HC2 demonstrated a sensitivity of 96.6% with a specificity of 89.1% for detecting HR HPVs, while Abbott RealTime HR presented a specificity of 99.2% with a sensitivity of 78.3%. The sensitivity and specificity of the Roche Cobas HPV for detecting HR HPVs were 91.7% and 97.0%. Abbott RealTime HR showed better sensitivity for HPV 16, whereas Roche Cobas HPV demonstrated better sensitivity for HPV 18 (Table 1).

Conclusions: The new real-time PCR assays exhibited comparable clinical performances to HC2, but discrepancies among the assays arose mainly in detecting HR HPVs other than HPV 16 and 18. The newly introduced assays would be useful for simultaneously identifying HPV 16 and 18 from clinical samples.

Table 1 Sensitivities and specificities of the HPV detection assays for high risk genotypes

Assay	HPV genotype	Sensitivity (%)	95% CI (%)	Specificity (%)	95% CI (%)
HC2	All HR	96.6	91.4 to 99.1	89.1	84.3 to 92.8
RealTime HR	All HR	78.3	69.9 to 85.3	99.2	97.0 to 99.9
	16	100.0	86.8 to 100.0	99.7	98.3 to 100.0
	18	90.9	58.7 to 99.8	100.0	98.9 to 100.0
Cobas HPV	All HR	91.7	85.2 to 95.9	97.0	94.0 to 98.8
	16	88.5	69.8 to 97.6	99.1	97.4 to 99.8
	18	100.0	71.5 to 100.0	99.4	97.9 to 99.9

Abbreviations: CI, confidence interval; HC2, Hybrid Capture 2; HR, high risk.

E-110

Diagnostic Performance Evaluation of the Elecsys Anti-HCV II assay in comparison with Elecsys and Vitros anti-HCV assays

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Background: Detection of anti-HCV antibodies has been widely used for screening HCV infection. We evaluated the diagnostic performance of a newly developed Elecsys Anti-HCV II assay in Korean patients.

Methods: A total of 500 (400 anti-HCV negative and 100 positive) sera were collected after tested by Elecsys Anti-HCV assay (Roche Diagnostics, Germany). All those samples were also tested with Vitros Anti-HCV (Ortho-Clinical Diagnostics, UK) and Elecsys Anti-HCV II (Elecsys II) (Roche Diagnostics, Germany) assays. Specimens positive or negative for all the three assays were regarded as positive or negative for anti-HCV, and medical records of the patients including previous results for HCV tests were reviewed to determine final results for anti-HCV when there were discrepancies among the results of the anti-HCV assays.

Results: Among the 500 samples, four (0.8%) showed discrepant results between the three anti-HCV assays. Sensitivity and specificity were 98.0% and 100.0% for the Elecsys II and 100.0% and 99.5% for the Vitros assay, and they were statistically not different between the assays. Concordance rates between the results by any two assays among the three were equal or greater than 99.2% with kappa coefficient of 0.98 or more ($P<0.0001$) (Table 1).

Conclusions: Sensitivities and specificities of the anti-HCV assays evaluated in this study including the new Elecsys II assay were high enough for use in clinical laboratories, and the results by the three assays agreed well with each other. However, weak positive results would need to be retested considering the discrepancies between the anti-HCV assays.

Table 1 Agreement between results by the anti-HCV assays

Comparative tests	Concordance rate (%)	Kappa coefficient	95% CI	P-value
Elecsys II vs Elecsys	99.6	0.99	0.97 to 1.00	<0.0001
Elecsys II vs Vitros	99.2	0.98	0.95 to 1.00	<0.0001
Elecsys vs Vitros	99.6	0.99	0.97 to 1.00	<0.0001

Abbreviation: CI, confidence interval.

E-111

Evaluation of a Novel Multiplex Real-Time PCR Assay for the Concurrent Detection of Hepatitis A, B and C Viruses in Patients with Acute Hepatitis

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Background: Acute infection of hepatitis B and C viruses (HBV and HCV) usually causes chronic liver diseases. These viruses have the common epidemiology of spreading via blood, thus the infection of both viruses has been a worrisome public health problem worldwide. Early detection and proper management of acute hepatitis would be one of the effective ways to control infections with hepatitis viruses. Therefore, effective assay for the identification of the pathogen would be essential in the differential diagnosis of acute hepatitis. We evaluated the recently developed novel multiplex real-time PCR assay for the concurrent detection of nucleic acids of HAV, HBV and HCV.

Methods: Among the 31,433 specimens which were requested for the PCR assays for detecting HBV DNA or HCV RNA during 2009 to 2011, a total of 651 serum samples from patients who were suspected of having acute hepatitis were collected and assayed using Magicplex HepaTrio Real-time Test (Seegene Inc., Seoul, Korea).

Results: Among the 651 samples, 645 (99.1%) were positive for any of the nucleic acids of HAV, HBV and HCV, and 459 (70.5%), 196 (30.1%) and 18 (2.8%) samples were positive for HCV RNA, HBV DNA and HAV RNA, respectively (Table 1). HBV DNA and HCV RNA were simultaneously detected from 21 (3.2%) cases. HAV RNA and HBV DNA were also concurrently identified from 7 (1.1%) specimens.

Conclusions: The newly developed multiplex real-time PCR assay was able to detect nucleic acids of HAV, HBV and HCV from most of the samples drawn from patients being suspected of having acute hepatitis, and thus the assay would be useful for screening acute viral hepatitis at the same time. This assay also has an advantage of identifying co-infections with the three types of hepatitis viruses.

Table 1 Result of the HepaTrio test using samples from patients being suspected of having acute hepa

Detected hepatitis virus	No. of cases (%)
HCV only	438 (67.3%)
HBV only	168 (25.8%)
HAV only	11 (1.7%)
HBV and HCV	21 (3.2%)
HAV and HBV	7 (1.1%)
HAV and HCV	0 (0.0%)
None	6 (0.9%)
Total	651 (100.0%)

E-112

Stabilized Amplification Technology: a New Real Time PCR Screening Test for Mycobacteria Infections

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Objectives. Tuberculosis is an infectious disease affecting lung and other organs that represents one of the most important cause of morbidity and mortality in developing countries and a restart disease in western countries. Since more than 50 years tuberculosis has been treated with a number of drugs that, due to a wide use of the therapies, caused the selection of antibiotic-resistant Mycobacteria. Recently, *DprE1* enzyme of Mycobacterium tuberculosis has been described as the target of Benzothiazinones (BTZ), a new class of antitubercular drugs (Makarov *et al*., 2009). The aim of the present study is to develop a ready-to-use screening and drug-resistance

molecular assay (based on *dpr1* gene) for *M. tuberculosis* diagnosis, storable at room temperature, that can be easily and rapidly performed also in developing countries. Design. The assay was developed in a Real-Time PCR format. Each test tube contains in a ready-to-use mixture all the necessary components (reaction buffer, dNTPs, $MgCl_2$, Hot Start DNA Polymerase, preservatives, stabilizers, primers, probes) in the freeze-dried form. A single couple of degenerated primers was designed both for the screening and the drug resistance analysis. Three fluorescent probes, one for the screening and two for the BTZ resistance or sensitivity, were designed and included in the mix. Further a couple of primers for the amplification of the Human Beta-Globin gene was used as an internal control.

Methods. For the validation of the assay we considered a panel of 24 mycobacterial strains obtained from the ATCC (*M. tuberculosis, M. bovis M. leprae,, M. avium, M. canetti M. marinum,, M. ulcerans, Nocardia farcinica, M. africanum, M. abscessus, M. kansasii, M .intracellulare, M. paratuberculosis, M. fortuitum, M. scrofulaceum, M. chelonae, M. xenopi, M. malmoense, M. gordonae, M. genavense, M. simiae, M. celatum, M. chimera and M. gastri)*. *DprE1* gene was amplified by PCR from the strains and cloned in pGem-Teasy vector (Promega). Amplified products were analyzed by electrophoresis, before sequencing analysis (Applied Biosystems 3730 DNA Analyzer). These clones have been used as positive controls for Real-Time PCR experiments.

Results and Conclusions. The system is able to properly detect all the bacterial strains with no cross-amplification. The sensitivity of the assay is one genome equivalent. The freeze-dried format guarantees high stability and reproducibility of the assay, allowing the storage at room temperature up to one year. In conclusion, the ready-to-use format shows the capacity to identify all the Mycobacteria strains that can affect humans and, simultaneously, the ability to identify the mutation responsible for the described drug resistance. Clinical isolates carrying mutations can also be detected due to a failure in the detection with the described probes. The robustness of the test, the easiness of shipment and storage, the reduced time needed for the preparation of the amplification mix, and the reduced risk of contamination drive for a reliable application in all the *Mycobacterium* diagnostic needs. Further, during the development, eight new sequences were obtained from the different Mycobacteria strains in the *dprE1* gene; they will be shortly available in databanks. A phylogenetic study will follow.

E-113

LATE-PCR assay cross-reactivity, inhibitory effects, and complexity versus performance of monoplex, 3-plex, and 9-plex configurations with and without whole-blood sample matrix

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Objective. Our goals were 1) to compare the effects of increasing assay and sample matrix complexity on limits of detection (LoD) and 2) to assess cross-reactivity and inhibitory effects on linear-after-the-exponential polymerase chain reaction (LATE-PCR). LATE-PCR detects 16 pathogens commonly associated with septicemia and may facilitate early goal-directed therapy: *Staphylococcus aureus*, coagulase negative staphylococci, *Enterococcus* species, *Acinetobacter baumannii, Klebsiella* species, *Enterobacter aerogene*s, and *Candida* species.

Methods. For LoD, whole blood collected from healthy adult volunteers and pure culture suspensions were inoculated with target organisms at concentrations of 10^8-10^3 CFU/mL for bacteria and 10^6-10^2 CFU/mL for fungi. For cross-reactivity and inhibition, 500uL of each target serial dilution was inoculated with 500uL of related pathogens at 10^8 CFU/mL. Initial concentrations were compared to a 0.5 McFarland turbidity standard and verified by spectrophotometry. Aliquots (100uL) of the three lowest dilutions were plated on sheep blood agar and incubated at 37ºC for 24hrs for quality control. DNA was extracted using mechanical and chemical lysis. Five uL of DNA eluate (1/40 of final volume extracted) were added to 20uL of PCR mastermix. Reactions were run in duplicate in monoplex, 3-plex, and 9-plex configurations for LoD evaluation and triplicate in 3-plex and 9-plex for cross-reactivity and inhibition testing. Purified gDNA from American Type Culture Collection was used as a positive control and water as a negative control. Experiments were performed twice to demonstrate reproducibility. LoDs is defined as the lowest concentration in CFU/mL detected 100% of the time.

Results. Bacterial and fungal DNA replicates were detected in monoplex down to 10^3 CFU/mL (equivalent to ~10^1 copies/reaction) and 10^4 CFU/mL (equivalent to ~10^2), respectively, 95-100% of the time when extracted from whole blood and pure culture. As the complexity of the multiplex configuration increased with addition of primer and probe sets, LoDs of DNA extracted from whole blood increased with complexity, while LoDs for DNA extracted from pure culture increased, but remained similar for 3-plex and 9-plex configurations. No cross-reactivity or inhibitory effects from related organisms were observed for *Candida* sp. The LoDs remained unchanged in the presence of high concentrations of related fungal organisms. Cross-reactivity and inhibition experiments are in progress. We expect there to be no inhibitory effects on LoD from related organisms in the 3-plex and 9-plex assays. However, based on preliminary studies, cross-reactivity likely will be observed between *Enterococcus* sp. and *Staphylococcus* sp., as well as *Enterobacter aerogenes* and *Klebsiella* sp. at concentrations greater than 10^7 CFU/mL.

Conclusion. Increased assay and sample matrix complexity negatively impacts the performance of the LATE-PCR sepsis assay. The monoplex assays can detect bacterial organisms in whole blood down to 10^2 CFU/m. A point-of-care instrument designed for parallel processing of monoplex assays or conservatively multiplexed assays (versus complexly multiplexed) could function as an adjunct to blood culture in diagnosing bloodstream infections by eliminating the possibility of cross-reactivity causing false positive results. Multiplex results warrant reagent optimization and further analytical and clinical validation.

E-114

The Development of a Mass Coding based Pathogen Detection Platform for Clinical Research

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Introduction: A mass coding based multiplex-PCR System for clinical research enables the accurate and sensitive detection of several different pathogens in a multiplex method. When conducting research into disease states, distinguishing the pathogens present at the site of infection from the large number of potential causative agents is a key to understanding disease. The most widely used method for pathogen nucleic acid detection is real-time PCR, but it is limited to interrogating only a few pathogens in a single reaction. The ability to test for dozens of divergent pathogens in a single multiplex-PCR reaction provides a distinct advantage when screening for infectious agents. The experimental system was successfully tested with archived biological samples previously identified to contain viral or bacterial pathogens.

Methods: Pathogen-specific primers labeled with small tags of variable mass are used to amplify RNA or DNA. The mass coding tags (MassCode) are cleaved from pathogen-specific amplicons by UV light and detected by mass spectrometry (MS). For each step in the workflow we developed novel reagents and protocols to maximize system performance. The key steps include reverse transcriptase (RT), PCR amplification, DNA purification, and UV-cleavage of the tags and detection of cleaved tags by a single quadrupole MS.

Preliminary Data: Initial application of the technology was to detect respiratory pathogens from nasal swabs. By employing a 20-plex respiratory pathogen panel over 69 wells (47 test samples and 22 controls) we detected 16 of 16 synthetic positive controls, 55 of 55 internal extraction and amplification controls, and 36 respiratory pathogens from archived biological samples. The pathogens identified corresponded with the pathogens previously identified using 1-3 plex biological assays. Because the mass coding panels interrogate for more pathogens in a single assay than low-plex assays we were able to identify previously unknown co-infections as well as solitary infections missed in the initial testing by reference labs. The respiratory panel employed in this research study interrogated viruses from the influenza, corona, parainfluenza, entero, and metapneumo groups as well as bacterial strains.

Using an enteric panel to test biological samples suspected of harboring bacterial or viral enteric pathogens we detected strains of salmonella, norovirus, campylobacter, and clostridium. The experimental system has been used in several additional studies with a large number of samples to identify both respiratory and enteric pathogens.

Novel Aspect: Mass coding tags are used in multiplex PCR employing novel enzyme formulations for pathogen detection with good signal-to-noise via MS

E-115

Interferon gamma mRNA detection by real-time reverse transcriptase PCR using whole blood of TB patients

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Background: Tuberculosis (TB) continues to be one of the most critical infectious disease and causes three million deaths annually. About one-third of world population is latently infected with *Mycobacterium tuberculosis* (MTB). Recently, new immunodiagnostic tests for diagnosis of latent TB infection (LTBI) called interferon gamma (IFN-γ) release assay (IGRA) have been developed. These tests employ monoclonal antibodies to detect IFN-γ level in the bloods which have been stimulated by MTB-specific antigens. Commercially available IGRA tests have shown higher specificity and sensitivity than conventional tuberculin skin test (TST).

Methods: In this study, the levels of the IFN-γ in the whole bloods of the TB patients were detected by using real-time RT-PCR and the test results were compared with those detected by commercial IGRA. Total of 69 subjects that include 18 active pulmonary TB patients, 30 close contacts with TB patients, and 21 normal healthy donors were enrolled for this study. All subjects were diagnosed by various clinical laboratory tests such as AFB sputum smear, mycobacterial culture, chest radiographs, histopathologic technique, TB/NTM real-time PCR and TST. Whole bloods of all subjects were stimulated by TB specific antigens (ESAT-6, CFP-10, TB7.7) for 24 hours, and then the plasma were used for commercial IGRA and the cell pellets were used for real-time RT-PCR to detect IFN-γ mRNA level.

Results: The levels of IFN-γ detected by real-time RT-PCR after 24-hour TB antigen stimulation showed over 80% consistence rate with those detected by using commercial IGRA when bloods from active pulmonary TB patients and normal healthy donors were used. However, the results by two tests using bloods from close contacts showed 50% coincidence rate. Therefore, the results seemed to suggest that IFN-γ mRNA levels of close contacts do not seem to represent IFN-γ levels. Subsequently, other cytokines were evaluated to ask whether any of them could represent the IFN-γ levels in the blood of close contacts.

Conclusion: The results from this study clearly showed that CXCL-10 (IP-10) mRNA levels may be useful for monitoring IFN-γ levels in the blood of close contacts as well as active pulmonary TB patients and normal healthy donors when 24-hr TB antigen stimulated bloods were used. Since the real-time RT-PCR using multiple targets such as IFN-γ and IP-10 can reduce cost and turn-around time, it may be more useful for monitoring massive number of population.

Key word: MTB infection, IFN-γ mRNA, IP-10 mRNA, IGRA, real-time RT-PCR

E-116

Sensitive Multiplex Test for the Typing of Fungi Using Beads Array in a Single Tip Assay

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Fungal infection is opportunistic infection which occurs in patients with serious underlying diseases and in immunologically compromised patients who are under the immune-suppressive therapy after organ transplantation or under anti-cancer chemotherapy. Fungal infection can be life-threatening, if the patient were not treated immediately with appropriate antibiotics. Thus, detection and typing of causative fungi at early stage of infection are critical for making appropriate therapeutic decision. However, diagnosis of fungal infection in its early-stage is hampered by the slow growth of fungi in microbial culture from patient's blood and the low diagnostic sensitivity of tests currently available. To overcome the obstacles in diagnosis of fungal infection, PCR-based nucleic acid amplification tests (NATs) have been developed and showed its usefulness for early detection of fungi in patients. In the preset study, we have developed the novel PCR-based multiplex NAT for typing three fungi species, *Aspergillus/Penicillium* (ASP/PEN), *Candida albicans* (CAN) and *Candida glabrata* (C. glab) using beads array in a single tip (BIST) technology. BIST tip is one of the platforms for multiplex testing and contains beads array in a capillary tip. Each bead in a BIST tip is coated with different oligo-nucleotides sequence tag; therefore it is easy to develop detection and typing tests of multiplex PCR products (up to 20 species) using the BIST tip. Primer sets that specifically amplify ASP/PEN, CAN or C. glab, respectively, were designed, one primer of which was fused with complimentary sequence of the tag sequence on the bead in BIST. Following PCR amplification, the resultant PCR products were denatured, hybridized with the tag sequence on the bead in BIST, and then hybridized PCR product on the bead was detected by chemi-luminescence. The newly-developed PCR based multiplex NATs were tested with DNA extracted and purified from plasma containing different numbers and species of fungi. Test result demonstrated that less than 100 cells of each ASP/PEN, CAN or C. glab in a sample were detected and no cross reactivity among three species was observed, indicating that the test has high sensitivity and specificity. In addition to the accuracy of test results, the whole process of the test (extraction/purification of DNA from sample, PCR setup/reaction, hybridization and detection) could be fully-automated and need only 3 hours. These results suggest that the newly developed PCR-based NAT is an easy, fast and sensitive assay for early detection and typing of fungi in clinical samples.

E-117

Comparative Analysis of Detection Methods and Features of Respiratory Viruses Isolated from Cheonan, South Korea

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Background: Respiratory viral infections occur in people commonly, and each virus is associated with individual respiratory diseases. Respiratory viruses are major causes of mortality and morbidity especially in infants, the elderly and immunocompromised patients. Therefore, an accurate diagnosis of respiratory virus in laboratory is meaningful and the long-term epidemiologic studies are important. This study is for epidemiologic investigation of respiratory viruses during 8 years in Cheonan area and comparative evaluation study of rapid isolation by multiple RT-PCR test.

Methods: We tested the specimens of 8,866 nasopharyngeal aspirates which were inhaled through the nasal cavity and were originated from the patients who were hospitalized with respiratory diseases in Dankook university hospital and referred to the Department of Laboratory Medicine for respiratory viral infection tests from September 2003 to September 2011. Until November 2006, those samples were incubated and indirect immunofluorescence method using monoclonal antibody was carried out. Since November 2005, multiplex RT-PCR was performed and the combination tests of above two methods were accomplished with the some samples and the results were confirmed. Since February 2011, real-time PCR was performed.

Results: The identification rate of respiratory viruses was 58.74% (5208/8866) as a whole. The isolation rate of viral culture was 29.15% (646/2216), and the identification rate of nucleic acid amplification test was 63.26% (4745/7501), respectively. According to ages, the infants under 5 years showed a high positive infection rate and the highest infection rate as 63.26% (2350/3420) in less than 1 year. The lower positive rate was shown with advancing years. The positive rate in more than 20 years was 23.71% (276/1164). According to months, the highest positive rate was shown in November and according to seasons, the highest in Spring, respectively. According to viruses, the isolation rate of HRV and HRSV-A was the highest as HRV (15.2% 1143/7501), HRSV-A (12.7% 956/7501), respectively. The tendency of coinfection of HRV, HAdV was high. The agreement rates between multiplex real-time RT-PCR assay and conventional multiplex RT-PCR assay were 98%.

Conclusions: We researched the epidemiologic studies on respiratory viruses for 8 years in Cheonan area and the comparative evaluation of rapid isolation by multiplex RT-PCR as major test method. This study can provide important basic information for epidemiologic aspects of many kinds of viruses, especially determining pattern of coinfection, which previous studies were difficult to present. It is thought to help to predict the type of disease and to determine the treatment methods.

E-118

False Positive Results on the Mediace TPLA and RPR Caused by Lipemic Sera

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Background: Mediace RPR (Rapid Plasma Reagin, "RPR") (Sekisui Chemical Co., Ltd, Osaka, Japan) and Mediace TPLA (*Treponema pallidum* Latex Agglutination, "TPLA") (Sekisui Chemical Co., Ltd, Osaka, Japan) have been used in many laboratories for the serologic tests for syphilis. It has been reported that some immunoturbidimetric assays give false results due to interference from bilirubin, lipid or hemoglobin. We recently

found false positive reactions in TPLA and RPR caused by highly lipemic sera. This study was aimed to evaluate the effect of lipid on RPR and TPLA.

Methods: Thirty nine sera with a triglyceride (TG) concentration over 500 mg/dL (500-599 mg/dL, 3; 600-699 mg/dL, 9 ; 700-799 mg/dL, 3; 800-899 mg/dL, 2; 900-999 mg/dL, 4; ≥1000 mg/dL, 18) were included in the evaluation of false positives for RPR and TPLA. The TG concentration, lipemic index, RPR and TPLA were measured using Hitachi 7600 chemistry autoanalyzer (Hitachi, Tokyo, Japan). All TPLA positive samples were tested again using TPLA neutralizing reagent to exclude nonspecific (latex binding) reactions. To reduce lipid concentration, high speed micro-centrifugation (centrifuged at 12,470 x g for 10 min) was done and the RPR or TPLA were performed on the samples showing positivity.

Results: The positive rate of RPR and TPLA was 7.7% (3/39) and 38.5% (15/39), respectively. All three RPR positive samples showed TPLA positivity. All 15 TPLA positive samples turned negative after a high speed micro-centrifugation, which indicates the false positive results. Among the 15 TPLA positive samples, the TG concentrations of 12 samples (80%) were over 1,000 mg/dL and the TG concentrations of the other three samples were 698, 870, 996 mg/dL, respectively. The lipemic indices of the TPLA positive samples ranged from 516 to 1,453. Out of 18 samples with TG level over 1,000 mg/dL, 12 (66.7%) samples were TPLA false positive. Nine (81.8%) samples out of 11 samples with lipemic index exceeding 500 were TPLA false positive.

Conclusions: The TPLA and RPR assay is affected by the TG concentration. Many samples with TG concentration ≥1,000 mg/dL or lipemic index ≥500 revealed false positive TPLA results. Due to the lack of facilities in many laboratories, lipemic index is not a widely used.Therefore, we suggest that the TPLA needs to be measured after a high speed micro-centrifugation in case of all TPLA positive samples with a TG concentration exceeding 600 mg/dL or lipemic sera.

E-119

Blood culture pathogens isolated from private hospitals in São Paulo - Brazil, from 2009 to 2011

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Background: Severe sepsis accounts for 20% of all admissions to intensive care units (ICUs). Bloodstream infections (BSI) due to bacterial and fungal pathogens affect over 200,000 individuals annually in the United States alone and are an important cause of morbidity and mortality worldwide. The mortality of BSI is approximately 27% and detection of microorganisms in blood cultures is considered an indicator of disseminated infection and has been shown to be a valid marker for surveillance in BSI. The objective of the study is to identify the etiology of blood cultures among patients and its stratification by gender, age and hospital ward.

Methods: During the timeframe of this study, 18,382 positive blood cultures were observed in the laboratory routine carried out by incubation in Bact Alert 3D ® with a protocol of five days for aerobe and anaerobe. The identification and sensibility testing of positive strains were processed by Vitek 2 system ® (bioMérieux). The epidemiological data were collected by the laboratory system Motion ® from 17 private hospitals where DASA provides laboratory services.

Results: Among the positive blood cultures, 9,254 (50.34%) were from female and 9,127 (49.65%) from male. The majority of positive cultures were from patients over 80 years - 5,067 (27,56%), and 3,434 (18,68%) samples from 70-79 years. From pediatric population, infants with less than 1 year were the most positive population - 2,402 (13.16%) samples, followed by 1 to 4 years old range that counted with 818 (4.45%) samples. Clinical ward was the major source of positive blood cultures - 6,027 (32.78%) followed by adult ICU - 5,909 (32.14%), emergency room - 3,922 (21,33%) and Neonatal units - 1,010 (5.49%). Gram-positive cocci accounted for 56.80% of the positive samples, Gram-negative bacilli to 30.54 % and yeast to 6.93% of the isolates. The most frequent isolated microorganism was coagulase-negative *Staphylococcus* (CNS) - 7,328 (39.86%) followed by *S. aureus* - 1,527 (8.30%), *Eschericia coli* - 1,328 (7.22%), *Klebsiella pneumoniae* 1,090 (5.92%), *Candida spp* 1,257 (6.83%) with *Candida albicans* accounting for 515 (40.97%) of the isolated *Candida* species. *Pseudomonas aeruginosa* was found in 841 isolates (45.75%), while *Enterobacter* species were identified in 611 (3.33%) isolates being the main specie *E. cloacae* (68.58%). *Enterococcus spp* were presented in 605 (3.29%) of the cultures, and *E. faecalis* was the main representative (79.67%).The medium time of positivity of the blood cultures was three days. Specifically for CNS, positivity was mainly found in the fourth day of incubation.

Conclusions: The study underscore the prominence of gram-positive cocci and among Gram negative organisms, *E. coli* was the main etiologic isolated agent. Because there was no available data on previous admissions to the hospital and treatment during current hospitalization, the definition of the presence of CNS as a contaminant or a true pathogen was hampered. However it was the main bacterial pathogen isolated from blood cultures and its role as a true pathogen cannot be underestimated. The study showed us also the high prevalence of Candida non albicans in blood cultures.

E-120

Laboratory Diagnosis of Urinary Tract infection in Children collected at the emergency room of a private children hospital, São Paulo, Brazil

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Background: Febrile illnesses are common, especially in children under 5 years of age. On average, young children experience three to six febrile illnesses per year. Febrile illness is the single most common reason for young children to be seen by primary care practitioners and to present to emergency departments for acute care. The references standard for most serious bacterial diseases is a positive microbiological culture from a sterile sample such as blood, urine, or cerebrospinal fluid. Although urinary tract infection affects at least 3.6% of boys and 11% of girls, establishing the diagnosis is difficult in early childhood owing to the lack of specific urinary symptoms, difficulty in urine collection, contamination of samples and to the delay obtaining the result which usually takes 24-48 hours.

Methods: All the urine cultures of a private children hospital at São Paulo, Brazil from July to December 2011 were included in the study. The urine was collected following the laboratory guidelines. The samples were collected by the laboratory staff and the nursing team, processed by conventional methods and the identification and susceptibility testing of the isolates were processed by Vitek 2 system®.

Results: In the period of the study 4,859 samples were processed for microbiological culture. Ninety percent (4,413) of this total were collected on the emergency room, with a positivity of 14%. The main isolate was *E. coli* in 384 samples (64%), *Proteus spp* 131 (22%) and *K. pneumoniae* 21 (3%). Of the positive samples, 320 (53%) were from children with 1 to 4 years old, 443 (74%) from female and 155 (26%) from male. As a quality marker of the collection standard, the results were divided into monomicrobial 566 (95%) and 16 polymicrobial with no more than two strains isolated per culture.

Conclusions: *E. coli* remains the most important agent isolated from children´s urine. The majority of positive samples in the emergency room were from urine collected from girls with 1 to 4 years old.

E-121

Prevalence of Community-Associated Methicillin-Resistant Staphylococcus aureus isolated from blood cultures of patients attended at private hospitals in São Paulo Brazil from 2010 to 2011.

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) strains had become a major concern for health care systems. The hospital epidemiology of MRSA has changed in the past few years due to the encroachment of community-associated MRSA (CA-MRSA) strains into health care settings. Since the late 1990s, strains of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) had emerged as important pathogens in both community-associated and health care-associated settings. CA-MRSA strains differ from hospital-associated MRSA (HA-MRSA) strains in several aspects, especially in terms of antimicrobial susceptibility.

Methods: Out of all blood culture processed in 2010 - 2011 from 28 hospitals in São Paulo (Brazil), 20,163 turned out to be positive after incubation in Bact Alert 3D ® with a five days incubation protocol for aerobe and anaerobe culture bottles. The identification and sensibility testing of positive strains were processed by Vitek 2 system ® and the data were collected by Observa® system (bioMèrieux) . The epidemiological data were collected by the laboratory system Motion ®. The susceptibility testing for Clindamycin and trimethoprim with sulfamethoxazole was used to define the phenotype of CA- MRSA.

Results: *Staphylococcus aureus* were isolated from 1,231 (6.10%) positive blood culture. Methicillin-Resistant Staphylococcus aureus (MRSA) were isolated from 759 (61.60%) of these positive cultures - 543 (71.54%) of them showed resistance to clindamycin being susceptible to trimethoprim with sulfamethoxazole (TPS), 160 (21.08%) of the isolates showed susceptibility to clindamycin and TPS and 53 (7%)

showed resistance to clindamycin and TPS. The distribution in the hospital wards of the CA-MRSA was 530 (43%) from clinical wards, 374 (30.40%) from Adult ICU and 255 (20.73%) from emergency room.

Conclusions: The study showed, as expected, a high prevalence of MRSA, however, almost ninety percent of the isolates were suggestive of CA-MRSA, particularly those isolated from patients from the clinical wards setting. These data are important to reassess antibiotic guidelines protocols for treatment of *S. aureus* blood stream infection in São Paulo, Brazil.

E-122

Diagnostic and Prognostic Value of Soluble CD14 Subtype (sCD14-ST) in Emergency Patients with Early Sepsis Using the New Assay PATHFAST Presepsin

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Background: CD14 is expressed on the membrane of monocytes/macrophages and activates the TLR4-specific inflammatory reaction against infectious agents whereby soluble CD14 is released yielding sCD14-subtype (presepsin) by proteolysis. Presepsin serves as a mediator of the response to lipopolysaccharid from infectious agents. First evidence suggested that presepsin may be utilized as sepsis marker.

Methods: Presepsin, procalcitonin and APACHE II score were determined at admission, after 24 and 72 hours in 140 septic patients admitted to the emergency department (ED). 119 healthy individuals served as control group. Primary endpoint was death within 30 days. The combined endpoint “major adverse event” (MAE) consisted of at least either the primary or at least one of the secondary endpoints (intensive care, mechanical ventilation or dialysis).

Results: Mean presepsin concentrations of the control group and the patient group were 159 (90% CI: 148-171) pg/ml and 2563 (90% CI: 1458-3669) pg/ml, respectively. In contrast to procalcitonin, presepsin as well as APACHE II score differed highly significant between patients with sepsis and severe sepsis or septic shock ($p<0.0001$). The 30-day mortality was 16.4% in total, ranging from 2.7% to 39.4% between the 1st and the 4th quartile of presepsin concentration. Presepsin demonstrated a stronger relationship with 30-day mortality compared with procalcitonin as displayed in the table. Receiver operating curve analysis of presepsin, procalcitonin and APACHE II score revealed AUCs of 0.878 (95% CI: 0.801-0.934), 0.668 (95% CI: 0.570-0.757) and 0.815 (95% CI: 0.709-0.895), respectively. Presepsin concentration increased significantly in patients with MAEs during the first 72 hours, whereas presepsin concentration decreased in patients without MAEs.

Correlation between plasma concentration at admission and 30 day mortality				
Quartile	1st	2nd	3rd	4th
Presepsin (pg/ml)	177-512 (N=37)	524-927 (N=35)	950-1810 (N=35)	1859-1575 (N=33)
Survivors/non-survivors (N)	36/1	32/3	29/6	20/13
Mortality (%)	2.7	8.6	17.1	39.4
PCT (ng/ml)	0.10-0.38 (N=30)	0.40-1.73 (N=37)	1.76-6.99 (N=36)	8.12-292 (N=37)
Survivors/non-survivors (N)	22/8	34/3	33/3	28/9
Mortality (%)	36.0	8.8	9.0	32.1

Conclusion: Presepsin demonstrated a strong relationship with disease severity and outcome. The prognostic accuracy was superior to procalcitonin. Presepsin provided more reliable prognosis and early prediction of 30-day mortality already at admission. Presepsin values were related to the course of the disease.

E-123

Comparison of false positive results for three automated treponemal antibody assays

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Background: Increasing availability of automated assays for detection of treponemal antibodies (TrepAb) has caused some laboratories to adopt a “reverse algorithm” for syphilis screening. The reverse algorithm begins with a treponemal specific test (e.g. TrepAb) and follows up positive results with a treponemal nonspecific test (e.g. RPR). If both tests agree then an accurate diagnosis can be made with confidence. However, if a positive TrepAb is followed by a negative RPR then the diagnosis is less clear. Patients who have been previously infected with *Treponema sp.* (whether treated or untreated) can have detectable TrepAb with a negative RPR. Alternately, the initial antibody result could be a false-positive. As such, a confirmatory test, which also detects TrepAb, is needed to resolve the discrepancy (e.g. FTA-ABS, TP-PA). Review of historical data showed that approximately 3% of samples showed the TrepAb positive, RPR negative pattern. This study was undertaken to determine how often this pattern was due to previous infection or potential analytic error.

Methods: Initial screening for TrepAb was performed using the BioPlex 2200 (Bio-Rad Laboratories, Hercules, CA). A reflexive panel was in place which automatically ordered FTA-ABS on all samples with equivocal or positive TrepAb and negative RPR. Aliquots of these samples, along with a similar number of TrepAb negative samples, were stored at -20 °C for later testing by alternate methods (Immulite 2000 [Siemens Healthcare Diagnostics, Deerfield, IL], Liaison [Diasorin, Saluggia, Italy]). Samples positive for both TrepAb and RPR were not included. For the purpose of this study, equivocal results from the BioPlex 2200 were considered positive as either result would require additional testing. Other testing methods did not have equivocal results. Limited sample volume prevented some samples from being tested by all 4 methods. A consensus result for the presence of TrepAb was reached if the majority (3/4 or 2/3) of antibody results were in agreement.

Results: A consensus result for TrepAb was possible for 86 samples with 36 positive and 50 negative. For 3 samples, no clear consensus could be reached with available data. The number of samples tested, agreement with consensus, PPV and NPV for each methods was as follows: FTA-ABS n=62, 91.9%, 100%, 83.9%; BioPlex n=86, 69.8%, 58.1%, 100%; Immulite n=69, 98.6%, 100%, 97.7%; Liason n=82, 100%, 100%, 100%.

Conclusions: The current practice of not confirming negative TrepAb results from an automated platform with a second antibody test seems warranted as the NPV was essentially 100% for all methods. The number of false-positive results varied across platforms with the BioPlex method having a lower PPV than the Immulite or Liason methods. Given the high PPV of the Immulite and Liason assays, routine confirmation of positive results is likely unnecessary. If confirmation testing is needed, the performance of the FTA-ABS shown in this study suggests it may not be the ideal choice.

E-124

HPV infection pattern and genotype distribution in normal, low-grade, and high-grade cervical lesions.

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Background: HPV is proven cause of cervical cancer and its precursor lesions in women. These lesions are screened by cervical cytology, and according to Bethesda system, they are classified in normal cytology, atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and cancer. The HPV genotype is an important risk factor for grade progression of these celular abnormalities, since there are high-risk and low-risk oncogenic genotypes. The aim of this study was to describe the HPV infection pattern and genotypes distribution in cervical cytology with normal, low-grade, and high-grade cervical lesions by assessing our clinical laboratory results database.

Methods: We assessed patients who had undergone cervical cytology and HPV genotyping with no more than one month appart between the tests from February 2009 to May 2011. 1913 women samples met this condition, 1715 with normal cytology, 131 with AS-CUS, 55 with LSIL, and 12 HSIL. Positivity, type of infection, oncogenicity, and genotypes were identified and presented. In addition, we correlated these parameters with different cytological abnormalities evidenced by cytology (Bethesda system). Genotyping was performed by PapilloCheck (GreinerBio-One).

Results: Positivity was 38,37% and single infections account for 58,5%. Of 1311 found viruses, 73,7% was high risks genotypes. Moreover, positivity increased with the degree of cell injury: normal cytology 34.05%, ASCUS 66.41%, LSIL 92.73%, HSIL 100.0% (Ptrend<0,0001). The same trend was also observed for high-risk genotypes: normal cytology 71.94%, ASCUS 79.67%, LSIL 79.38%, HSIL 85.0% (Ptrend=0,0087). But not for simple or multiple infections: normal cytology 59.93%, ASCUS 49.43%, LSIL 54.9%, HSIL 75.0% (Ptrend=0,51). Beside that, the eight most common genotypes in normal cervical cytology were 16, 56, 44, 53, 6, 39, 42, and 68; In ASC-US 16, 52, 56, 31, 53, 39, 73, and 51; In LSIL 16, 56, 66, 68, 53, 31, 51, and 39; In HSIL 16, 58, 73, 52, 56, 39, 68, and 82; Finally, the prevalence of HPV16 increases, and HPV6 and HPV44 decreases with worsening of cervical

lesions. HPV16 frequency was 29.09%, 13.74%, 11:34%, 35.0% for normal cytology, ASCUS, LSIL and HSIL, respectively (Ptrend= 0.0029); HPV 6 frequency was 6.42%, 3.30%, 3.09%, and 0% for normal cytology, ASCUS, LSIL and HSIL, respectively (Ptrend=0.03); HPV 44 was 7.41%, 3.85%, 3.09%, 5% for normal cytology, ASCUS, LSIL and HSIL, respectively (Ptrend=0.0029).

Conclusion: The proportion of HPV positivity and the frequency of high risk genotypes increase with the degree of cell injury in cervical cytology. This trend was not observed simple or multiple infections. Also, genotype distribution is heterogeneous in the different grades of cervical lesions. Moreover, HPV16

increases whereas HPV6 and HPV44 decreases its frequency with the degree of cell injury in cervical cytology.

E-126

A case of Analytically and Clinically Discrepant Hepatitis B Testing Results

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Background: In most cases, patients appear to recover from acute hepatitis B virus (HBV) infection and do not exhibit the surface antigen (HBsAg), but are positive for hepatitis B surface antibody (HBsAb). Chronic carriers are those who are positive for HBsAg for more than 6 months. Some chronic carriers eventually become HBsAg negative - so called "delayed clearance." Usually, HBsAb is not measurable in chronic infection. Detection of both HBsAg and HBsAb is unusual.

Case summary: Many discrepancies in hepatitis B testing results are either due to analytical or clinical discordances. Here, we report a patient whose results were both analytically as well as clinically discrepant. A 40-year old female patient presented to the University of Louisville Hospital being positive for HBsAg and HbsAb over a period of two years. The virus load remained low, at 300-2800 copies/mL over a period of one year. The liver enzymes AST and ALT stayed within the normal range at 27 U/L and 29 U/L respectively. Hepatitis delta and core IgM antibody were negative for this patient.

Discussion: There are three potential reasons for this result: 1) Interference from endogenous antibodies in the two-step HBsAb assay. 2) HBsAg is from one strain and HBsAb is from another. 3) Endogenous neutralization of low levels of HBsAb or HBsAg follows from the biological uniqueness of HBV. During the early stages of HBV infection, the HBsAb is low so that it is neutralized and not recognized by the solid-phase HBsAg in the assay. Likewise, later HBsAg may be neutralized by HBsAb. It remains unclear whether all HBsAg is cleared after acute infection. Thus, very low copy levels of HBV viral DNA can be found in the liver, blood mononuclear clear cells and even in serum of persons who have apparently recovered from an acute infection. It is also unclear to what extent HBsAb develops during chronic infection. This neutralization hypothesis is currently under active investigation.

Conclusions: 1) Different testing platforms may produce different results depending on the kinetic and exposure of the capture HBsAg and the extent of endogenous HBAg/HBsAb. 2) This relationship may have consequence for new quantitative HBsAg assays.

E-127

Identification of spurious signal in immunoassays due to serum antibodies against blocking proteins

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Background: Many immunoassays are currently performed using various forms of bovine casein to block the non-specific binding of sample constituents and other assay reagents to the underlying substrate. However, the possible presence of immunoglobulins against casein in test samples is often not considered during assay development and can lead to erroneous results.

Methods: A serological assay was developed for the detection of serum antibodies against the intestinal nematode *Strongyloides stercoralis* using a novel diffractive optics-based biosensor platform. This technology enables the label-free detection of molecular interactions in real- time and thus allows for the monitoring of each reagent incubation step during sample analysis. The steps in this assay consisted of: 1) blocking of sensors using a commercially available casein-based blocking solution; 2) immobilization of a Strongyloides recombinant antigen on the sensors; 3) incubation of patient serum diluted 20-fold in a commercially available serum diluent designed to eliminate low- and medium-affinity interactions; and 4) amplification of antibody binding using goat anti-human IgG coated gold nanoparticles.

Results: An analysis of over one hundred patient sera by this method led to the identification of a subset of samples, representing 7% of the total, that displayed inexplicable reactivity to the assay surface. This reactivity was determined to be caused by the presence of anti-bovine casein antibodies in the subset population which bound to the casein used to block non-specific binding sites on the sensors. The addition of free to the serum diluent successfully eradicated this unexpected reactivity.

Conclusions: The use of a label-free, real-time biosensor technology enabled the detection of aberrant signal resulting from the binding anti-bovine casein antibodies in a subset of patient sera to casein used to block the assay substrate. In standard plate-based enzyme-linked immunosorbent assays using a casein-based blocking method, samples containing antibodies to bovine casein may lead to artificially elevated signal and false positive results. These results highlight the potential problems posed by the presence of antibodies against blocking proteins in patient sera and the importance of developing strategies to eliminate their reactivity when developing immunoassays.

E-128

Genotyping of rs12979860 and rs8099917 near the IL28B locus in plasma samples: an alternative material to perform the SNP detection.

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Background: Recent genome-wide association studies have identified two single nucleotide polymorphism (SNPs), near the IL28B gene (rs12979860 (C/T) and rs8099917 (G/T), that are associated with sustained virological response (SRV) in patients with chronic hepatitis C virus (HCV) infection who are under therapy with pegylated interferon (IFN) α and ribavirin. The favorable variants of the two most widely studied *IL-28B* polymorphisms are C/C for rs12979860 and T/T for rs8099917. These genotypes are strong pretreatment predictors of early viral clearance and SVR in patients with genotype 1 HCV infection. Therefore, the presence of the T allele for rs12979860 and G allele for rs8099917, are predictors of nonresponse to treatment. The genetic tests are usually performed in whole blood samples.

Objective: The aim of this study was to evaluate and validate plasma samples as an alternative material to detect IL28B polymorphisms. Therefore, the test could be performed in the same clinical sample of the viral load test or qualitative PCR for HCV avoiding the needing of new blood draw.

Methods: The results of 40 whole blood samples were compared with the results of the same test carried out in plasma that was obtained from whole blood and genomic DNA extracted using DNA Mini Kit (Qiagen, Valencia, CA). The two SNPs were tested using TaqMan® SNP Genotyping Assays (Applied Biosystems., Foster City, CA) to rs12979860 and rs8099917 polymorphisms.

Conclusions: All samples could be genotyped and in both materials and the results were consistent despite the DNA quantification and fluorescence signal of plasma samples were lower than the blood samples. Regarding to rs12979860, homozygous C/C was present in 45% (18/40) and 55% (22/40) of the samples were heterozygous C/T. For rs8099917 SNP, homozygous T/T was detected in 72.5% (29/40) and heterozygous G/T was present in 27.5% (11/40) of the samples.

E-129

Increase Prevalence of Proteus Mirabilis Drug Resistant Bacteria in Long-Term Care Facilities.

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Introduction: *Proteus Mirabilis* (*P.mirabilis*) are aerobic, gram negative rods that form "swarming colonies" when plated on non-inhibitory media. Proteus are the second most common enterbacteriaceae after E.coli, it has been linked to several pyogenic infections especially in the urinary tract of catheterized patients or those with an anatomical abnormality of the urinary tract. This bacterium is becoming a concern as a cause of nosocomial infections and because of the rise in multidrug resistant *P.mirabilis*.

Methods: 433,834 culture specimens collected from residents in Long-Term Care Facilities from 2007 to 2011 were analyzed. Cultures were done using MicroScan Walkaway 96 conventional panels; No growth or <10,000 CFU/mL was considered negative, > 50,000 CFU/mL were considered positive. The positive cultures were separated further by gram stain, and organisms isolated. Statistic calculations were done using *Analize-It.*

Results: No significant change in the % total positive cultures and the total proteus identified over the 5 years was found. We found slight increase with the total gram negative drug resistant cultures, the increase in the drug resistant *P.mirabilis* was significant, table 1.

	2007	2008	2009	2010	2011
Total # of culture	79,170	79,441	93,702	92,169	89,252
% Total positive	54.7%	55.15	57.7%	55.9%	56%
%Total gram negative	65.1%	69.7%	67.9%	68.85	68.6%
% gram negative drug resistant/total gram negative	11.9%	12.5%	13.0%	13.1%	13.3%
% P.Mirabilis/total gram negative	16.3%	17.1%	16.8%	16.3%	16.4%
% P.Mirabilis drug resistant/total gram negative drug resistant	1.0%	5.7%	6.5%	6.9%	8.4%

Conclusion: Although there was no change in the prevalence of P.mirabilis over the period tested, a significant increase in the multidrug resistant P.mirabilis in our population was noticable. This rapid rise may be due to the spread of the strains producing enzyme which cause resistance to multiple antibiotics. Cautious and appropriate use of antimicrobial therapy for the treatment of suspected infections in residents of long-term care facilities are very important because P.mirabilis are resistant to the most frequently prescribed antimicrobials. Healthcare providers should check the susceptibility profiles of isolates and adjust their choice of antimicrobial drugs accordingly. In addition, an infection control priority should be used to identify the risk factors for multidrug resistant which would help to limit the spread of these bacteria in the geriatric population.

E-130

Detection and genetic characterization of Human T-lymphotropic virus subtype 1b (Central Africa) in a clinical laboratory routine in Brazil.

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Background: Human T-lymphotrophic virus type-1 (HTLV-1) was the first human retrovirus to be discovered and is supposed to infect around 20 million people worldwide, with endemic areas in South America, Japan, the Caribbean, and Africa. It has been recognized as the cause of adult T-cell leukemia/lymphoma (ATL), HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) adult T-cell leukemia (ATL). HTLV-1 can be grouped in 6 major genetic subtypes (a-f), some widespread as HTLV-1a, the Cosmopolitan subtype, and others restricted, so far, to geographic regions as HTLV-1b in Central African countries. Blood donor screening is mandatory in Brazil since the 1990s with enzyme immunoassays (EIAs) for antibodies to HTLV, and the positive samples later confirmed with HTLV-I/II Western blot (WB). Laboratory testing for HTLV-1 and -2 infections has become also routine in transplantation and clinical diagnoses in many countries. However the high proportion of indeterminate results of the HTLV screening test (WB) is still a challenge all over the world.

Methods: In a clinical laboratory diagnosis routine samples were screened to HTLV by Abbot Architect rHTLV-I/II Immunoassay (Abbott Laboratories, Wiesbaden, Germany). The reactive samples were submitted to HTLV Western Blot and/or PCR analysis using generic primers to HTLV tax gene and further specific PCR to LTR region from HTLV-1 and HTLV-2. All amplicons were further sequenced. The sequence analysis was performed using MEGA V.4 software and Neighbor-joining/Kimura-2-parameter.

Results: The sequence of the *tax* region obtained in the PCR confirmatory test of one serological reactive sample showed a high identity with the rare HTLV-1b, the Central Africa subtype. This was confirmed by the sequence analysis of LTR region that showed 100% similarity with Z311662 sequence from HTLV-1 MWMG from Zaire, Central Africa.

Conclusions: Our work revealed that the serological reagents currently used to screen the HTLV-1 infection, mostly from HTLV-1a subtype, is also sensitive to rare and diverse HTLV-1 subtypes. This analysis showed, by the first time, the presence of HTLV-1b subtype in Brazil and in the Americas.

E-131

A Multicenter Performance Evaluation of the ADVIA Centaur HBsAgII Assay on the ADVIA Centaur Immunoassay System

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Background: The ADVIA Centaur® HBsAgII Assay* is a sandwich immunoassay using direct chemiluminescent technology for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum and plasma. The currently marketed ADVIA Centaur HBsAg assay has been enhanced to create the HBsAgII assay that allows for the detection of common mutants of HBsAg. The purpose of this study was to evaluate the HBsAgII assay performance on the ADVIA Centaur and ADVIA Centaur XP systems, including the expanded claims for the testing of pediatric and neonatal samples.

Materials and Methods: The ADVIA Centaur HBsAgII Assay was evaluated at a Siemens internal site and at two external sites. Reproducibility was evaluated with three reagent lots using an eight-member panel including two nonreactive serum samples, two low-reactive serum samples, and four reactive serum samples, along with low and high calibrators and positive and negative controls. A hierarchical precision analysis of variance (replicates nested in runs, runs nested in days) was done for each site and extended across all sites and lots. A total of 2022 prospectively collected samples from subjects with signs and symptoms, at high risk for hepatitis B infection, or undergoing kidney dialysis, as well as transplant recipients, were evaluated among the three sites. In addition, unique populations were also evaluated: acute and chronic, pediatric, and neonatal subjects, in addition to seroconversion panels. A 95% Clopper-Pearson confidence interval was calculated for reactive and nonreactive percent agreement where applicable.

Results: Reproducibility estimates were calculated across all lots and sites and for those panel members with an Index > 2.0. The CVs ranged from 1.9% to 4.6% for the within-run precision and from 3.7% to 7.4% for the total precision. For the adult subject population, the reactive percent agreement between the ADVIA Centaur HBsAgII Assay and the reference assay was 96.5% (95% CI: 92.0%-98.8%) and the nonreactive percent agreement was 99.8% (95% CI: 99.5.0%-100.0%) For both the acute and chronic populations, reactive agreement was 100.0%. For the pediatric population, the reactive and nonreactive percent agreements between the ADVIA Centaur HBsAgII Assay and the reference assay were each 100.0%. For the neonatal population, the nonreactive percent agreement between the ADVIA Centaur HBsAgII Assay and the reference assay was 100.0%. There were no reactive neonatal samples. In all cases, the HBsAgII assay detected seroconversion on at least the same bleed date as did the reference assay, and earlier in 5 out of 10 panels examined.

Conclusions: These results demonstrate that the ADVIA Centaur HBsAgII Assay is a precise and accurate immunoassay for the qualitative in vitro determination of hepatitis B surface antigen in human subjects, including pediatric and neonatal populations.

* Under FDA review. Not available for sale in the U.S. This assay is CE marked.

E-132

A study of Clinical Isolates of different strains of yeasts from positive blood cultures collected in private Hospitals in São Paulo, Brazil from 2009-2011

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Background: Recently, it has been seen an increased frequency of non-albicans species of Candida, such as C. glabrata, C. krusei, C. tropicalis, and C. parapsilosis, as the cause of candidemia. The increasing proportion of candidemia due to Candida non albicans has important implications for therapy. Community-onset (CO) candidemia, defined as a positive blood culture represents a distinct clinical entity associated with substantial morbidity and mortality. Invasive candidiasis (IC; candidemia and other deep-seated infections, including disseminated candidiasis, endocarditis, and meningitis and hepatosplenic infection) now is widely recognized as an important public health problem, with considerable morbidity, mortality, and associated health care costs.

Methods: An episode of positive blood culture was defined as the isolation of any pathogenic species of Candida from at least one blood culture specimen. All samples of blood culture processed between 2009 and 2011 which represents a total of 244.761 samples were included on this study. 1254 blood culture positive for yeast were divided in the various strains of Candida spp. All blood cultures were incubated in

Bact Alert 3D ® with protocol of five days of incubation for aerobe, anaerobe yeast cultures samples. The identification of the strains was processed at Vitek 2 system ®. The data was collected by the system manager Observa ®.

Results: Of 1254 strains of Candida spp, 514 were Candida albicans and 740 non Candida albicans. 903 (72, 01%) positive cultures were obtained from peripheral puncture and 351 (27,99%) catheter puncture. The distribution of the species were divided into the local hospital wards, 567 (45,22%) were isolated of patients from Adult ICU, 431 (34,37%) from general wards, 181 (14,43%) from Neonatal ICU, 45 (3,59%) from Emergency room and 30 (2,39%) from Pediatric ICU. The positive isolates were very representative under age 1 (15,47%) and aged over 40 years 67,94% distributed in different division of ages. The main species isolated were Candida albicans 514 (40,99%), Candida parapsilosis 312 (24,88%), Candida tropicalis 216 (17,22%) and Candida glabrata 126 (10,05%). The prevalence of the two main species isolated on the study was for Candida albicans in the Adult ICU 54%, and 46% for Candida parapsilosis in Neonatal ICU 46%.

Conclusions: The predominant yeast blood stream infection in the studied area was caused by Candida non-albicans (59%). Except by the Neonatal ICU the positivity was related with peripheral puncture. Candida albicans was commonly isolated in adult care units and Candida parapsilosis was isolated mainly in the Neonatal ICU unit. These data are relevant considering that Candida non albicans are usually more resistant to fluconazole than Candida albicans.

E-133

Validation of confirmatory TPPA syphilis testing in the reverse sequence algorithm.

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The traditional approach to syphilis screening is rapid plasma reagin (RPR) testing, followed by confirmatory treponemal antibody testing when the RPR is positive. Many experts propose shifting this paradigm to initial treponemal antibody testing, followed by RPR to confirm probable active disease. This new approach (sometimes called "reverse sequence") allows specimens to be screened using automated enzyme immunoassay (EIA) instruments and avoids the problem of false-positive RPR results. However, the problem of false-positive EIA results (e.g. not confirmed by traditional confirmatory tests such as Treponema pallidum particle agglutination or TPPA) has not been extensively evaluated.

We reviewed all patients from 2008-2011 whose initial screening EIA treponemal antibody test (TREP-SURE©, Phoenix Bio-Tech Corp., Mississauga, Ont., Canada) was positive (index >1.2) according to manufacturer's instructions (n=302) to determine concordance with RPR (Macro-Vue™, Becton Dickinson & Co., Sparks, MD) and TPPA (Serodia®, Fujirebio Diagnostics, Inc., Malvern, PA). If the RPR was also positive (n=96), TPPA was positive (+) or equivocal (+/-) in 100%. If the RPR was negative (n=206), the EIA result was similarly confirmed by TPPA in 176 patients (85%). Clinical review of patients in whom the EIA result was not confirmed failed to reveal any history of syphilis or significant pre-test probability. Receiver Operator Characteristic analysis of EIA-positive, RPR-negative patients (with TPPA positivity defined as true positive) revealed three distinct intervals of performance for the EIA assay. Results with low indices (1.3-4.1) were likely to be TPPA-negative and to represent false-positive results, while results with indices above 4.1 (especially above 11.5) had a high likelihood of being true-positive results.

We conclude that reverse sequence syphilis screening programs may characterize EIA-positive, RPR-positive specimens as "positive" without needing to perform TPPA testing. However, EIA-positive, RPR-negative specimens require TPPA confirmatory testing, especially if the EIA indices are less than 4.2.

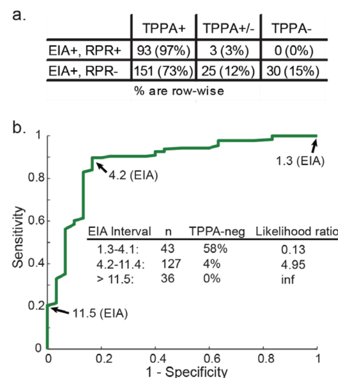

Fig a. Table showing sample distribution using the reverse sequence. **Fig b.** ROC curve demonstrating classification of confirmatory TPPA by TrepSure™ EIA index value (RPR negative data only). The n column represents number of samples in the interval, the TPPA-neg column represents percentage of samples in the interval that were TPPA-negative.

E-134

A Simplified Protocol for Rapid Sequence-Based Fungal Identification from Culture or Formalin-Fixed, Paraffin Embedded (FFPE) Tissues.

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Background: Identification of fungi is often a challenge in clinical microbiology, especially in small samples. Routine culture often results in unidentifiable sterile hyphae or no growth and histologic evaluation is hampered by variable tissue morphotypes. We developed a sequence-based assay using available reagents, kits and equipment familiar to any molecular pathology laboratory. The strengths of this approach include: simplicity, definitive results, and species-specific data in less than 12 hours.

Methods: Culture fungi were selected from in-house ATCC stocks and sterile hyphae cultured fungi. FFPE specimens for analysis were selected randomly based on tissue source from archival tissues retrieved from pathology files. Prior diagnoses were blinded. DNA was extracted (QIAamp mini kit, Qiagen) and amplified using published primers ITS3 and ITS4, hybridizing to conserved sequences between the 5s and 28s rRNA genes of all fungi. DNA was purified (QIAquick PCR cleanup kit, Qiagen), sequenced (BigDye3.1, ABI) and used to interrogate the NCBI BLAST nucleotide-to-nucleotide database. Species identification was based on best sequence alignment.

Results: DNA was successfully amplified from cultures and FFPE tissues. ATCC isolates of Cryptococcus laurentii, Candida glabrata, Candida parapsilosis, and Candida tropicalis were all verified: 99%, (gb FJ153214.1); 99%, (gb CR380958.2), 100%, (gb HQ263346.1), 98%, (gb JF300164.1) match, respectively. Sterile hyphae from four cases identified: Ajellomyces dermatitidis, 99%, (gb EF592163.1); Pichia kudriavzevii, 98%, (gb HM771638.1); Cladosporium cladosporioides, 99%, (gb JN253511.1); and Coprinellus micaceous, 98%, (gb GU227721.1) match, respectively. Trichophyton rubrum, Cladosporium cladosporioides, and Cladosporium tenuissimum were identified in toe nail with 96% (gb FM178326.1); 96% (gb JN253511.1); 89% (gb Y15966.1) match, respectively. In lung, a tissue diagnosed as Cryptococcus sp.was identified as Ajellomyces dermatitidis by sequencing with 99% match (gb EF592163.1). Candida sp. with previous histological diagnosis in lung, skin, and esophagus identified as Candida albicans with 93%, (gb HQ014713.1); 99%, (gb HQ014713.1); 96%, (gb HQ014723.1) match, respectively.

Conclusions: We have established an assay for fungal identification based on DNA sequence that is both faster and more definitive than culture or tissue morphology with a unified workflow for fresh, frozen or FFPE fungi in less than 12 hours.

E-136

IDbox™: A Sample-to-Answer Molecular Diagnostic Platform for Multiple Sample Types

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Background: The GenturaDx IDbox™ system is a fully automated integrated molecular detection platform capable of multiplexing a variety of sample types. The IDbox single-use cassette facilitates all steps of the assay process: cell lysis, nucleic acid extraction and purification, amplification, and target detection. Cassettes are pre-loaded with assay specific reagents to minimize hands-on time, risk of errors, and contamination thereby minimizing the need for highly trained operators in the clinical laboratory. The only manual step is addition of sample to the IDbox cassette. The standardized cassette design enables processing diverse sample types for detecting multiple analytes simultaneously.

Several studies were performed to demonstrate the IDbox system's flexibility for diverse sample types and target types. Flexibility was tested by examining the system's ability to detect bacteria, viruses (DNA and RNA), and human genomic DNA from various matrices including culture media, cerebral spinal fluid (CSF), serum, urine, and blood.

Methods: Diverse targets were tested with analytical samples containing varying concentrations of analyte. Using culture media, two DNA viruses, herpes simplex virus 1 and 2 (HSV 1 and 2) and one RNA virus, influenza A (Flu A), were tested. HSV 1 was also tested using CSF. Another viral DNA target, cytomegalovirus (CMV), was tested using serum. A bacterial target, *Chlamydia trachomatis* (CT), was tested using urine. Human genomic DNA was extracted from whole blood (WB) and tested for the detection of beta actin (β-actin).

Results: Using transport media, HSV 1 was tested over a range of 62.5 to 7.81 $TCID_{50}$/mL with 100% detection at 15.6 $TCID_{50}$/mL, HSV 2 was tested over a range of 0.3 to 0.003 $TCID_{50}$/mL with 100% detection at 0.03 $TCID_{50}$/mL and Flu A was tested over a range of 100 to 1 $CEID_{50}$/mL with 100% detection at 10 $CEID_{50}$/mL. Using CSF, HSV 1 was tested over a range of 2.5 x 10^2 to 3.13 x 10^1 $TCID_{50}$/mL with 100% detection of all replicates. For detection of CMV in human serum, concentrations from 5 x 10^6 to 5 x 10^2 $TCID_{50}$ /mL were tested; all replicates were detected with average Ct values from 24.33 to 35.83, R^2 = 0.9697. For detection of CT in urine, analyte concentrations from 5 x 10^4 to 5 x 10^0 $TCID_{50}$/mL were tested; all replicates were detected with average Ct values from 20.40 to 34.68, R^2 = 0.9992. For detection of β-actin in WB, average Ct values were 29.60 with 5% WB, and 28.1 with 10% WB and all replicates were detected.

Conclusions: The fully integrated IDbox system successfully extracted nucleic acids from multiple sample types and detected the target DNA and RNA sequences over a range of concentrations, thereby indicating its ability to handle various sample types and targets, and demonstrating flexibility of the system.

E-137

Performance Evaluation of a Prototype Rubella G2 (RubG2) Assay on the ADVIA Centaur System

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Objectives: *Rubella virus* is a member of the family Togaviridae. Primary infections during pregnancy can lead to fetal death or congenital rubella syndrome (CRS). Babies born with CRS typically exhibit low birth weight, deafness, eye disease, mental retardation, and cardiac abnormalities. We report the evaluation of a fully automated prototype Rubella G2 (RubG2) assay* in a chemiluminescence immunoassay format on the ADVIA Centaur® system (Siemens Healthcare Diagnostics, Tarrytown, NY, U.S.). The assay is for the quantitative and qualitative detection of IgG antibodies to rubella virus in human serum or plasma and may be used in the assessment of immune status to rubella in individuals including women of childbearing age.

Methods: Specificity and sensitivity were determined by testing approximately 1000 patient samples in singlicate. The assay was calibrated to yield a cutoff value of 10 IU/mL using serial dilutions of WHO 1st International Rubella Standard run in triplicate using two assay kit lots. Calibration was verified using a 1-in-2 dilution of the CDC low-titer rubella standard. Precision was determined according to the CLSI EP5-A2 protocol, 2 runs/day for 20 days. Samples containing ANA, CMV, EBV, HAMA, HBV, HSV, measles virus, mumps, parvovirus B19, RF, rubella IgM, toxoplasma, and VZV, as well as flu vaccine recipient samples, were tested for cross-reactivity. Interference was evaluated according to the CLSI EP7-A2 protocol.

Results: Sensitivity and specificity were 100% and 99.5%, respectively. Linear regression for the WHO (IU/mL) versus RubG2 demonstrated that RubG2 = 0.99 (WHO 1st IRP) - 0.65 IU/mL; R^2 = 1.00. The mean result of a 1-in-2 dilution of CDC low-titer standard (commercialized at ~21.0 IU/mL) was 14.9 IU/mL. The RubG2 assay is standardized to the WHO through the range of 1.0-400 IU/mL. The RubG2 assay had within-run and total CVs of less than 9.9% and 14.2%, respectively, over the assay range in the 20-day precision study. This assay was evaluated for potential cross-reactivity in other viral infections and disease states, and no change in clinical interpretation was observed. No clinically significant changes were observed for the common interferents tested.

Conclusions: The results demonstrate that the prototype ADVIA Centaur RubG2 assay is a sensitive and specific assay for detecting IgG to rubella virus. The performance is competitive with currently marketed assays.

* Under development. Not available for sale.

E-138

Digital ELISA of HIV P24 capsid protein with sensitivity of nucleic acid amplification tests

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Background: Nucleic acid amplification techniques such as PCR have become the mainstay for ultimate sensitivity for detecting low levels of virus, including human immunodeficiency virus (HIV). As a sophisticated technology with relative expensive reagents and instrumentation, adoption of Nucleic Acid testing (NAT) can be cost inhibited in some settings. We report a simple low cost digital immunoassay for the p24 capsid protein of HIV. Single Molecule Arrays (SiMoA, or digital ELISA) technology enables three logs greater sensitivity than conventional immunoassays. Comparable sensitivity to NAT for detection of acute HIV infection is demonstrated.

Methods: SiMoA assay used similar reagents as in conventional ELISA. P24 specific capture antibody was immobilized on paramagnetic bead and detection antibody was biotinylated. Sandwich immunocomplexes were formed by incubating P24 antigen and antibodies together and then labeled with streptavidin conjugated beta-galactosidase. Labeled immunocomplex on beads were isolated and sealed in individual micro well containing fluorescent substrate on array. Beads with enzyme label that converting substrate into fluorescent product over time therefore were considered "on" in digital counting. Well arrays were imaged with a CCD camera. The whole range of signal was determined using imaging analysis software to get Average Enzyme per Bead (AEB, the unit of measurement of SiMoA). The output was related to a standard curve and converted to a p24 concentration of the sample. The digital p24 immunoassay was evaluated for recovery and analytical sensitivity using recombinant p24 and cultured HIV lysate. Clinical sensitivity for first detection of HIV infection was evaluated with HIV-1 seroconversion panels, and compared with commercially available NAT methods, immunoassays for p24, and 4th generation HIV combo immunoassays.

Results: Limit of detection (3SD method) was estimated as 4.87 fg/mL across 11 runs. This represents 2000-3000 fold greater sensitivity than commercial immunoassays for p24 detection, and equates to approximately 120 RNA copies/mL. Assaying serial samples (Seroconversion panel) from 10 HIV-infected individuals, the digital p24 immunoassay detected acute HIV infection as early as NAT methods, and 8-10 days earlier than conventional immunoassays. Comparison of assay results between the digital immunoassay and a quantitative NAT method from HIV infected sera exhibited a linear correlation R2 > 0.99.

Conclusions: The data suggest that the prototype SiMoA digital p24 immunoassay has comparable sensitivity to NAT for acute HIV detection.

E-139

Fewer false positive and false negative results with 4th generation HIV antigen/antibody combo assay compared to 3rd generation HIV antibody assays in a low prevalence setting

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Objective: FDA approved 4th generation HIV antigen/antibody combo assays are now increasingly used to screen for HIV disease. Although 4th generation HIV assays can reduce early diagnostic window their overall benefits over widely used 3rd generation

HIV antibody assays in low prevalence settings have not been well studied. A recent study suggests that 4th generation HIV rapid assays may not provide any additional advantage over 3rd generation HIV assays. The goal of this study was to evaluate the performance of an automated, random access 4th generation Abbott ARCHITECT HIV antigen/antibody combo assay (Abbott Combo test) against two commercially available 3rd generation automated HIV assays (Ortho Clinical Diagnostics VITROS Anti-HIV 1+2 assay (Vitros test) and Siemens ADVIA Centaur HIV 1/O/2 enhanced assay (Centaur test) in a low prevalence clinical setting.

Methods: A total of 123 patient samples received from 3 clinical laboratories in Dallas (Parkland Memorial Hospital, UT Southwestern Medical Center and Dallas county Department of Health and Human Services) were included in this study. First, screen positive samples by our current screening method (Vitros test) were tested against Abbott Combo and Centaur tests with confirmation by IFA/WB assays. Then, acute HIV cases positive by qualitative NAAT assay only and negative or indeterminate by WB were tested by the above automated, random access assays to determine true and false negative results.

Results: Out of 99 screen positive samples (by our current screening method) (Vitros test) selected for the initial study, 10 were confirmed positive, 82 confirmed negative (false positive by Vitros test) and 7 indeterminate by confirmatory IFA/WB tests. All 10 confirmed positive samples were reactive by the Abbott Combo test as well. In contrast, one of the true positive samples was nonreactive by the Centaur test. Compared to 82 false positive results by Vitros test, only 4 were false positive by Abbott Combo test and 10 by the Centaur test. 1 out of 7 IFA/WB indeterminate samples was reactive and remaining 6 were nonreactive by Abbott Combo and Centaur tests. Of the 24 samples positive by NAAT assay, 18 were negative by WB assays, 4 were indeterminate and 2 had unknown WB status. Out of these 18 confirmed acute infection cases (NAAT positive, WB negative), 13 were reactive by Abbott combo test whereas only 8 were reactive by Centaur test. 4 NAAT positive, WB indeterminate samples and 2 NAAT positive, WB unknown status samples were all reactive by both Abbott Combo and Centaur tests.

Conclusions: Our targeted study performed in a low prevalence setting clearly indicates that automated, random access 4th generation Abbott Combo test generates fewer false positive and false negative results compared to automated, random access 3rd generation HIV assays. Further, this study shows that there is a significant difference in performance among the automated, random access 3rd generation HIV assays as well.

Thursday AM, July 19, 2012

Poster Session: 9:30 AM - 12:00 PM
Clinical Studies/Outcomes

E-140

Cystatin C/creatinine ratio according to the degree of renal impaiment

S. Cho, M. Kim, J. Yang, Y. Jeon, H. Lee, J. Suh, E. You. *Kyung Hee University Hospital, Seoul, Korea, Republic of*

Background: Creatinine (Cr) is the most popular and traditional renal marker, however, age and muscle mass dependency limits its use in measuring renal function in pediatrics. Cystatin C (CysC) is a 121-amino-acid and a non-glycated 13-kilodalton basic protein. CysC is produced at a constant rate in all nucleated cells and less influenced by muscle mass, gender or age than Cr. Over- or underestimation of renal function is very critical to form a scheme for treatment or a follow up plan. In previous study, we suggested that serum CysC concentrations could underestimate renal dysfunction in pediatric patients. In this study, we planned to investigate how the CysC/Cr ratio changed according to the increase of these markers and the quantitative relationship between these 2 renal markers in adult patients with renal impairment.

Methods: For the patient group, a total of 209 individuals with renal impairment were enrolled from the Department of Nephrology at Kyung Hee Medical Center from August 2009 to October 2010 (Table 1). Serum CysC was measured using HiSens Cystatin-C LTIA (HBI Co., Anyang, Korea) on a Toshiba chemical analyzer (Toshiba, Nasushiobara, Japan). Cr tests using an enzymatic method (CICA, Tokyo, Japan) were performed on the same chemical analyzer.

Results: CysC ranged from 1.0 to 7.8 mg/L and Cr presented a broader range from 0.4 to 21.5 mg/dL. CysC/Cr ratio shows the siginificant negative correlation with Cr (Fig.1, $P<0.001$).

Conclusions: Therefore, it is possible that serum CysC concentrations could underestimate renal dysfunction in adult patients, too. According to this study, we suggest that the combined use of two renal markers can be more helpful to estimate renal function over use of any single marker. Future studies including the analysis with body mass index and proteinuria need to be performed for more exact assessment of the compatibility of these renal markers.

Table 1. Characteristics of patients

Total number of patients/specimen	209/231
Mean age (range)	59.29 (18-91)
Male/Female	116/115
Mean serum creatinine (mg/dL)	4.54
Mean serum cystatin C (mg/L)	3.22
Disease entity	
Chronic kidney disease	45
Unspecified kidney failure	22
Non-insulin-dependent diabetes mellitus	22
Renal failure	15
Other chronic renal failure	11
Dialysis	10
Kidney transplant status	9
Nephrotic syndrome	7
Diffuse mesangial proliferative glomerulonephritis	6
Disorder of kidney and ureter, unspecified	5
Hypertension	5
Hematuria	5
Gastroenteritis and colitis of infectious origin	4
End-stage renal disease	4
Unspecified edema	3
Acute tubulo-interstitial nephritis	2
Congestive heart failure	2
Chronic nephritic syndrome	2
Other disease*	30

*acute tubular necrosis, benign neoplasm of renal pelvis, hypertensive renal disease without renal failure, multiple myeloma, Grand mal seizures, fever, unspecified, other disorders of kidney and ureter in infectious and parasitic diseases, polycystic kidney, sepsis, etc.

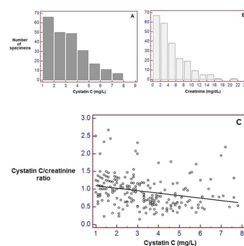

Fig.1. Distribution of cystatin C, creatinine and cystatin C/creatinine ratio, which shows the significant negative correlation with Cr ($P<0.001$).

E-141

Alzheimer's disease diagnosis using ELISA test to detect Antibodies to activated Microglial cells in the CSF

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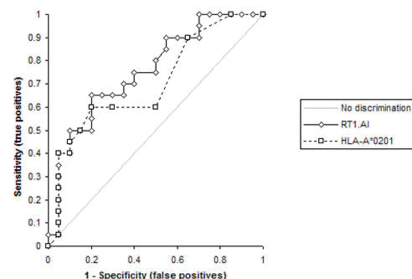
Background: Testing for biomarkers to assist in the diagnosis of Alzheimer's disease (AD) in the CSF has mainly focused on Total tau protein, phosphorylated tau, and β-amyloid isoforms. Immunohistochemical studies have previously demonstrated that activated microglia are a distinct feature of AD pathology and that autoantibodies are produced that recognize these cells.

Objective: To identify the antigen associated with anti-microglial cell autoantibodies in CSF, and to establish whether the presence of these auto-antibodies could be used as a diagnostic test for AD. Method: We identified major histocompatibility complex 1 (MHCI) as the microglial surface antigen to which autoantibodies are directed in AD patients. ELISAs were established using two distinct forms of MHCI as the antigen. One form was HLA.A*0201, the most commonly expressed form of MHCI in humans, whilst rat RT1.A[l] was also used, to provide a more direct comparison with the rat brain cross-sections previously employed for immunohistochemistry. 20 CSF samples from individuals with confirmed AD and 20 CSF samples from non-AD controls were tested. Autoantibodies were detected using an alkaline phosphatase conjugate of goat anti-human IgG. The AD CSF samples were collected at post-mortem so as to provide definitive confirmation of AD from the presence of brain amyloid plaques. Living donors were used as a source of asymptomatic CSF samples.

Results: Data was analysed by constructing receiver operator characteristic (ROC) curves, shown in the figure. Using RT1.A[l] as the antigen in the ELISA, the area under the curve (AUC) was 0.756 (p = 0.0004) and when using HLA-A*0201 as the antigen AUC was 0.705 (p = 0.0071).

Conclusions: There is a statistically significant association between antibodies recognizing MHCI in CSF and the presence of AD.



E-142

Mean platelet volume in patients with chronic alcohol consumption

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Background: Mean platelet volume (MPV) is a parameter generated by fully-automated blood count analyzers as a routine complete blood count (CBC), and a useful platelet function index that can show platelet activation and its production rate in bone marrow. Moreover, MPV has been studied in various disease groups other than hematologic disease such as cardiovascular, hepatic and rheumatic diseases. However, because it had not been investigated whether alcohol consumption could influence to MPV, we planned to study this platelet index in patients with chronic alcohol abuse. We tested carbohydrate-deficient transferrin (CDT), the sum of a- and disialotransferrin, to identify patients with chronic alcohol consumption.

Methods: A total of 62 patients, who were suspected to drink alcohol in interviews, were enrolled in this study at Kyung Hee University hospital, a tertiary teaching hospital in Seoul, Korea, between November 2010 and May 2011. MPV was measured using EDTA blood in Advia 2120 (Bayer Diagnostics, Tarrytown, NY, USA). Gamma-glutamyl transferase (GGT) test was done in TBA-200FR NEO (Toshiba, Tokyo, Japan). CDT test was done in capillary zone electrophoresis (Sebia, Lyss, France).

Results: In total patients, 39 patients showed normal CDT results (range 0.6~1.2%) while 23 patients presented increased CDT levels (range 1.3~17.9%). Mean of MPV levels showed no significant difference between normal CDT group and increased CDT group (Fig.1). Mean of GGT showed no statistical difference between these 2 groups, however, GGT presented the positive correlation with MPV ($P<0.001$).

Conclusions: Therefore, this study suggests that MPV is associated with the degree of liver damage to be estimated by GGT instead of the effect through the direct alcohol intake. However, because there was the trend that MPV levels were slightly higher in increased CDT group than normal CDT group, the further study with more patients should be followed for the clear conclusion of the alcohol effect on MPV.

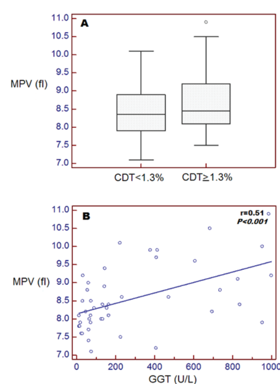

Fig.1 Mean platelet volume (MPV) levels showed no significant difference between normal carbohydrate-deficient transferrin (CDT) group and increased CDT group (A). Gamma-glutamyl transferase (GGT) presented the positive correlation with MPV (B, *P*<0.001).

E-143

Assessment of cardiovascular risk in Nepalese individuals with chronic kidney disease

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Background: Chronic kidney disease (CKD) has become a major public health problem worldwide and 10.2% prevalence is reported in Nepal. Studies conducted in different parts of world indicate that risk of cardiovascular disease (CVD) is greater in patients with CKD compared to their non-CKD counterparts and CVD are the leading cause of death in CKD. Till date, no documented study was found on this topic in Nepalese individuals. So, this study aims to find out risk of future CVD and to examine CVD risk factors in Nepalese individuals with CKD.

Methods: This cross-sectional study, conducted in TU Teaching Hospital, Nepal included 150 pre-dialysis CKD patients and 150 healthy controls (75 males and 75 females both), with mean±SD estimated glomerular filtration rate (GFR) 18.1±7.9 & 91.2±16.2 ml/min respectively. Mean±SD age of cases and controls was 55.3±13.1 & 40.2±9.8 years respectively. CKD was defined as per National Kidney Foundation-Kidney Disease Outcome Quality Initiative guideline and GFR was estimated by Chronic Kidney Disease-Epidemiology Collaboration formula. Blood Pressure and other anthropometric parameters were measured while fasting blood was analyzed for different serum biochemical risk factors. Framingham risk scores were used to predict the CVD risk. Data were analyzed by SPSS program using Chi-square test, students t-test, one way ANOVA and logistic regression. P-value of <0.05 was considered significant.

Results: This study found the clustering of several traditional and novel CVD risk factors in CKD. 39% were current smokers, 52% were diabetic, 65.3% were hypertensive, 31% had the history of acute chest pain, 39% had the left-ventricular hypertrophy in Electrocardiogram, 86.6% had elevated homocysteine, 94.6% had elevated hsCRP, 94.6% had high anti-oxLDL, 84% had dyslipidemia, 53.3% were anemic, 45.2 % had metabolic syndrome and 66.6% had multiple CVD risk factors as defined by NCEP-ATPIII. Severity and duration of CKD had positive association with high cholesterol (P=014), high LDLc (P=0.006), low HDLc (P= NS) , high TG (P=0.01), high Lp(a) (P=0.002), high anti-oxLDL (P<0.001), high hsCRP (P=0.006), hyperhomocysteinemia (P=0.001), low hemoglobin (P<0.001), low serum albumin (P<0.001), presence of metabolic syndrome (P=0.02) and presence of multiple risk factors (P=0.004). CKD cases had significantly higher Framingham predicted risk for CVD. After adjustment for age, gender, diabetes, smoking and hypertension compared to healthy controls CKD cases had 5.2 times higher risk for coronary heart disease in 10 Yr (P<0.001) , 5.0 times higher risk for general CVD in 10 Yr (P<0.001), 4.4 times higher risk for stroke in 10 Yr (P<0.001).

Conclusion: CKD patients have very high prevalence of CVD risk factors and very high predicted risk for CVD. CVD risk and prevalence of risk factors increases with CKD severity and duration. So, screening should be done in order to indentify the individuals at high risk for future CVD.

E-144

Maternal plasma prolactin levels in normal and preeclamptic pregnancy

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Background: Preeclampsia is a pregnancy specific complication characterized by hypertension, proteinuria, edema, and activation of the homeostatic system. It is present in 5-8% of all pregnant women, and remains as a major cause of maternal and perinatal morbidity and mortality worldwide. The major function of prolactin is its role in lactation during pregnancy, but many authors have claimed that this hormone is also involved in angiogenesis. There is also evidence that prolactin causes renal retention of electrolytes and elevate arterial pressure in rabbit. In this context, our study aims to compare the serum prolactin level between preeclamptic and healthy pregnancies. In addition, we compared the value of serum prolactin between mild and severe preeclampsia cases and also correlated it with mean arterial pressure (MAP) and 24 hr-urinary total protein (UTP).

Methods: A total of 54 pregnant women diagnosed with preeclampsia were recruited in this hospital based prospective study. Pre-eclampsia was defined as per Australasian Society Consensus Statement research definition. Pre-eclampsia was regarded as serious if severe hypertension was associated with proteinuria or if hypertension was associated with severe proteinuria. The criteria for severe hypertension and severe proteinuria were respectively (1) systolic blood pressure ≥160 mm Hg or diastolic ≥110 mm Hg and (2) proteinuria >5 gm in 24 hours. Sixty age and gestational weeks matched healthy pregnant women were taken as controls. 8 mL of blood was drawn from preeclamptic cases as soon as the disease was diagnosed and prolactin level was analysed on the same day from an aliquot of serum by sandwich chemiluminescent assay principle (Bio-Ekon, Beijing). Shapiro-wilk test was used to see the distribution of prolactin and data were expressed in median. Median was compared between groups using Mann-Whitney U test and Spearman rank correlation coefficient was used to show correlation. All the P- values were two- tailed, and those < 0.05 (95% Confidence interval) were considered statistically significant.

Results: Among 54 preeclamptic women, 41 had mild preeclampsia and 13 had severe preeclampsia. The mean age of the preeclamptic cases and pregnant controls were respectively 26.4±3.23 and 26.13±3.35. The difference in this mean was statistically insignificant (P=0.658). The mean gestational weeks of the preeclampsia cases and pregnant controls were respectively 32.01±3.08 and 31.21±2.92 weeks, the difference being statistically insignificant (P=0.157). This study showed that the median concentration of prolactin was significantly higher in preeclampsia than in normal pregnancies (156.6 Vs 129.8 ng/mL, P=0.012). Though the median concentration of prolactin was higher in severe preeclampsia in comparison to mild one, the difference did not reach the significant level (228.3 Vs 152.9 ng/mL, P=0.061). No significant correlation of prolactin was found with mean arterial pressure and 24-hr UTP.

Conclusion: Prolactin was markedly elevated in preeclampsia cases than in healthy pregnant but no such difference was found between mild and severe cases. Also, lack of correlation with MAP and 24-hr UTP suggests that prolactin is not associated with severity of disease and hence does not play significant role in pathogenesis of disease.

E-145

Creating awareness about Good Clinical Laboratory Practices in Mumbai, India - the road ahead

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Background: In India, the National Accreditation Board for Testing and Calibration Laboratories (NABL) has been providing accreditation services to medical laboratories since 1998 and is currently following ISO 15189; 2007 standards. Guidelines have also been published for Good Clinical Laboratory Practices (GCLP) by Indian Council of Medical Research (2008). However, accreditation of clinical laboratories is still not mandatory. It is therefore, necessary to create awareness about GCLP amongst not only the laboratorians but also amongst students who may take up laboratory science as a vocation.

Methods: The Department of Biochemistry and Clinical Nutrition at the Seth GS Medical & KEM Hospital has been conducting a yearly, four day course in GCLP since 2005. For the first three days, the importance of the pre analytical phase, analytical phase and post analytical phase is elaborated. The participants subsequently return to their laboratories and solve a problem using a set of specific performance indicators. After a period of two months, they return to present their findings. The

students who do not have a laboratory placement are assigned a laboratory where they carry out their project.The training given in the course was evaluated using Kirkpatrick's four levels of training.

Results: All four levels of Kirkpatrick's evaluation were achieved by the participants. Reaction was obtained by feedback from participants. Learning was evaluated by providing pre / post - tests. Behaviour was observed by noting a change in the knowledge, skills and attitude of the participants which was evident when the participants presented their projects. Both laboratorians and students reported an alteration in their performance. Many of the participants said that the course motivated them to apply the principles of accreditation and also start preparation for it. Others from accreditated laboratories opined that some principles of GCLP became clear to them after having undergone the course. This confirmed that the fourth level of Kirkpatrick's evaluation was achieved, as it examined the impact on the individual and the laboratory.

Conclusions: During the last 7 years, this GCLP training has had a very good clinical outcome as it has given the laboratory staff confidence to work in the laboratory and implement GCLP. The course is becoming an important source of laboratory training and helping the students and laboratorians to understand and apply the principles of accreditation. It has made small albeit definite inroads in the dissemination of knowledge and in educating the stakeholders.

E-146

An association between serum uric acid and Cerebrovascular disease (Ischemic stroke and Vascular dementia)

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Background: At our study we have investigated uric acid in 200 patients diagnosed with the first ischemic brain stroke, where blood samples were taken during the acute phase and post acute phase, 200 patients diagnosed with vascular dementia developed as a consequence of ischemic brain stroke, i.e. of many small ischemic focus of various age and 200 subjects at healthy group.

Methods: Uric acid was determined by DIMENSION LxR automatic analyser of DADE BEHRING Company. Determination of uric acid based on the enzymatic method, range 155-428 mmol / L. Collected data were statistically analyzed using programs SPSS version 11.0 and Microsoft Office Excel 2003.

Results: The ischemic stroke patients had an average age of 70.12 years, in control group was 69.34 years, and group with vascular dementia was 73.74 years. The patients with ischemic stroke had hyperuricemia in 30 % and vascular dementia in 8%. Our results showed that uric acid increased to 7 days after ischemic stroke by 5.3%, after 14 days of 9.5%. Although we found an increase of uric acid in acute and post acute phase after ischemic stroke Wilcoxon signed ranks test ($Z = -1.569$, $p=0.117$) showed that this difference was not significant for serum uric acid after 24-48 hours and 7 days of ischemic stroke ($p < 0.05$). According the same test ($Z = -0.599$, $p=0.549$) for the significance level of $p < 0.05$ no significant difference between serum uric acid after 7 and 14 days of ischemic stroke. Using the Wilcoxon signed ranks test ($Z = -2.736$, $p=0.006$) was found statistically significant difference between the average concentration of uric acid after 24-48 hours and 14 days of ischemic stroke. Using the Mann-Whitney test ($Z = -1.837$, $p=0.066$) it was not a significant difference between concentrations of uric acid in the acute phase (24-48 hours of ischemic stroke) and control groups. According to the same test ($Z = -2.837$, $p=0.005$; $Z = -2.734$, $p=0.006$) it was a significant difference between concentrations of uric acid after 7 and 14 days of ischemic stroke and control groups. Using the same tests, no significant difference between the average concentrations of uric acid in acute ($Z = -0.458$, $p=0.647$; $Z= -0.614$; $p=0.539$) and post-acute phase ($Z = -0.700$, $p=0.484$) of ischemic stroke and vascular dementia groups with the significance level of $p < 0.05$. Using the Mann-Whitney test ($Z=- 2.241$, $p= 0.025$) was significant difference between the average concentrations of uric acid in vascular dementia and control group with the significance level of $p < 0.05$. The Spearman correlation test was not found significant correlation between the number of strokes and uric acid in serum of patients with vascular dementia for significance level of $p < 0.05$ ($r = 0.114$, $p=0.425$).

Conclusions: Uric acid concentration is higher in the group with ischemic brain stroke and vascular dementia than in control group. It is possible that increases uric acid reflect renovascular atherosclerosis and tissue hypoxia. Therefore monitoring of uric acid at patients with ischemic stroke is important because uric acid is more harmful than protective.

E-147

Diagnostic usefulness of NEUTROPHIL GELATINASE-ASSOCIATED LIPOCAIN (N-GAL) at patients in Intensive Care after Cardiac surgery

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Background: The renal injury is connected with increased N-GAL concentrations in urine. The kidney epithelia express and excrete massive quantities of N-GAL within 30 minutes in the urine when damaged by ischemia-reperfusion injury nephro-toxins, sepsis and chronic progressive changes. N-GAL is a biomarker that can predict development of acute kidney disease 1-3 days earlier than serum creatinine. At our study in 150 patients we have investigated N-GAL in urine after cardiac surgery and we wonted to proof if possible acute kidney injury (AKI) will develop.

Material and **Methods:** The concentrations of N-GAL in 450 urine samples were determined using CMIA (chemiluminesecent microparticle immnoassay) Architect i 2000 Abbott diagnostic. All of 150 patients were hospitalized at Department of Cardiac surgery in Intensive Care Unit at the University Clinics Center of Sarajevo. We have collected samplers of urine after 3, 6 and 12 hours of cardiac surgery with cardiopulmonary bypass (CPB). The normal serum range of N-GAL was between 0,0 - 135 ng/mL. The urine samplers have not any infection contamination. We have measure creatinine in serum by means of DIMENSION LxR (DADE BEHRING Company). Collected data were statistically analyzed using programs SPSS version 11.0 and Microsoft Office Excel 2003.

Results: The mean concentration of N-GAL 3 hours after cardiac surgery was 52.25 ± 91.86 ng/mL and after 6 hours 14.56 ±31.13 ng/mL and after 12 hours 14.15±16.3 ng/mL. The only 5 % (8 patients) have N-GAL concentration > 135 ng/mL 3 hours after surgery. The N-GAL concentration rises already 3 hours after cardiac surgery and fall down after 6 and 12 hours. The patients with high concentration of N-GAL (> 135 ng/mL) after 3 hours have developed AKI. The N-GAL concentration for these patients after 6 hours was 110 ±16.5 ng/mL and 12 hours 110±26.3 ng/mL. The concentration of creatinine in serum rice, more then reference range after 12 hours. The correlation of urine N-GAL after 3 and 6 hours has good correlation coefficient $r = 0.755$. We identified regression line with slope of 0.2558 and a y axis intercept of 6.3152. After 6 and 12 hours correlation coefficient for N-GAL in serum was low $r = 0.212$ The correlation coefficient for N-GAL after 3 and 12 hours was 0.035, so we have not good connection of results.

Conclusions: N-GAL could be one of the predictive markers for AKI at patients with cardiac surgery with measurement after 3 hours. Therefore it should be measured at all patients after cardiac surgery because it is marker for more early detection of AKI than creatinine.

E-155

Correlations between Bone Specific Alkaline Phosphatase (BAP), Tartrate-Resistant Acid Phosphatase (TRAP-5b) and Magnesium, Calcium, and Phosphate ions in osteoarthritic patients

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Background: Mg(2+) magnesium ions stimulate the secretion in osteoblasts. Ca(2+) calcium and PHOS(2-) phosphate ions have implications in secretion of the tartrate-resistant acid phosphatase (TRAP-5b) in osteoclasts. These markers play an essential role in bone turnover of osteoarthritic patients.

Objectives: to determine the serum levels of BAP and TRAP-5b, and Mg(2+), Ca(2+), PHOS(2-) ions in serum and synovial fluid in patients with osteoarthritis (n=12) versus control group (n=5).

Material and Methods: The serum levels of the enunciated markers were measured by ELISA technique. The ion serum levels were measured by a Vitros250 biochemistry analyzer, whereas the synovial fluid levels were measured by bone flame atomic absorption spectrometry (FAAS).

Results: Serum levels of BAP increased in study group (2.45±0.38U/L; $p<0.0003$) versus control group (2.2±0.33U/L). Serum levels of TRAP-5b increased in study group(2.86±0.25U/L; $p<0.0007$) versus control group (1.9±0.16U/L). In serum: levels of Mg(2+) decreased in study group (1.31±0.25mg/dl; $p<0.002$) versus control group (2.12±0.73mg/dl); levels of Ca(2+) increased in study group (5.42±1.17mg/dl; $p<0.005$) vs. control group (4.28±0.73mg/dl); and [PHOS(2-)] increased in study

group (5.04±1.03mg/dl) vs. control group (3.46±0.51mg/dl). In synovial fluid: Mg(2+) decreased in study group (3.65±1.12μg/ml) vs. control group (5.12±1.04μg/ml), p<0.001; Ca(2+) decreased (13.73±2.17μg/ml) vs. control group (16.45±3.62μg/ml), p<0.001; and PHOS(2-) decreased (6.32±1.86μg/ml) vs. control group (9.55±2.25μg/ml), p<0.003.

Conclusions: Serum levels of BAP were significantly increased in patients with osteoarthritis versus control group, attesting osteoblastic activation. Serum levels of TRAP-5b were significantly higher in patients with osteoarthritis versus control group which attest osteoclastogenesis activation. The serum levels of Ca(2+), Mg(2+), and PHOS(2-) ions in osteoarthritis cases were increased as a result of bone demineralization through hidroxiapatite microcrystal solubilization and mobilization of those ions in the circulating torrents, or decreased in synovial fluid secondary to stimulation of osteoblastic apoptosis. A rate of resorption exceeding the osteoblastic synthesis induces the progression of ostheolisis.

E-157

Performance Evaluation of a Redesigned ARCHITECT LH Assay for the Quantitative Determination of Luteinizing Hormone

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Background: Determination of the concentration of human luteinizing hormone (LH) plays an important role for the predication of ovulation, evaluation of infertility, diagnosis of pituitary and gonadal disorders and in the differential diagnosis of puberty disorders. The ARCHITECT LH assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of human luteinizing hormone in human serum and plasma to serve this clinical utility. The assay was redesigned to improve robustness in terms of better lot-to-lot consistency, allowing users to discontinue current practice of matching reagent, calibrators and controls. The assay is referenced to the WHO 2nd International Standard 80/552 for LH and uses a 6-point Standard Calibration curve, compared to the 2-point calibration curve adjustment performed on the predicate LH assay.

Methods: The imprecision of the assay was determined across the measuring range based on guidance from CLSI protocol EP5-A2 using serum and plasma based panels. All panels were assayed on two reagent lots and two instruments, in replicates of three at two separate times per day over a 20 day period. Correlation with the preceding ARCHITECT LH assay (predicate device), was evaluated on 107 unique specimens across the range of 0 to 250 mIU/mL using the Passing-Bablok regression method (CLSI EP9-A2-IR). Sensitivity (e.g. Limit of Quantitation (LoQ, CLSI EP17-A), Limit of Blank (LoB) and Limit of Detection (LoD)), Linearity (CLSI EP6-A) and accuracy by recovery were determined. Expected values were established for normal males (n=199), normal cycling females (n=64) and post-menopausal females (n=124). Lot-to-Lot reproducibility was assessed by testing a BIO-RAD Liquicheck Control, composed of three different LH levels (3, 15, and 45 mIU/mL) across three reagent and calibrator lots resulting in nine different combinations per level.

Results: The total imprecision (%CV) of the assay was determined using samples with LH concentrations across the claimed measuring interval (0.09 mIU/mL to 250.00 mIU/mL) and ranged from 2.4% to 8.9%. Limits of Quantitation, Blank and Detection were determined with 0.09 mIU/mL, 0.01 mIU/mL and 0.03 mIU/mL, respectively. Correlation slope to the predicate device was 1.04. The assay demonstrated linearity for the entire measuring interval with an absolute deviation from linearity of <=1 mIU/mL for samples within LOQ and 10 mIU/mL, <=11% for samples within 10 and 70 mIU/mL, and <=15% for samples above 70 mIU/mL. Mean recovery determined on 15 specimen spiked with known LH concentrations was 101.2%. Process capability indices demonstrated excellent (>6 Sigma) lo-to-lot reproducibility.

Conclusions: The assay showed excellent sensitivity and precision across the entire measurement range. In addition a very good correlation with the predicate device was shown. The high lot-to-lot reproducibility demonstrates an outstanding assay run performance (> six sigma quality process) and allows the end-user more flexibility in managing inventory. The redesigned ARCHITECT LH assay is a valuable tool in clinical laboratories for the accurate and precise determination of human luteinizing hormone. The redesign improved performance as well as process and run capability compared to the predicate device.

E-158

Associations of Arginine and its Methylated Derivatives with Biomarkers of Kidney Function

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Background: Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are turnover metabolites of methylated arginine (ARG) containing proteins. SDMA, an emerging biomarker for renal function, has been shown to outperform creatinine-based equations for estimated glomerular filtration rate (eGFR). In this study, our goal was to investigate the relationship between plasma levels of ARG, ADMA, SDMA, or ratios of these analytes and known renal biomarkers (creatinine and estimated glomerular filtration rate [eGFR]) in a selected patient population.

Methods: This study was approved by our Institutional Review Board. Left-over patient plasma samples (n=132) were selected to cover a wide range of creatinine concentrations that ranged from 0.30 to 12.54 ng/dL (2.83 ± 2.45 ng/dL). All samples were analyzed for ADMA, SDMA, and ARG using a published liquid chromatography-tandem mass spectrometry method. A linear regression model was applied to each pair of variables and the slope coefficient was calculated. Creatinine and the two eGFR calculations (MDRD and CKD-EPI) had skewed distributions, and log-transformations were performed before correlation. Data shown for creatinine and eGFR was adjusted for age, gender and race (Table 1). A P-value <0.05 indicated significance.

Results: The variables ARG, SDMA, ARG/SDMA, ARG/ADMA and SDMA/ADMA were significantly associated with creatinine and the two eGFR formulae, while ADMA was not. However, the strongest correlation with both creatinine and eGFR were SDMA, ARG/SDMA and SDMA/ADMA.

Conclusions: SDMA and some ratios of these derivatives were found to be significantly associated with established biomarkers of renal function in the selected patient population. Further investigation of clinical application of these markers is warranted.

Table 1		Pearson correlation	Slope	P-value		Pearson correlation	Slope	P-value
Log Creatinine	ARG	-0.14	-0.004	0.006	SDMA	0.64	0.441	<0.001
Log eGFR MDRD		0.18	0.005	0.005		-0.66	-0.511	<0.001
Log eGFR CKD epi		0.17	0.005	0.007		-0.66	-0.496	<0.001
Log Creatinine	ADMA	0.03	0.171	0.44	ARG/SDMA	-0.66	-0.006	<0.001
Log eGFR MDRD		-0.05	-0.179	0.485		0.68	0.006	<0.001
Log eGFR CKD epi		-0.04	-0.160	0.518		0.66	0.006	<0.001
Log Creatinine	ARG/ADMA	-0.14	-0.002	0.006	SDMA/ADMA	0.67	0.336	<0.001
Log eGFR MDRD		0.19	0.003	0.006		-0.67	-0.392	<0.001
Log eGFR CKD epi		0.19	0.003	0.008		-0.67	-0.381	<0.001

E-159

Interpretation of Hemoglobin A1c values among different health care professionals

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Background: We assessed the daily routine regarding the use of HbA_{1c} measurement techniques, expected precision of HbA_{1c} value, and the magnitude of HbA_{1c} changes possibly eliciting treatment change advices. Therefore, we surveyed a large group of diabetes care professionals regarding these aspects.

Methods: A questionnaire with 10 questions was developed and was sent to participants through a website serving health care professionals. The survey focused on internists, pediatricians, and general practitioners (‘doctors’); and diabetes specialist nurses, and primary health care practice nurses specialized in diabetes.

Results: In total 104 doctors, 177 diabetes specialist nurses and 248 primary care practice nurses responded to the survey. The majority of diabetes specialist nurses (70 %) and the primary care practice nurses (79%) consider an HbA_{1c} value as an absolute value (uncertainty at an HbA1c of 53 mmol/mol (7.0% DCCT) of ≤ 1 mmol/mol (≤ 0.1% DCCT)) and are not aware of the fact that every HbA_{1c} result has an inherent uncertainty based on the analytical performance of the used method. Both nurses groups intended to change therapy of the patient based on very small changes in HbA_{1c} concentrations. A decrease of at least 5 mmol/mol (0.5% DCCT) or 11 mmol/mol (1.0% DCCT) at an

HbA_{1c} value of 75 mmol/mol (9.0% DCCT) after adjustment of therapy, is considered as sufficient by all health care professionals to allow the conclusion that glucose regulation has improved. In contrast, even a very small or no increase in HbA_{1c} is considered by the majority of the diabetes specialist nurses and primary care practice nurses as significant enough to allow the conclusion that glucose regulation has worsened. Most of the doctors adhere to change of 5 mmol/mol (0.5% DCCT) as a clinically meaningful cut-off point. Approximately 35% of the health care professionals use HbA_{1c} in combination with fasting glucose for the diagnosis of diabetes.

Conclusions: There is a significant ($p<0.001$) difference in interpretation of changes in HbA_{1c} results between doctors and diabetes specialist nurses/primary care practice nurses. In general, nurses consider therapy changes based on very small changes in HbA_{1c}, whereas doctors preferably agree to the clinically relevant change of 5 mmol/mol (0.5% DCCT). Changing therapy based on relatively small changes in HbA_{1c} might lead to undue adjustments in the treatment of patient with diabetes, also in the light of the fact that the analytical performance of some of the HbA_{1c} methods is not precise and reliable enough to warrant changes in therapy based on differences less than 5 mmol/mol (0.5% DCCT).

E-162

Serum 25-OH vitamin D3 levels in elderly

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Aim: In the present study, we measured serum levels of 25-OH vitamin D3 (25-OH vitD3) in elderly.

Material-method: 855 old persons (690 women, 165 men, age range: 61-99 years) were included in the study. 25-OH vitD3 levels below 10 ng/mL were accepted as absolute deficiency, levels between 10 - 25 ng/ml as vitamin D deficiency and those between 25 - 80 ng/ml as normal values. Serum levels of 25-OH vitD3 were measured in Roche Modular Analytics E170 Immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany) using electrochemiluminescence immunoassay (ECLIA) method.

Results: The mean age of the group was 69.32 ± 6.41 years (mean ± SD). 25-OH vitD3 serum levels were within the absolute deficiency range in 19.1% (218 persons) of the study group, at vitamin D deficiency levels in 55.4% (474 persons) and in recommended reference range only in 25.5% (163 persons).

Conclusions: It was determined that the serum 25-OH vitD3 levels were below recommended reference levels in 75% of the studied elderly population. It was concluded that it is necessary to measure vitamin D levels at certain intervals in this age group in order to prevent the unfavorable effects of low vitamin D levels and vitamin D supplementation might also be useful.

E-163

Lack of correlation between urinary and whole blood Neutrophil gelatinase-associated lipocalin (NGAL).

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Background: Neutrophil Gelatinase Associated Lipocalin (NGAL) has recently been identified as a promising biomarker in the diagnostics of acute kidney injury (AKI). The current techniques for NGAL assessment comprise manual enzyme linked immunoassays (ELISAs), a urinary fully-automated immunoassay developed for use on Abbott ARCHITECT®, as well as the Triage® NGAL whole blood point of care immunoassay. The analytical performance of Abbott ARCHITECT® NGAL with that of Triage® NGAL were recently compared, concluding that the former immunoassay should be preferred in routine practice for several analytical advantages, including the lower analytical imprecision. To the best of our knowledge, however, no previous studies have compared the concentration of NGAL in paired whole blood and urine samples (i.e. collected simultaneously from the same patient).

Methods: A comparison study was performed on 25 consecutive, paired patient's fresh urine and EDTA blood samples referred from the emergency department for urgent urinalysis and blood testing for polytraumas. All samples were tested within 2 h from collection. Results of NGAL on Triage® NGAL Device (Biosite-Inverness Medical, Waltham, MA, USA) were compared with those obtained on the urine specimen collected from same patient on Abbott ARCHITECT (Abbott Park, IL, USA). Urinary creatinine was assessed on ARCHITECT by Jaffe, rate blanked and compensated assay. The statistical evaluation was performed with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK). The study was carried out in accordance with the Declaration of Helsinki and under the terms of all relevant local legislation.

Results: No statistically significant correlation was found between whole blood NGAL and either urinary NGAL (r=-100; p=0.636), or urinary NGAL after correction for urinary creatinine (r=-0.129; r=0.540), whereas a significant correlation was observed between urinary NGAL and urinary NGAL corrected for urinary creatinine (r=0.797; $p<0.001$).

Conclusions: There is still controversy as to whether the ideal biological matrix for NGAL assessment should be urine instead of blood, serum or plasma. The former biological matrix presents several advantages, including the higher increase of concentration during progression to AKI as well as the overall better diagnostic performance as recently assessed in a large meta-analysis of published studied. The use of blood, serum or plasma might be however more advisable in anuric patients, and does not obviously require the adjustment for urinary creatinine concentration. Regardless of which is to be considered the most suitable sample between blood and urine and despite the limited number of subjects included in this study, these results demonstrate a lack of correlation between the concentration of NGAL as measured in paired blood and urine specimens in a population of patients admitted to the emergency department for polytraumas. Further studies are indeed warranted to establish how the relationship between blood and urinary NGAL might vary in health and disease.

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Comparison between two diagnostic criteria for Gestational Diabetes Mellitus in women assisted at Clinical Analysis Laboratory in Brazil

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Introduction: Gestational Diabetes Mellitus (GDM) is defined as glucose intolerance first diagnosed during pregnancy, which may or not persist after childbirth. The screening test for gestational diabetes should be performed at the first visit of prenatal care in pregnant women with risk factors, using standard criteria for diagnosis. In pregnant women whose presence of diabetes is unknown, screening tests should be started between 24 and 28 weeks gestation, using the oral glucose tolerance test with 75-g (OGTT). In 2010, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) adopted new criteria for diagnosis and classification of hyperglycemia during pregnancy. The diagnosis of GDM is confirmed by the 75-g OGTT when one of plasma glucose value overtakes: fasting plasma glucose ≥ 92 mg/dL, 1-hour ≥ 180 mg/dL or 2-hour ≥ 153 mg/dL. In Brazil, some services still use the old criteria of the Brazilian Diabetes Society (BDS) for diagnosis GDM (fasting plasma glucose ≥ 110 mg/dL or 2 hours after 75-g of glucose overload ≥ 140mg/dL). *Objectives:* To compare two diagnostic criteria for Gestational Diabetes Mellitus (IADPSG and old BDS) in women assisted at Clinical Laboratory in Brazil.

Material and Methods: We evaluated 1154 pregnant women with mean age of 27 years (range: 14 - 44 years) assisted in a Clinical Analysis Laboratory, that provides service to a segment of the public health system in Brazil during 2011. The patients were submitted to a 75-g oral glucose tolerance test (OGTT) in the times zero (fasting plasma glucose), one hour (1-h) and two hours (2-h) according to medical request for GDM diagnosis. The 75-g glucose overload was performed by a commercial product (Glutol - lemon flavored, ready to use). The plasma glucose was measured by Hexokinase method - Dimension. To assess the degree of agreement between diagnostic criteria was used the Kappa method as statistical analysis.

Results: Using the criteria of IADPSG we identified 94 (8.1%) patients with GDM through fasting plasma glucose, 67 (5.8%) by the 1-h post overload and 113 (9.8%) by the 2-h value post 75-g glucose overload. We detected 189 (16.4%) women with GDM by one or more altered plasma glucose values. Using the old BDS criteria, we identified 8 (0.7%) patients with GDM through the fasting plasma glucose and 207 (17.9%) by the 2-h post 75-g glucose overload. The agreement the two diagnostic criteria for GDM low (kappa = 0.02).

Conclusions: The low agreement between the two diagnostic tests for GDM supports the need for more investigations about its diagnosis, and targeting the maternal/fetal health and the future development of diabetes mellitus. In 2011, the BDS has adopted the IADPSG criteria for diagnosis of GDM. The standardization of the GDM diagnostic criteria is important for making comparisons among different regions and to analyze temporal tendencies. Also, the correct diagnosis of GDM is important because early interventions can lessen the adverse effects of pregnancy.

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Decreased resistin and VCAM-1 levels in Gilbert's syndrome: A possible prevention against atheroclerosis?

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Background: Individuals with Gilbert's syndrome (GS) were found to have mild elevations in fasting serum unconjugated bilirubin concentrations and a marked reduction in coronary heart disease risk. However, the mechanism of decreased frequency of atherosclerotic disease in GS was not entirely known. The aim of this study to examine resistin, sVCAM-1, sICAM-1 and hs-CRP levels in individuals with GS.

Methods: A total of forty male patients with Gilbert's syndrome referred to the outpatient clinic of the Department of Gastroenterology, Gulhane School of Medicine were enrolled to the study. Age, sex and body mass index (BMI) matched forty male healthy volunteers were studied as control group. The diagnosis of GS was made by unconjugated hyperbilirubinemia (> 17.1 μmol/L) on at least two occasions. In this case control study, we investigated the circulating resistin, sVCAM-1, sICAM-1 levels with ELISA method and hs-CRP levels in individuals with Gilbert's syndrome who had no confounders such as hypertension, diabetes mellitus, obesity or dyslipidemia and controls.

Results: Age, gender and BMI distributions were similar between the two groups. HDL-C were higher in subjects with GS when compared to controls p=0.005). Resistin, sVCAM-1, and hs-CRP levels were lower in GS than the healthy controls (p<0.001, p=0.001, and p<0.001 respectively). Resistin, sVCAM-1 and hs-CRP were negatively correlated with unconjugated bilirubin (r= -0.474, p<0.001; r=-0.362, p=0.001; and r= -0.565, p<0.001 respectively). HDL-C levels were positively correlated with unconjugated bilirubin (r= 0.308, p=0.005).

Conclusions: In this preliminary study, the data suggest that reduced resistin, sVCAM-1 and hs-CRP levels may have a role in the mechanism of protection against atherosclerosis in Gilbert's syndrome.

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Has the Incidence of Intravenous Contrast Induced Nephropathy Been Overestimated?

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Background: Administration of iodinated contrast media has been causally associated with the development of transient, acute kidney injury (AKI), known as contrast induced nephropathy (CIN), particularly among individuals with pre-existing renal dysfunction. In an effort to reduce the incidence of CIN, providers routinely screen patients by measuring serum creatinine (SCr) before administering contrast. This practice frequently results in withholding contrast in patients with elevated SCr and other risk factors, often at the expense of diagnostic accuracy and clinical care. Notwithstanding the widely held belief in the nephrotoxic potential of iodinated contrast, the true incidence and clinical significance of CIN is still debated, predominantly because of a lack of controlled studies in the field. In the current study we sought to compare the incidence of AKI between contrast-enhanced and noncontrast CT scan recipients using a rigorous statistical analysis of SCr trends over time while accounting for presumed CIN risk factors.

Methods: All abdominal, pelvic, and thoracic CT scans performed at our institution between 2000 and 2010 were identified. Scans were stratified into those where intravenous iodinated contrast was (contrast group) and was not (noncontrast group) administered. Patients were stratified by baseline SCr into low (<1.5mg/dL), medium (1.5-2.0 mg/dL), and high (>2.0 mg/dL) risk groups. Post-scan temporal trends in SCr were analyzed using sixteen interpolation models of summary measures of area under the SCr-time curve (SCr_{AUC}), maximum post-scan SCr (SCr_{MAX}), and intra-individual SCr variation (SCr_{VAR}). AKI was defined as an increase in SCr ≥ 0.5mg/dl. Incidence of AKI was determined using Cochran Mantel Haenszel estimates.

Results: We identified 315,612 scans among 106,342 unique patients with 4,714,988 SCr results. Summary measure analysis revealed that the contrast group experienced greater declines in SCr (SCr_{AUC}; p < .0001) and smaller SCr_{MAX} increases (p < .0001) following scanning compared to the noncontrast group. In both groups SCr_{VAR} increased with declining baseline renal function and was highest among individuals with AKI. After adjusting for baseline renal function, AKI incidence was lower among the contrast group compared to the noncontrast group (O.R.=0.70 (95% CI 0.61-0.82), p < .0001).

Conclusions: Contrast-enhanced CT scan recipients are, paradoxically, at a lower risk of SCr-defined AKI as compared to noncontrast CT scan recipients, even after accounting for baseline renal function. Increased pre-scan SCr variability is associated with an increased incidence of post-scan AKI independent of contrast administration, suggesting that cases of CIN may be partially explained by dynamic changes in renal function. These results question the utility of SCr as a marker for CIN, as physiologic variability in this analyte likely confounds its diagnostic accuracy, contributing to a high false positive rate and resulting in an overestimation of the incidence of contrast-dependent renal injury.

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Association of Tumor Necrosis Factor-alpha and oxidative stress in diabetic nephropathy

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Background: Chronic kidney disease (CKD) is a major cause of morbidity and mortality worldwide. It results from varied etiologies, diabetes being a frontrunner amongst them. Type 2 diabetes mellitus (DM) is a proinflammatory state with increased oxidative stress which could tremendously increase the risk of micro and macrovascular complications. Diabetic nephropathy is the commonest chronic complication of DM and the leading cause of end-stage renal disease. This study was planned to explore the relationship between tumor necrosis factor-alpha (TNF-α), high-sensitivity C-reactive protein (hsCRP) and different oxidative stress parameters in Type 2 diabetes (DM), diabetic and non-diabetic chronic kidney disease (DM-CKD and non DM-CKD).

Methods: Age and sex matched patients of DM, DM-CKD, non DM-CKD and healthy controls (n=50 each) were recruited from Diabetes and Nephrology Clinics and staff of UCMS & GTB Hospital. Plasma TNF-α and hsCRP levels as inflammatory mediators were measured by ELISA. Blood glutathione (GSH), plasma glutathione-S-transferase (GST), ferric reducing ability of plasma (FRAP) as antioxidant parameters and malondialdehyde (MDA) as marker of oxidative stress were measured spectrophotometrically.

Results: Mean plasma TNF-α (pg/mL) and hsCRP (mg/L) levels were significantly higher in DM-CKD (p<0.01) compared to other study groups. Lipid peroxidation, measured as MDA (nmol/mL) was highest in patients with DM-CKD, 5.19±0.39; followed by non DM-CKD, 3.68±0.29; and DM, 2.60±0.35 (p<0.01), whereas antioxidant capacity of blood measured as FRAP (μmol/L) showed a progressive decline in patients with DM, 409±55.54; non DM-CKD, 256.8±44.12; and DM-CKD, 170.78±142.27 respectively (p<0.01) as compared to healthy controls. Similarly, GSH (mg/g Hb) showed a progressive decline in patients with DM, 1.89±0.13; non DM-CKD, 1.53±0.12; and DM-CKD, 0.90±0.17 (p<0.01). Activity of GST (nmol/min/mL) showed a different pattern with respect to other oxidative stress parameters, being highest in non DM-CKD (p<0.01). Moreover TNF-α correlated positively with hsCRP (r = 0.400; p<0.01) and MDA (r = 0.423; p<0.01) and negatively with GSH (r = -0.370; p<0.01) and FRAP (r = -0.344; p<0.01).

Conclusions: The present study suggests that oxidative stress is highest in DM-CKD as compared to other study groups, possibly due to additive effects of DM and CKD, since both these diseases are associated with impairment of the oxidant-antioxidant equilibrium. However, there are subtle alterations in the behavior of antioxidant parameters in all study groups, suggesting different etiology of oxidative stress. Increased TNF-α and hsCRP levels reveal that hyperglycemia leads to the activation of certain signaling pathways which in turn cause inflammation. Hence, interplay between inflammation and oxidative stress may play equally important role in the pathogenesis and progression of diabetic nephropathy.

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Psychosis susceptibility gene ZNF804A and anti-psychotic response in schizophrenic patients

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Background: The high-risk ZNF804A variant has an impact on brain functional dysconnectivity between dorsolateral prefrontal cortex (DLPFC) and hippocampal formation during an N-back memory task in healthy subjects. This altered connectivity between DLPFC and hippocampal formation might be a basis of human

memory function. Patients with schizophrenia have pronounced deficits in the aspects of neurocognitive function such as speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving and social cognition. Memory deficits are prominent trait markers of schizophrenia with impairments also observed in first-degree relatives. Previous studies showed that ZNF804A gene variant has a pharmacogenetic importance. We therefore replicate the same study in North Indian schizophrenia patients to identify whether this gene variant influences the response of positive or negative schizophrenia symptoms to antipsychotics.

Methods: 50 unrelated schizophrenia patients (30 male and 20female; mean age: 52.8±11.6 years) and 80 normal controls (42 male and 38female; mean age: 54.9±6.9 years) were enrolled in our study. Diagnosis of schizophrenia patients was according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria with consensus based on the clinical interviews and case records. We assessed the response of positive and negative symptoms by PANSS at the start of treatment and after six weeks. PCR-Snap Shot technique was used for genotyping.

Results: Significant differences were seen in the genotype distribution ($\chi 2 = 6.10$, d.f. = 2, $p = 0.04$) and allele frequencies ($\chi 2 = 5.14$, d.f. = 1, $p = 0.02$; odds ratio = 0.57 95% confidence interval =1.09-3.48) between the patients and controls.

Conclusions: A significant ZNF804A (rs1344706 T>G) genotype and allelic frequency was found in North Indian patients having schizophrenia. Patients with high risk T/T genotype showed significantly poorer improvement of positive symptoms (p =0.03) compared to patients with a protective allele (T/G and G/G).

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One year follow up of Lebanese smokers using Nicotine Metabolites and C-Reactive Protein.

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Background: Nicotine metabolite (NM), such as cotinine is tobacco specific and has a long half-life; its measurement is used for smoking detection. Serum NM, C-reactive protein (CRP) and respiratory function (FEV1/FVC) were monitored in Lebanese smokers enrolled in a smoke cessation program.

Methods: The study included 295 adult smokers (males:160 and females:135; age:18-88 years). Behavioral therapy, respiratory function and Fagerstrom questionnaire for nicotine dependence score (NDS) were assessed. Nicotine replacement therapy (NRT) was administered for 6 weeks depending on baseline serum NM determined by Siemens Immulite 2000. The analytical range was 10-500 ng/ml; a level below 25 ng/ml indicates non-smoker. Precision was 11.5% and 7.4% at 14.5 and 49.2 ng/ml, respectively. Recovery was 92.8%. CRP was determined using Siemens BNProspec with a range of 0.16-10.2 mg/L and precision of 7.1% and 7.4% at 0.475 and 5.59 mg/L, respectively. Patients were followed at one month, three months and one year. All measurements were done at baseline and one year.

Results: NDS highly correlated to NM levels at baseline (r= 0.435, p= 0.0001). 98 out of 295 smokers, continued the one year follow up: 75 (25.4%; male/female = 36/39; age: 18-72 years) did not quit and 23 (7.8%; male/female = 11/12; age: 18-67) succeeded in quitting. Non-quitters and quitters had NDS distributed as: mild/moderate/severe 14/38/23 and 8/11/4, respectively. Descriptive statistics are shown in the table below (WRST: Wilcoxon signed rank test; *statistically significant p<0.05).

	Non-quit Median (Minimum - Maximum)	Quit Median (Minimum - Maximum)	WRST, p
Baseline NM (ng/ml)	359 (11-923)	262 (10-566)	0.018*
1 year NM (ng/ml)	348 (40-675)	10 (10-24)	0.0001*
WRST (NM: baseline vs 1 year), p	0.121	0.0001*	
Baseline CRP (mg/L)	1.43 (0.15-19.6)	1.56 (0.2-16.6)	0.879
1 year CRP (mg/L)	1.62 (0.2-16.4)	1.88 (0.2-8.4)	0.941
WRST (CRP: baseline vs 1 year), p	0.710	0.119	
Baseline FEV1/FVC (%)	72 (40-90)	72 (46-88)	0.747
1 year FEV1/FVC (%)	71 (44-97)	70 (52-85)	0.322
WRST (FEV1/FVC: baseline vs 1 year), p	0.799	0.064	

Conclusion: NM levels correlated significantly with NDS. No difference was observed at one year for CRP and lung function in quitters versus non-quitters. NM level in non-quitters was the same at baseline and one year. Quitters had significantly lower baseline NM level. Consequently, serum NM is a useful tool in smoke cessation programs, and more specifically to identify smokers with higher probability of quitting.

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Effectiveness of Vitamin D2 versus Vitamin D3 in the Treatment of Pre-dialysis Chronic Kidney Disease Patients following Kidney Disease Outcomes Quality Initiative (K/DOQI) Guidelines

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Background: Vitamin D deficiency is very common in the pre-dialysis chronic kidney disease (CKD) population and is treated according to the 2003 K/DOQI Clinical Practice Guidelines for Bone Metabolism and Disease in CKD. These guidelines recommend administration of a variable high dose vitamin D_2 regimen based on the severity of vitamin D deficiency. Some studies have shown that these guidelines may not be adequate, and that vitamin D_3 therapy may be better than vitamin D_2. In this study, our objectives were to evaluate the current K/DOQI guidelines for the effectiveness in treating hypovitaminosis D in CKD patients, and to compare it with the effectiveness of vitamin D_3 therapy following the same guidelines.

Methods: All subjects (n=16) enrolled after consent were adults with stage 3 or 4 CKD and vitamin D deficiency (25-hydroxyvitamin D [25OHD] < 30 ng/mL). Subjects were randomized to receive either vitamin D_2 or vitamin D_3 50,000 IU once per week for 4 weeks then monthly thereafter for those with 25OHD between 5 and 15 ng/mL or 50,000 IU once per month for those with 25OHD between 15 and 30 ng/mL. Subjects were followed for a total duration of 6 months of treatment. The treatment groups were balanced (n = 8 in each treatment arm). The 25OHD was measured by an FDA-approved chemiluminescence immunoassay. The primary endpoint was percentage of subjects with vitamin D in normal range (25OHD > 30 ng/mL) after 6 months of treatment. The study was double blinded and patients were enrolled over the course of 10 months.

Results: Only 50% of subjects in either treatment groups reached the 25OHD level > 30 ng/mL after 6 months of treatment. Two subjects in each treatment group had no response, defined as < 5 ng/mL increase.

Conclusions: The 2003 K/DOQI guidelines were found inadequate in treating vitamin D deficiency in pre-dialysis CKD subjects with stage 3 or 4 CKD. There was no significant difference in percentages of vitamin D deficiency correction in this patient population between vitamin D_2 and vitamin D_3 treatments at the end of 6 months following K/DOQI guidelines. Further statistic analysis to compare the two groups at multiple points during treatment will be performed after unblinding the two groups upon completion of the study.

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Glial Fibrillary Acidic Protein (GFAP) levels on admission differentiate between ischemic stroke (IS) and intracerebral hemorrhage (ICH) and correlate with severity and outcome among ICH patients

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Background: A simple and accurate method of differentiating ischemic stroke (IS) from intracerebral hemorrhage (ICH) could facilitate acute therapeutic management. GFAP is a highly brain-specific intermediate filament protein maintaining astroglial cell structure and migration. Under physiological conditions, GFAP is not actively secreted from cells, and is typically not detectable in the plasma of healthy individuals. Studies have shown that GFAP is released rapidly in acute ICH, whereas a more delayed release can be observed in patients with ischemic stroke. This delayed release reflects the more gradual occurrence of necrosis and cytolysis found histopathologically in IS, in contrast to the immediate cell destruction in case of ICH. GFAP may therefore function as a biomarker indicating ICH in patients with symptoms indicative of acute stroke. In this study we tested the diagnostic accuracy of plasma GFAP, as measured by biochip array technology, for the differentiation between IS and ICH and its ability to serve as predictor of severity and outcome.

Methods: In a prospective study we included 98 patients with acute stroke, 73 IS and 25 ICH. Twenty healthy individuals served as controls. Blood samples were taken at the time of admission and at 24, 48 and 72 hours thereafter. A final measurement was

performed on day 7. The mean time from the onset of stroke symptoms and hospital admission was 3.2 hours. Stroke severity was measured at the time of admission with the Scandanavian Stroke Scale (SSS). Functional outcome was measured with the modified Rankin scale (mRS) on day 7 and acute stroke patients were categorised into three severity groups (mild, moderate and severe) according to their mRS-score: mild (mRS-score:0-2), moderate (mRS-score:3-4) and severe (mRS-score:5-6). GFAP levels were quantified in EDTA plasma samples employing Randox-BAT technology on the Evidence Investigator analyser.

Results: The mean age (SD) of the patients was 75.2 (9.4) years. Forty-two patients (42.86%) died during a follow-up period of 1 year. The mean time (SD) between the onset of neurological symptoms and hospital admission was 3.22 (1.58) hours. At admission, mean plasma GFAP levels were significantly elevated in ICH (10.40ng/ml) compared to IS patients (0.17ng/ml) and healthy controls (0.12ng/ml) (P<0.0001 anova-test). The diagnostic accuracy of a single GFAP measurement upon hospital admission for the differentiation between patients with ICH and those with IS, is high [AUC=0.87 (95%CI 0.72-0.95), P<0.0001]. Further analysis revealed that GFAP values within the ICH group were significantly higher among patients with a higher severity score (14.91ng/ml) compared to moderate (1.29ng/ml) and mild (0.37ng/ml) groups (p<0.0001 anova-test). No such differences were observed within the IS group 0.21 vs 0.17 vs 0.14ng/ml respectively). Furthermore, within the ICH group GFAP levels were significantly lower among survivors (1.27ng/ml) when compared to non-survivors (14.70ng/ml) (p<0.005) while no such differences were observed within the IS group of patients (0.17 and 0.16 respectively).

Conclusions: Our data suggest that the determination of GFAP levels upon admission can be used to differentiate between IS and ICH as well as serve as a predictor of severity and mortality among ICH patients.

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Diagnosis of subarachnoid hemorrhage by spectrophotometric detection of blood-derived pigments in the intrathecal space

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Background: Blood leakage occurs in subarachnoid hemorrhage (SAH) into the space between the arachnoidea and the soft membrane (pia mater) surrounding the brain. The single most important symptom of SAH is a sudden severe headache. A large number of headache patients seek medical attention in the emergency departments, but only a small part turn out to have SAH or the so-called warning leak into the intrathecal space. Mortality in SAH is as high as 40-50 %. Accurate, immediate diagnosis is therefore imperative to expedite the endovascular or surgical treatment of the possible arterial aneurysm. Computed tomography (CT) of the head is the principal diagnostic method but may give a false negative result in small warning leaks or if the patient is coming to the emergency department after a delay of days or even weeks. Spectrophotometric analysis of the cerebrospinal fluid (CSF) is a sensitive diagnostic technique under these conditions.

Methods and Results: We report a series of 772 spectrophotometric CSF analyses performed in possible SAH cases in the Helsinki University Central Hospital during 2007-2011. Physicians had been instructed to submit CSF samples for spectral analysis when the patient was suspected to have SAH or a warning leak despite negative or unclear CT findings, or when it was deemed necessary to rule out an underlying SAH in unequivocal or nonspecific symptomatic clinical diagnoses such as sudden headaches. We used for the analyses an autoscaling Agilent 8453 spectrophotometer by scanning the absorbance between 600 and 350 nm. The concentrations of bilirubin (peak absorbance at 450-460 nm) and oxyhemoglobin (peak absorbance at 415 nm) were determined. In SAH, the CSF concentrations of both bilirubin and oxyhemoglobin are usually elevated. Elevated bilirubin is clinically significant even alone, but small elevations of oxyhemoglobin alone usually suggest a puncture artifact. The upper reference limit for bilirubin in the CSF is 0.17 micromol/l; we could reliably assay bilirubin concentrations well below that. In the majority of the cases (90 %) the result was clearly negative. In thirtythree cases (4.3 %) the diagnosis of SAH was made. Possible SAH was encountered in fifteen additional cases (1.9 %). In 20 additional cases (2.6 %) CSF bilirubin was increased but was explained by the increased level of CSF protein or serum bilirubin or both.

Conclusions: Visual examination of the valuable CSF sample, only performed by many laboratories, is much less sensitive than the spectrophotometry and is discouraged.

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Search for a novel salivary biomarker candidate for with chronic fatigue syndrome

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Objective: Identification of a salivary biomarker candidate that could be explored further for its ability to diagnose chronic fatigue syndrome (CFS). Relevance to clinical laboratory medicine: At present, there is no objective method to diagnose CFS. The current method of diagnosis requires the physician to rule out all other possible causes of chronic fatigue before assigning a diagnosis of CFS. An objective and rapid method to diagnose CFS would reduce the time required to arrive at a definitive diagnosis.

Methods: Saliva was obtained from an archive of samples originally collected as part of a study conducted in the State of Georgia, USA by the Centers for Disease Control and Prevention (Reeves, et al. BMC Health Services Research, 2009, 9:13) to determine prevalence of CFS in the community. Briefly, 10,837 households were contacted through telephone survey. A total of 780 individuals participated in clinical assessment. Based on clinical assessment, 112 participants were found to have CFS. Healthy controls were composed of 147 subjects from the study that did not report signs of fatigue on initial interview and later were found to be free of symptoms of CFS or other significant medical conditions. As part of the clinical assessment saliva samples were obtained for biochemical assessment. Saliva samples were maintained at -80C until evaluated. Raw saliva samples were first processed by spin-filtering to acquire the small-molecular-weight (sMW) fraction (nominally, <5kDa). An analysis of ionizable components in the sMW fraction was performed using liquid chromatography with mass spectrometric detection (LC-MS). LC-MS results were compared first within groups to identify ions common to each group, i.e. a significant ion peak present at a particular combination of retention time and mass-to-charge ratio. A list of promising biomarker candidates was reduced to a single biomarker of interest based upon the significance level of the difference observed for a non-parametric comparison of the group samples. This biomarker candiate was found to be 3 times more abundant in CFS subjects than controls (p<0.001, Wilcoxon rank sum test). The Receiver Operating Characteristic (ROC) Area-Under-the-Curve (AUC) is 0.94 (95% CI 0.86 to 0.98). High-resolution mass spectrometry was used for chemical identification of this candidate as a 2.6kDa peptide of the 42-kDa basic Proline-Rich-Protein 4 (PRB4), a known salivary protein. MS-fragmentation pattern and retention time of a synthesized peptide are identical to the native biomarker candidate identified in saliva, confirming its identity.

Conclusions: We have identified a 2.6kDa peptide of salivary protein PRB4 associated with CFS patients compared with healthy controls. The clinical significance and reproducibility of this finding can now be explored in future studies by comparing levels of the specific candidate biomarker in a blinded fashion in a variety of patients populations, including those with other fatiguing illnesses.

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Characterization of biochemical parameters, trace elements and hormones concentrations from a healthy elderly population of a medical school hospital.

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Background: Nowadays, it has been seen the phenomenon of aging population in worldwide. However, there are few data about reference values to be used in laboratory tests for elderly. This fact encouraged us to evaluate some laboratory parameters concentrations present in blood samples from ambulatory elderly people.

Methods: A group of elderly population without clinical evidence of serious chronic diseases was evaluated. The study was submitted and approved by our Internal Review Board (IRB). The patients were selected based on the SENIEUR protocol

(*SENIor EURopean Protocol*). The blood samples of 120 elderly people (34 men and 86 women), aging 72 ± 8 years, were analyzed. The blood, after 12 hours fast, was collected by venipuncture using sterile standard metallic needles. It was collected in two types of evacuated tubes (Vacutainer Systems - Becton Dickinson, EUA): SST II *Advance* gel and clot activator tube and a specific tube for trace elements analysis, without heparin. Biochemical analyses were carried out on Roche/Hitachi MODULAR *ANALYTICS* PP (Roche Diagnostics GmbH, Germany). The neutron activation analysis (NAA) was applied for trace elements determination. Short and long irradiations were carried out under a thermal neutron flux of about 4 x 10^{12} n cm^{-2} s^{-1} for Br, Rb, Se and Zn determinations. After adequate decay times, the irradiated samples and standards were measured using a Hyperpure Ge detector Model GX2020 coupled a gamma-ray spectrometer. The radioisotopes measured were identified according to their half-lives and gamma-ray energies and the element concentrations were calculated by comparative method. The thyroid hormones were evaluated on AutoDELFIA automatic immunoassay system (Perkin Elmer, USA). The HPLC method certified by NGSP was used to measure the glycated hemoglobin level on Variant II turbo (Bio-Rad Laboratories, Inc., USA).

Results: Biochemical parameters: uric acid: 5.1±1.4 mg/dL, total bilirrubin:0.71±0.26 mg/dL, Na:141±3 mEq/L, K:4.5±0.4 mEq/L, Ca:9.5±0.5 mg/dL, ionized Ca:5.1±0.5 mg/dL, P: 3.5±0.5 mg/dL, Mg:2.10±0.28 mg/dL, glucose:93±10 mg/dL, glycated hemoglobin:5.7±0.5%, urea:37±13 mg/dL, creatinine:0.84±0.19 mg/dL, Fe:105±31 µg/dL, total iron-binding capacity:301±38 µg/dL, ferritin:183±155 ηg/mL, total protein:7.3±0.5 g/dL, albumin:4.4±0.3 g/dL, total cholesterol:211±36 mg/dL, HDL-cholesterol:59±15 mg/dL, LDL-cholesterol:128±32 mg/dL, triglycerides:122±61 mg/dL, AST:22±7 U/L, ALT:20±11 U/L, alkaline phosphatase:80±25 U/L and GGT:23±13 U/L. Trace elements: Br: 3.46± 0.85 mg/L, Rb: 320.0± 56.9 µg/L, Se: 77.0±25.3 µg/L and Zn 95.2±13.6 µg/L. Thyroid hormones: TSH:2.09±1.60 µU/mL, total thyroxine:8.9±1.7µg/dL, total triiodothyronine:122±19 ηg/dL and free thyroxine:1.08±0.21ηg/dL.

Conclusions: The results suggest the need to establish specific reference values for elderly. The population evaluated do not present deficiency or excess of trace elements.

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Lower sex hormone-binding globulin (SHBG) is independently associated with metabolic syndrome in middle-aged and elderly men in China

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Objective: A low level of testosterone in men has been shown to be related with an increased risk of metabolic syndrome. Free testosterone, the main active form of testosterone, is not always tested directly. Because sex hormone-binding globulin (SHBG) increases with age while testosterone declines, we examined the relationships between SHBG and the metabolic syndrome in middle-aged and elderly men in China.

Methods: A cross-sectional study was done among 437 men, aged 45 to 94, from the health checkup population of Zhongshan Hospital Fudan University. Patients who were under treatment with hormone replacement therapy (HRT), or diagnosed with thyroid disease, chronic renal failure, chronic hepatopathy or cancer, were excluded from the study. Early morning fasting sera were assayed for total testosterone, SHBG, and other biochemical markers such as fasting glucose, fasting insulin, TC, HDL-C, LDL-C, TG. SHBG was measured using a chemiluminescent immunoassay (Beckman Coulter). Free testosterone was calculated using the Vermeulen equation (Vermeulen, JCEM 1999). Complete medical history was taken and reviewed for each patient.

Results: Metabolic syndrome was defined using the criteria of the Chinese Diabetes Society (CDS 2004). There were 82 men with metabolic syndrome (18.7%). The SHBG level of the metabolic syndrome group was significantly lower (39.27±26.54nmol/L, p=0.030) than that of non-metabolic syndrome group (45.49±20.32nmol/L). SHBG correlated significantly with systolic BP (Spearman r=-0.020), diastolic BP(r=-0.100), BMI(r =-0.350), and FBG(r=-0.096), with all p-values < 0.05. Analyzing the effect of SHBG on parameters of the metabolic syndrome in a linear multivariate regression analysis, adjusted for age and insulin resistance index (HOMA-IR = Fasting Glucose (µU/ml) ×Fasting Insulin (mmol/L)/22.5), SHBG was significantly and inversely associated with systolic BP, diastolic BP, BMI and FBG, while HDL-cholesterol and triglycerides showed no significant relationship to SHBG after adjusting for HOMA-IR and age. In a logistic regression taking metabolic syndrome as the dependent variable, age, SHBG and HOMA-IR were included in the final model with statistical significance.

Conclusions: Lower SHBG is independently associated with metabolic syndrome among middle-aged and older men. SHBG may be an independent predictor of metabolic syndrome, but the mechanism of how SHBG is involved in the metabolic syndrome needs to be further studied.

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Nuclear factor-kappa B activity in the peripheral blood lymphocytes measured with the novel quantitative system using the fluorescent correlation spectroscopy correlates with intra-abdominal fat area in patients with essential hypertension

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Background: Etiology of essential hypertension is extremely complex, and obesity and insulin resistance are its important risk factors. They are now widely recognized as chronic inflammatory diseases. The transcription factor, nuclear factor-kappa B (NF-κB) is known as a key regulator of many inflammatory processes. NF-κB regulates the expression of various inflammatory cytokines involved in metabolic diseases. We hypothesized that NF-κB activity could be the useful indicator of systemic 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 fluorescent-labeled molecules in a confocal area on the basis of the fluctuation of fluorescent intensity by their Brownian motion in solutions. The principle of NF-κB quantitation was to analyze the difference of the fluctuation of fluorescent intensity between the fluorescent-labeled DNA probe and fluorescent-labeled DNA probe NF-κB complex. 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/1.73m2), 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: 0.18±0.16 ng/µg of nuclear protein) was positively correlated with daytime systolic blood pressure, diastolic blood pressure, serum total cholesterol levels, triglyceride levels, serum lactate levels (The Pearson correlation coefficients = 0.319, 0.357, 0.310, 0.428, 0.438, and P values = 0.047, 0.026, 0.038, 0.003, 0.004, respectively). Multiple stepwise regression analysis revealed that intra-abdominal fat area was independently correlated with NF-κB activity (p< 0.05, R2=0.66, AICc=-9.86). Eighteen out of 45 patients were diagnosed with metabolic syndrome. NF-κB activity in patients with or without metabolic syndrome was 0.26±0.19 and 0.12±0.12 ng/µg of nuclear protein, respectively. In logistic regression analysis, NF-κB activity was significantly correlated with patients with metabolic syndrome ($\chi 2$=9.42, p<0.05).

Conclusions: These results suggest that the elevation of NF-κB activity reflects the systemic inflammation caused by active visceral fat 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.

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Assessment of Cystatin C as an Index of Allograft Function in Kidney Transplantation

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Background: Management of renal transplant patients requires periodic measurement of renal function, which is usually assessed by measuring the glomerular filtration rate (GFR). Serum concentration of Cystatin C (CyC) has been proposed as a marker to determine a reduced GFR, but the data of its value in renal transplant patients are conflicted. Urine CyC can be considered a marker of proximal tubular function and has been described its utility for predicting allograft function. This prospective observational study aimed to assess the relevance of serial postoperative serum and urinary CyC measurements for predicting allograft function after kidney

transplantation.

Methods: Serum and urine CyC levels were measured by particle-enhanced nephelometric immunoassay using a BN II nephelometer in 50 patients scheduled for kidney transplantation from living donors, immediately before and after surgery, and at day 1, day 7, month 1 and month 3. Serum creatinine levels were analysed by modified Jaffe method in Cobas 8000 analyser. GFR was estimated by Modified Diet in Renal Disease (MDRD) equation. Data were expressed as mean ± Standard errors. SPSS was used to statistical analysis (SPSS for Windows, 16.0).

Results: Mean serum and urine CyC, creatinine levels and GFR values were statistically different among these patients at all time points. Of the 50 patients enrolled, all had a high pretransplant serum CyC (5.93 ± 0.26 mg/L) and urinary CyC (5.00± 0.74 mg/L) correlated significantly with GFR (r= -0.709, r= -0.547, p <0.001, respectively). Serum and urine CyC levels demonstrated consistent significantly decreases after renal transplantation while GFR values had consistent increases. Most strong correlation was determined between serum and urine CyC with GFR at day 7 (r= -0.814, r= -0.602, p<0.001, respectively).

Conclusions: We conclude that CyC is an efficacious marker as much as creatinine to assess renal function. Urinary CyC levels return to lower levels as the filtered load and tubular function improves. These findings suggest that increased serum and urinary CyC levels early and accurately predicted allograft function after renal transplantation.

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Validation of the ADVIA Centaur Syphilis Assay on the ADVIA Centaur System

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Background: Syphilis is caused by the spirochete *Treponema pallidum*. It is primarily transmitted via sexual contact but can also be transmitted vertically to the fetus or through direct exposure to blood. Because many individuals with syphilis have no symptoms, many clinicians recommend routine syphilis testing for patients at risk. Syphilis is infectious mostly during the primary, secondary and early latent stages. During the latent stage, syphilis may progress into a tertiary infection if untreated. It is at this stage that syphilis can do the most damage to the body, affecting the brain, blood vessels, the heart and bones. It can eventually lead to death.[1,2]

Siemens Healthcare Diagnostics has developed an assay on the ADVIA Centaur® family of instruments for the qualitative detection of antibodies to *T. pallidum* in human serum and plasma. The assay is intended for use as an aid in the diagnosis of syphilis infections.*

In this study, we evaluated the performance of the ADVIA Centaur Syphilis Assay. The performance evaluation included a reproducibility study on the ADVIA Centaur system, a method comparison study versus the IMMULITE 2000® predicate assay, and an analytical sensitivity study using WHO materials.

Methods: Analytical sensitivity was determined in-house on the ADVIA Centaur system using two lots of WHO materials: 05/132 (1st IS for human syphilitic plasma IgG and IgM) and 05/122 (1st IS for human syphilitic plasma IgG). Reproducibility studies using two reagent lots, quality control materials, and serum sample pools, and a method comparison study comparing the ADVIA Centaur Syphilis Assay to the predicate IMMULITE 2000 Syphilis Screen Assay, were performed at three external clinical trials: two U.S. sites and one European site. Clinical samples were from high-risk populations, including HIV-positive patients.

Results: Based on internal evaluation, the estimated sensitivity at cutoff was 1.86 mIU/mL for WHO 05/132 (IgG/IgM) and 0.71 mIU/mL for WHO 05/122 (IgG)[†]. Reproducibility studies showed a total CV between 2.2% and 8.8%, and a within-run CV between 1.1% and 3.6%, depending on the sample. The negative percent agreement of the ADVIA Centaur Syphilis assay compared to the comparative assay was 99.4% and the positive percent agreement of the ADVIA Centaur SYPH assay compared to the comparative assay was 97.9%.[‡]

Conclusions: The results of this preliminary evaluation indicate that the ADVIA Centaur Syphilis Assay is a precise immunoassay for the detection of antibodies to *T. pallidum* and demonstrates good performance.

* In the U.S., the assay is not intended for blood and tissue donor screening.

[†] These data are based on research and are not commercially available.

[‡] Equivocal results obtained on the ADVIA Centaur system were assigned a clinical interpretation opposite that of the comparative assay result.

[1] Centers for Disease Control and Prevention. Syphilis_CDC Fact Sheet. Available at: http://www.cdc.gov/std/syphilis/STDFact-Syphilis.htm.

[2] www.hc-sc.gc.ca.

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Application of differential cutoff levels of Procacitonin might be useful for the detection of bacteremia among the patients of emergency department

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Background: Procalcitonin (PCT) has proved to be a useful diagnostic marker for discriminating bacterial infection from other inflammatory conditions in critically ill patients. Especially, emergency departments (EDs) are often the first point of contact and care for many patients, and early diagnosis of bacteremia is extremely important but a diagnostic challenge.

The aim of this study is to evaluate the usefulness of PCT in patients with bacteremia at emergency department by evaluating the predictive value of PCT at different cutoff levels, and to discriminate the different trends of predictive values according to the patient's age, body temperature, absolute neutrophil count (ANC) and C-reactive protein (CRP) concentrations which indicate bacterial infection.

Methods: We enrolled 3305 cases (between the ages of 20 and 90 years) and retrospectively analyzed their PCT concentrations and blood culture results performed for three years with body temperature, ANC and CRP results. PCT concentrations were measured by VIDAS® B.R.A.H.M.S PCT assay (bioMérieux, Marcy L'Etoile, France). Four levels (0.1, 1, 2, 5 ng/ml) were determined as possible cutoff levels and used for further analysis. The trends of positive predictive value (PPV) and negative predictive value (NPV) at each level were analyzed.

Results: A total of 3305 cases were enrolled in this study: 564 with bacterial infection (positive blood culture without contamination); 2741 with suspected local infection or other diseases (negative blood culture). PPVs of PCT cutoff at 5.0 ng/ml ranged from 27.4% to 41.7% with increasing trend as patients` ages increased, and those of PCT cutoff at 0.1 ng/ml increased from 12.1% to 22.9% according to patients` ages. Patients with neutrophilia (ANC >7,500/mm^3) had higher PPVs at all the cutoff levels of PCT subjects with increased age had higher PPVs. Patients with CRP over 10 mg/dl or body temperature above 38°C had higher PPVs compared to the patients with normal results also. NPVs of four cutoff levels 0.1, 1, 2, 5 ng/ml were 95.1, 92.2, 91.1, 89.0, respectively.

Conclusions: This study shows that PPVs at each cutoff level have increasing trends as the patients' age, body temperature, ANC and CRP increase in the subjects with bacteremia. Therefore, in patients with suspected bacteremia, application of differential PCT cutoff levels in different age, ANC, CRP and body temperature groups could be desirable for effective detection of patients with bacterial infection. Also, less than 0.1 ng/ml of PCT could be used as a cutoff level to rule out bacteremia.

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Utility of Serial Measurement of Serum Butyrylcholinesterase in Acute Poisoning with Organophosphate Compounds

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Background: Retrospective analysis of the utility of serial measurements of serum Butyrylcholinesterase (BChE) in predicting the duration of stay in the intensive care unit (ICU), duration of mechanical ventilation (MV) and outcome of the patient from MV in organophosphate (OP) compound poisoning patients.

Methods: The medical records of patients who presented to tertiary care hospital with symptomatic insecticidal poisoning from January 2009 to December 2010 were utilized for the study purpose. As a protocol in our study, all cases that presented with symptomatic organophosphate poisoning, whose serial BChE activity levels were available and who did not have any other underlying diseases were included in the study. Patients with history of other co-morbid conditions and chronic illness like diabetes mellitus, hypertension were excluded from the study. Data regarding clinical manifestation at presentation, BChE activity results and its interpretation, details of patient management, duration of stay in the ICU, duration of MV and data on outcomes of patients were noted. Statistical analysis was performed using the tabulated data collected from case records of patients with OP

poisoning. One way ANOVA was used to estimate the utility of serial measurements of serum BChE. Reciever Operator Characterisics were constructed to determine the cut off levels of acetylcholinesterse to determine the length of stay in ICU, number of days on MV and the outcome of the patient. Differences were considered significant if the p value was <0.05.

Results: Forty four patients with history of poisoning were admitted between January 2009 and December 2010 with an age range of 02 - 60 years. All of them were adults except a two year old girl where mother had consumed poison and fed it to the baby. Majority of the cases were males (83.8%). 48.6% of the patients were smokers and 45.9% of the patients were alcoholics. The mean serum butyrylcholinesterase levels were 2,346.5 IU/L±2,650.3, 2,161.8 IU/L±2,719.2, 2,648.6 IU/L±2,969.8, 3,104 IU/L±3,279.1, 3,617.2 IU/L±3,460.4 on day 1, day 2, day 3, day 4 and day 5 respectively. Two mg of atropine was given as a test dose followed by 2 mg every 10-20 min as required to keep the patient atropinized. A bolus dose of oxime was instituted in 7 patients. Thirty three patients improved and were discharged from the hospital however; four patients succumbed to the poisonous compound.

Serum acetycholinesterase levels below 1,250IU/L, 1,789IU/L and 2,764IU/L on day 3, day 4 and day 5 respectively indicates longer duration of stay in the ICU. Patients with serum BChE levels below 975IU/L, 876IU/L, 1,245IU/L, 1,395IU/L and 1,875IU/L on day 1, day 2, day 3, day 4 and day 5 respectively take a longer time to be out of MV. Levels below 870IU/L, 1,110IU/L, 1,020IU/L and 885IU/L on day 2, day 3, day 4 and day 5 respectively indicate poor prognosis of the patient and mortality.

Conclusions: We conclude that the serial measurements of serum butyrylcholinesterase levels can be useful in predicting the length of ICU stay, duration of MV and the prognosis of the patient with OP poisoning.

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Association Between Graft Function and Serum Interleukin-18 Levels in Patients with Kidney Transplantation

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Background: Acute graft dysfunction can be caused by ischaemic or immunological injury leading to serious consequences both in the short and long term. IL-18 is primarily a macrophage-derived cytokine; however, its expression has been reported in a wide range of cells. Recent studies have suggested that IL-18 may predict early graft function after renal transplantation. This prospective observational study aimed to assess the relevance of serial postoperative serum IL-18 measurements for predicting graft function after transplantation.

Methods: We studied 50 kidney transplant recipients (13 female, 37 male; mean age: 38.12 ± 13.67). Blood samples were collected immediately before and after surgery at day 1, day 7, month 1 and month 3. Serum IL-18 levels were measured by ELISA using a commercial kit (eBioscience Human IL-18 ELISA). Serum creatinine levels were analysed by modified Jaffe method in Cobas 8000 analyser. GFR was estimated by Modified Diet in Renal Disease (MDRD) equation. Patients were assigned to 2 groups: defined slow graft function (SGF) as a reduction in serum creatinine by <70% on day 7 and immediate graft function (IGF) as ≥70%. Data were expressed as mean ± Standard errors. We compared IL-18 levels with between patients with IGF and SGF using both Mann-Whitney U and Student's t tests.

Results: Among 50 recipients, 6 had SGF, and 44 had IGF, no patient had delayed graft function (DGF; required dialysis within 1 week of transplant). Serum IL-18 levels were statistically different between groups before transplantation (673.02 ± 75.70 pg/mL in IGF and 1074.59 ± 368.52 pg/mL in SGF (p<0.05). Serum IL-18 levels on day 7 were significantly higher in SGF compared to IGF. There were no significant differences in serum IL-18 levels between the groups on the first day of transplant (640.72 ± 93.48 pg/mL vs 675.53 ± 165.56, p>0.05).

Conclusions: Furthermore, our preliminary findings suggest that serum IL-18 might be considered a useful marker of predicting graft function after renal transplantation. The sequential monitoring of serum IL-18 in kidney transplant recipients might be recommended. Future larger studies are needed to confirm our findings.

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Incidence of chromosomal abnormalities in 13,920 samples from various regions of Brazil.

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Background: The prevalence of chromosomal abnormalities is of great importance to support clinical and appropriate genetic counseling. Brazil is divided in five geographic macroregions with cultural, racial, climatic and socio-economic patterns. The aim of this study was to evaluate the incidence of chromosomal abnormalities in samples sent from the five macroregions of Brazil.

Methods: We conducted a retrospective study of the karyotypes results from a database of cytogenetic studies of samples sent to a cytogenetic service during the period of January 2006 to January 2012. The karyotypes were previously carried out with the G-banding and the cells obtained from culture of peripheral blood lymphocytes.

Results: We evaluate the 13,920 samples from all Brazilian regions, 11,206 (80.5%) were classified as normal as 43.78% 46,XX and 36.72% 46,XY. 2714 (19.5%) samples showed results with some abnormalities and among them, the most frequent was trisomy 21 (57.59%). The male to female ration of the trisomy 21 was 1.3. Of the tested samples, 193 (1.39%) showed monosomy X - Table 1

Conclusions: Following the results obtained with the studied population, normality corresponded to 80.50%. Trisomy 21 was the most frequent autosomal abnormality with slightly higher male to female ratio than describe in the literature. There is no significant relationship with any discrepancy between the increase and decrease of chromosomal abnormalities and the Brazilian macroregions.

Retrospective analysis of 13,920 distributed by regions of Brazil

TABLE 1:	NORTH		NORTHEAST		MIDEAST		SOUTHEAST		SOUTH		TOTAL	
NORMAL	817	79,86%	2,584	77,64%	1,089	82,50%	3,301	83,17%	3,415	79,79%	11,206	80,50%
Female		41,84%		42,52%		47,58%		43,61%		44,21%		43,78%
Male		38,03%		35,13%		34,92%		39,56%		35,58%		36,72%
Trisomy 21	116	11,34%	447	13,43%	117	8,86%	361	9,10%	522	12,20%	1,563	11,23%
Female		4,59%		5,23%		4,02%		4,08%		5,70%		4,89%
Male		6,74%		8,20%		4,85%		5,01%		6,50%		6,34%
45,X	20	1,96%	68	2,04%	23	1,74%	34	0,86%	48	1,12%	193	1,39%
Others abnormalities	70	6,84%	229	6,88%	91	6,89	273	6,88%	295	6,89%	958	6,88%
TOTAL	1,023		3,328		1,320		3,969		4,280		13,920	

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Routine Reflex-Testing for Urinary Lipid Bodies is Not Justified

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Background: The presence of lipid bodies in urine specimens is considered an abnormal finding and requires additional investigation. Urinary lipid bodies are seen in patients with renal glomerular disease causing severe proteinuria e.g. nephrotic syndrome. Routine practice has been to automatically reflex urine specimens with protein concentrations of 300 mg/dL or greater for lipid bodies analysis by addition of Sudan IV stain to urine sediments and by polarization microscopy. We investigated the prevalence of urinary lipid bodies in urine specimens with protein concentration of 300 mg/dL or greater and compared laboratory findings with patients' clinical diagnosis.

Methods: Urine specimens with protein concentrations of 300 mg/dL or greater as determined by Multistix 10SG (Siemens) over a six month period were reviewed for the presence of lipid bodies. Patients' laboratory findings were reviewed for clinical correlation.

Results: 103 urine specimens with protein concentrations of 300 mg/dL or greater were reviewed during the study period. The majority of specimens (n=82) had protein concentrations between 300 mg/dL and 1000 mg/dL with only 18 specimens positive for lipid bodies (8 being free bodies), whereas the remaining 64 samples were free of lipid bodies. In the 21 specimens with protein concentrations greater than 1000 mg/dL, only 9 specimens showed the presence of lipid bodies. Overall, lipid bodies were present in 27 specimens (26%). The majority of the specimens 76 (74%) were negative for lipid bodies. There was no association between the extent of proteinuria and the presence of lipid bodies (P=0.5). Review of clinical diagnosis and other laboratory findings showed evidence for renal dysfunction in 26 of the 27 lipid bodies positive specimens.

Conclusions: Review of lipid bodies results following reflex testing of proteinuria specimens were negative for the majority of the specimens. All of those that were positive, with the exception of one patient, had an expected diagnosis of renal dysfunction. This low prevalence among patients with proteinuria does not justify routine reflex testing of all specimens for lipid bodies analysis. Discrete testing is therefore recommended.

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Beneficial Biological Effects of Coxib Therapy on the Progression of Periodontal Disease. A prospective Study in a Romanian Clinical Trial

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Background: Recent studies revealed that patients who regularly use anti-inflammatory drugs have a lower progression of periodontal disease.

Objective: To analyze the effects of new non-steroidal anti-inflammatory drugs (coxibs) on cyclooxygenase concentrations within the crevicular fluid in a cohort of patients with periodontitis from southwestern Romania.

Materials and Methods: The 77 patients from the study population (15-25 years), either non-smokers or previous smokers, with severe chronic local juvenile periodontal disease, were monitored for a period of 180 days. Exclusion criteria: subjects with cardiovascular disease and diabetes. Patients were treated with systemic etoricoxib or celecoxib, at baseline and 10 days/month. During the 6-month treatment phase, clinical parameters and blood smear from crevicular fluid (CF) samples were taken at 2-month intervals. Radioimmunoassay of CF samples were performed for prostaglandin E2 (PGE2) and thromboxane B2 (CF-TxB2), by RIA or ELISA.

Results: The analysis of variance revealed a highly significant increase in prostaglandin E2 at values of 3-fold over baseline by 6 month (P=0.005), and significant values were obtained for gingival index (P=0.048). Crevicular fluid thromboxane B2 levels reached a 4-fold peak over baseline at 2 month. Reduction in bleeding was found to be highly significant (P=0.005), and the rate of bone loss increased 44% during the 6-month period, as compared to baseline pretreatment.

Conclusions: The results of this study indicate that the cyclooxygenase pathway activation represents an essential pharmacological modulation involved in the progression of periodontal disease and bone destruction.

Key words: crevicular fluid, prostaglandin E2, thromboxane B2,coxib therapy, periodontitis.

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Role of S100β protein as an early predictor of Brain Death development after sever Traumatic Brain Injury.

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Background: To ascertain the role of S100β protein (S100B Pt) as an early predictor of Brain Death (BD) development after a severe Traumatic Brain Injury (TBI).

Methods: During 36 months 144 severe head injury patients were included. The clinical variables were: gender, age, reference GCS after resuscitation, presence of bilateral mydriasis on admission, prehospital hypotension and desaturation, CT findings, presence of other associated injuries, S100B-Pt levels, as well of the final result to Brain Death. Blood samples were obtained as soon as possible on admission and then every 24 hours until the fourth day of evolution, unless the patient died earlier.

Results: A total of 16 of the patients 11.1% progressed to BD. Median S100B-Pt levels from the group that evolved to BD were daily higher than non-BD group. Each 1µg/L increased in the S100B-Pt value on admission and in the second sample, showed an Odd Ratio of deterioration to BD of 2.625 (95%CI 1.296-5.316) p=0.07 and 3.742 (95%CI 1.172-11.94) p=0.026, respectively. To detect the cut-off point of S100B-Pt level (on admission and 24h) in those patients with a score of V-VI according to the Marshall-CT-Classification that may evolve to BD, we performed a ROC-analysis (p=0.013 and p=0.007 respectively). The cut off point of the admission-sample was 0,473µg/L (sensitivity of 80% and Specificity of 74.5%) and in the 24 hours-sample was 0.371µg/L(sensitivity of 80% and Specificity of 68.6%).

Conclusions: S100B-Pt may become an early and accurate biomarker to predict deterioration to BD after a severe TBI.

E-200

The effectiveness of a Methicillin-Resistant Staphylococcus Aureus (MRSA) rapid screening program for high risk patients in reducing hospital acquired infections (HAIs) in a community hospital

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Background: Hospital acquired infections (HAIs) are a major cause of morbidity, mortality, increased length of stay and excessive healthcare costs. Invasive MRSA infections occur in approximately 94,000 people annually and cause as many as 9,000 deaths per year, according to the CDC.

Objectives: To perform a four year retrospective impact review of our rapid screening program for MRSA to determine the clinical impact and cost effectiveness of detecting MRSA colonized and/or infected patients using real time rapid polymerase chain reaction (PCR), thereby providing clinicians with actionable information in less than one hour instead of days to reduce MRSA patient to patient transmission. An effective interventional surveillance program combined with strong 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: An integrated, comprehensive, 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 preventionists when MRSA is detected.

Results: Our screening strategy focused on high 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 four years after implementation of the rapid PCR MRSA screening program the infection rate in 2011 was .17/1000. Comparing MRSA infection rates from 2007 to 2011 there was an 82% reduction in MRSA HAI with a corresponding 85.5% reduction in associated infection costs. The four year MRSA surveillance screening costs for Laboratory PCR testing was $352,763. Based on the average cost of medical care for a MRSA infection incurred during a hospital stay of $35,000 dollars per infected patient, we decreased the cost of infection by $2,145,500 during this four year period and the length of stay (LOS) for the critical care units decreased by 21%.

Conclusions: A four year retrospective review of our MRSA rapid screening program for high risk patients using automated molecular detection PCR demonstrates a strong clinical impact and financial effectiveness. The Laboratory's rapid screening program supports prevention strategies that permit rapid identification and interventions that assure patient safety, improves bed management, decreases length of stay, saves millions of dollars in hospitalization and infection costs associated with HAIs, while enhancing patient outcomes and significantly reducing hospital acquired infections.

E-201

Fetuin-A and interleukin-6 levels as inflammation markers in hypertensive patients

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Background: Despite the evidence of importance of reducing blood pressure (BP), hypertension continues to be one of the most commonly occurring diseases in humans. White coat hypertension (WCH), isolated clinic hypertension, is defined as

the observation of BP levels > 140/90 mmHg in several visits to the clinic, while 24-hr ambulatory blood pressure monitoring (ABPM) levels are < 125/80 mmHg. Oxidative stress is thought to play a critical role in the pathogenesis of hypertension. Human fetuin-A (alpha-2-Heremans Schmid glycoprotein), a protein produced by the liver and secreted into serum in high concentrations, is a major serum-based inhibitor of vascular calcification and negative acute phase protein. The role of fetuin-A in cardiovascular disease is still controversial. Some studies stated that low concentrations of fetuin-A in serum is a more important marker at presence of cardiovascular diseases, where as others claimed that high concentrations to be more significant. The aim of this study was to evaluate serum fetuin-A, interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP) levels in essential, white coat and renal hypertensives (RH) and compare with normotensives (NT).

Methods: 22 essential hypertensive (EH) subjects (M/F:4/18) aged 51.59±10.01 years, 22 WCH subjects (M/F: 4/18) aged 51.4 ± 10.16 years, 22 RH subjects (M/F:7/15) aged 55.45±11.46 years and 22 normotensive control subjects (M/F:4/18) aged 49,6±8,26 years were recruited in this study. Serum fetuin-A, IL-6 levels were measured by ELISA; whereas hs-CRP levels were determined by nephelometric assay.

Results: Systolic and diastolic BP were not significantly different between the EH and RH patient groups. Levels of serum fetuin-A, IL-6 and hs-CRP did not differ between the EH and WCH groups. RH group had the lowest fetuin-A and the highest hs-CRP values. The levels of fetuin-A was higher in normotensives than the other groups. Normotensives had lower hs-CRP values than the other groups.

Conclusions: According to our results, low fetuin-A levels may cause atherosclerosis due to inflammation in RH. Fetuin-A and hs-CRP could also be a modulator of inflammation in RH. As it is known atherosclerosis is one of the main factors causing or leading hypertension. Further study is needed to clarify the relationship between fetuin-A, IL-6, and CRP levels or cardiovascular damage/inflammation in patients with hypertensive subjects.

E-202

Incremental Prognostic Value of High-Sensitivity Troponin T in Mortality Risk Prediction of Stable Coronary Artery Disease

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Background: N-terminal pro-B-type natriuretic peptide (NT-proBNP) and cardiac troponins are well established prognostic markers in patients with congestive heart failure and acute coronary syndromes. Recently, NT-proBNP and the new high-sensitivity cardiac troponin have been shown to support prognosis of stable coronary artery disease (CAD). We now evaluated the combined determination of NT-proBNP and the new high-sensitivity troponin T assay (TnT-hs) for risk of mortality prediction in patients with stable CAD.

Methods: We investigated 1469 patients with stable angiographic CAD (gender: 364 women and 1105 men; age: 27 to 89 years, mean 64 years) who participated in the LURIC study during a median follow up of 7 ½ years. Plasma was sampled prior to coronary angiography and stored at -80°C, pending analysis. TnT-hs and NT-proBNP were determined in baseline samples using immunoassays (Roche Diagnostics, Germany).

Results: A total of 525 (35.7%) patients died during 7 ½ years follow up. 263 (17.9%), 187 (12.7%) and 75 (5.1%) died later than 5 years, within 1 to 5 years and within 1 year, respectively, indicating an increasing mortality risk. TnT-hs and NT-proBNP median values of survivors and non-survivors were 8.0 versus 19.6 and 208 versus 757 ng/L, respectively. 34% of the1469 subjects exhibited TnT-hs ≥ 14 ng/L, the 99th percentile cut-off for AMI. Logistic regression found TnT-hs and NT-proBNP as independent risk markers. The incremental value TnT-hs in addition to NT-proBNP was examined in two statistical models (see table 1).

Tab. 1: Contribution of TnT-hs in addition to NT-proBNP for prediction of death

	Logistic Regression	Cut-point Model
AUC of NT-proBNP	0.742	0.680
AUC of TnT-hs	0.725	0.678
AUC of the combined model	0.761	0.742
Increase of AUC by TnT-hs	0.019	0.061
Additional contribution of TnT-hs	p<0.0001	p<0.0001
Net Reclassification Index (NRI*) %	32.4	54.0

*NRI estimates the patients reclassified in the right direction by inclusion of TnT-hs.

Conclusion: Many patients with stable CAD exhibited elevated concentrations of TnT-hs which were strongly associated with mortality. The combined determination of TnT-hs and NT-proBNP was superior for risk stratification compared to determining either marker alone especially in patients with high short-term mortality risk.

E-203

The identification of patients with the clinical diagnosis of fibromyalgia using a multiplex cytokine assay.

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Background: Fibromyalgia (FM) is a medical syndrome characterized by chronic pain and allodynia. Other clinical manifestations include debilitating fatigue, sleep disturbance, joint stiffness and cognitive dysfunction. FM is a diagnosis of exclusion as a test to confirm the diagnosis is lacking. Recent investigations suggest that aberrant expression of immune mediators, such as cytokines, may contribute to the symptoms of FM. Therefore, the cytokine responses to mitogenic activators of peripheral blood mononuclear cells (PBMNCs) isolated from large cohorts of fibromyalgia patients and healthy individuals were compared. The results strongly support an association of specific cytokine expressions with FM.

Methods: A total of 110 FM and 91 control subjects were studied. The study was approved by the IRB of UIC. All FM patients had been clinically diagnosed at least 1 year prior to the study and were off FM-approved drugs for 2 weeks. Suspensions of PBMNCs were isolated from anticoagulated blood and challenged in triplicate in RPMI medium overnight with no mitogen, PHA, or PMA and ionomycin. Cytokine concentrations in plasma as well as in culture supernatants were determined by using a multiplex immunoassay based on Luminex xMAP™ bead array technology. Fluorescence was measured using a Bio-Plex 200 fluorescence bead reader (BioRad Laboratories, Hercules, CA). A custom panel of antibody-conjugated beads for measuring human cytokines IL-6, IL-8, IFN-γ, IL-5, IL-10, MIP-1α, MIP-1β, and MCP-1 (BioRad Laboratories, Hercules, CA) was used according to the manufacturer's instructions. Concentrations were transferred into statistical software, with means and standard deviations calculated for each cytokine. Cytokine concentrations in patients were compared to those of healthy individuals by using two t-tests, Pooled (equal variance) and Satterthwaite (unequal variance) p-values. Initially, the F-test was used to test if the variances of the values in two groups were equal. Since all F-test p-values were below 0.1, except for IL-5 in PMA challenge, a t-test with Satterthwaite's estimate of the variance was used. The confidence level was set at 5%.

Results: Of 91 control subjects, unstimulated PBMC cultures produced low levels of cytokine. Cytokines in about half were below the lower detection limit. In contrast, with PHA and PMA/ionomycin challenges, extracellular expressions of all cytokines, except for IL-5 in PMA/ionomycin challenges, were significantly increased in supernatants. However, when cytokine concentrations of FM patient samples were compared to the control values, the concentrations of most cytokines were lower in FM samples. The decrease was statistically significant for all cytokines except for IL-5 in the PHA challenge.

Conclusions: Decreased PBMNCs responses to mitogen challenges were strongly associated with FM. The suppression of cytokine secretion was statistically significant for all cytokines tested except for IL-5. The cytokine assay used in this study may prove to be a valuable test modality to facilitate the diagnosis of FM.

E-204

Evaluation of serum paraoxonase and arylesterase activities in asthma and chronic obstructive pulmonary disease patients

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Background: Asthma and chronic obstructive pulmonary disease (COPD) are inflammatory lung diseases that are characterized by systemic and chronic localized inflammation and oxidative stress. Recent literature in this field reports conflicting findings. Some studies suggest that asthmatics and COPD patients have a decreased ability to respond to oxidative stress, while others find up-regulated antioxidative function. Paraoxonase-1 (PON-1), which has PON and arylesterase (AE) activities, is a high-density lipoprotein (HDL)-bound antioxidant enzyme that prevents LDL

oxidation. PON-1 also has both antiatherogenic and antiinflammatory properties. The aim of the present study was to determine serum PON-1 and AE activities in asthmatics and COPD patients and healthy subjects. We investigated whether serum PON1 and AE activities differ between asthmatics and COPD patients and demonstrated their relation with inflammatory markers, serum lipid and lipoproteins. Moreover, we also evaluated if the presence of comorbidities affects the activities of serum PON-1 and AE in COPD patients.

Methods: 40 asthmatics (F/M: 24/16; mean age: 39+/-14), 40 COPD patients (F/M: 4/36; mean age: 62+/-10) and 19 healthy controls (F/M: 3/16; mean age:44+/-5) were included in this study. COPD patients were divided in two groups; 20 pure COPD patients and 20 comorbid COPD patients. We excluded patients with hypertension, metabolic syndrome, diabetes mellitus, thyroid, renal, hepatic, rheumatic, cardiac, cerebrovascular, malign, infectious diseases to establish asthma and pure COPD groups. Patients using drugs which could affect PON-1 and AE levels were excluded in these groups. There were 11 hypertensive, 5 diabetic and 4 cardiac patients in the comorbid COPD group. Serum PON-1 and AE activities, leukocyte counts (WBC), erythrocyte sedimentation rate(ESR), high sensitive-CRP (hs-CRP) levels, eosinophil cationic protein (ECP), fasting blood glucose, LDL-cholesterol, triglyceride levels were determined in asthma patients, COPD patients and controls. Serum PON-1 and AE activities as well as blood glucose, LDL-cholesterol and triglyceride were measured by spectrophotometric method; hs-CRP and ECP levels were determined by nephelometric assay and ELISA, respectively.

Results: Mean age was higher, male gender was more prevalent in COPD than other groups. Total cholesterol, HDL- and LDL-cholesterol, triglyceride levels and leukocyte counts did not display any significant difference between COPD patients and healthy controls. In asthma patients LDL-cholesterol was significantly lower; whereas HDL-cholesterol levels were significantly higher compared to controls. Both hs-CRP and ESR values were significantly higher in COPD group than in asthma and control groups. Although PON-1 and AE levels were lower in patients than controls; no difference was found between asthma and COPD groups and also between pure and comorbid COPD patients.

Conclusions: Our findings suggested that the lower PON-1 and AE activities in asthma and COPD patients compared to healthy subjects might be due to imbalance of oxidant/antioxidant system. Although asthma and COPD are two different conditions, serum PON-1 and AE activities cannot be markers of differential diagnosis as they overlap. Due to the usage of drugs such as statins and aspirin, the enzyme activities may not be significantly different between comorbid and pure COPD patients.

E-205

Comparison of diagnostic accuracy for inflammatory bowel diseases (IBD): Promethrus IBD 7 panel versus combined measurements of ASCA and pANCA

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Background Inflammatory bowel disease is a group of chronic autoimmune mediated gastrointestinal inflammatory diseases which mainly compose of two subgroups: ulcerative colitis (UC) and Crohn's diseases (CD). The diagnosis of IBD is often difficult and frequently uses serology testing as pre-test to identify risk patients for further invasive diagnostic approach. We compared the predictive values for IBD of the Prometheus Inflammatory Bowel Disease (IBD) Serology 7 (IBD7) panel (Prometheus Laboratories, San Diego, CA) with that of measurements of only ASCA (IgG and IgA) and pANCA (named as IBD3 in this study) in serum from clinical patients referred for evaluation of suspected IBD.

Patients and Methods Results from 577 patients who had IBD7 ordered in 2010 to 2011 were evaluated. The IBD7 testing was performed in Prometheus Laboratories. The medical records of 264 patients who maintained medical care in Cleveland Clinic Healthcare System were reviewed. The diagnosis of IBD was established on the basis of clinical, laboratory, radiologic, endoscopic, and pathologic evaluations. IBD3 data was extracted from IBD7 reports obtained from Prometheus Laboratories.

Results A total of 120 patients had positive results in the IBD7 and 180 in the IBD3 panel. Three hundred and eighty patients appear testing negative and 104 positive in both IBD3 and IBD7 panels, indicating the concordance between the two approaches reaches 84%. Among 264 patients with medical records, 56 patients appear testing positive and 168 patients testing negative in both IBD3 and IBD7 panels. The concordance between the two approaches reaches 85%. In the discordant cases between IBD3 and IBD7, IBD3 appears negative in 11 cases that IBD7 was positive and actually only two cases truly had IBD (PPD 18%), as verified by reviewing medical records. However, IBD3 detected additional 29 cases as positive that IBD7 was negative, and among them, 10 cases were true IBD (PPD 34%). The PPD values were 59% in 56 patients with positive results in both panels, comparing to 49% in IBD7 and 48% in IBD3 only.

Conclusions Combined measurement of ASCA and pANCA (IBD3) showed similar prediction value for IBD as Prometheus IBD7 in clinical referred patient population. The PPD value is higher for patient with positive result in both panels than that with positive in any single panel.

E-208

6-Biomarker Algorithms Identify Alzheimer's Disease At High Accuracy

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Background: Late-onset Alzheimer's disease (LOAD) is a multifactorial disease. It is characterised by neurofibrillary tangles and amyloid plaques and can only be accurately diagnosed post-mortem. The ε4-allele of apolipoprotein E is a confirmed but insufficient risk factor.

Methods: To find new Alzheimer's disease biomarkers, platelets, which are peripheral model cells of neurons, were investigated. 50 Alzheimer's disease patients and 50 age- and sex-matched individuals were analysed by 2-dimensional differential gel electrophoresis and statistically evaluated. Algorithms of the most significant LOAD-biomarkers were generated using logistic regression and were assessed by Receiver operating characteristic (ROC) curves and Area under the curve (AUC).

Results: Several algorithms that contain five or six of the LOAD biomarkers apolipoprotein E4 (ApoE4), monoamine oxidase B, coagulation factor XIIIa, glutathione S-transferase omega wild type (wtGSTO-1) and mutant (mutGSTO-1), and tropomyosin showed AUC above 0.9 but differed in the weighting of these biomarkers. Best AUC were obtained when our finding that wtGSTO-1 is prominent in nonApoE4 LOAD patients was taken into consideration: Algorithm 1: AUC=0.938 (95% confidence interval 0.8839-0.9923) and Algorithm 2: AUC = 0.952 (95% confidence interval 0.8799-1.0000).

Conclusions: A combination of only six LOAD biomarkers is sufficient to differentiate between LOAD patients and controls at a considerably high accuracy.

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